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15th International Conference on Plant Growth Substances

July 14-18, 1995

Minneapolis, Minnesota USA

PROGRAM – ABSTRACTS

International Plant Growth Substances Association

Held in conjunction with the annual meeting of the
Plant Growth Regulator Society of America
July 18-20, 1995

Friday July 14	Saturday July 15	Sunday July 16	Monday July 17	Tuesday July 18
	8:00-8:30 am **Opening Ceremony** Nicollet ABC	8:00-11:45 am **Symposia** 3) Signaling Molecules I: Elicitors, Jasmonate, Systemin, Etc. Nicollet D1/D2 5) Hormonal Regulation of Membrane Functions Greenway FG 8) Hormone Metabolism Nicollet A 11) Hormones and Stress Nicollet BC **Workshop** 2) Role of Hormones in Root Growth and Development Greenway HIJ		
	8:30-12:00 pm **Plenary Session** 1) Signal Transduction I Nicollet ABC		8:30-11:50 am **Plenary Session** 3) Hormonally Regulated Gene Expression I Nicollet ABC	8:30-11:50 am **Plenary Session** 4) Generating Fundamental Knowledge and Applications Using Transgenic Plants Nicollet ABC
	1:20-5:00 pm **Symposia** 1) Signal Transduction II Nicollet A 6) Hormone Biosynthesis I Nicollet BC 12) Regulation of Flowering Nicollet D1/D2 13) Hormones and Cell Wall Metabolism Greenway FG **Workshop** 1) Auxin-Binding Proteins Greenway HIJ	1:00-3:00 pm **Plenary Session** 2) Integration of Growth Processes: Regulation of Cell Division and Cell Elongation Nicollet ABC	1:20-5:00 pm **Symposia** 2) Hormonally Regulated Gene Expression II Nicollet A 7) Sandoz Sym: Hormone Biosynthesis II Nicollet BC 14) Tropisms Greenway FG 16) Interaction of Hormones with Other Signaling Systems Nicollet D1/D2 **Workshop** 3) The Acid Growth Hypothesis: How to Reach a Consensus Greenway HIJ	1:20-5:00 pm **Symposia** 4) Signaling Molecules II: Phenolic Derivatives Greenway FG 9) Hormone Perception and Sensitivity Nicollet A 10) Hormones and the Cytoskeleton Greenway HIJ 15) Regulation of Growth and Development by Light Nicollet D1/D2 17) Biologically Active Natural Products from Plants and Microorganisms Nicollet BC
6:00-11:00 pm **Opening Reception** Nicollet ABC	5:00-7:00 pm **Odd-Numbered Posters** Exhibit Hall	3:15-10:00 pm **Tour of U of MN Landscape Arboretum, Conference Banquet, and Gospel Concert**	5:00-7:00 pm **Even-Numbered Posters** Exhibit Hall	5:30-10:00 pm **Closing Reception at the Minnesota Zoo**
			8:00-10:00 pm **Silver Medal Lecture** **Award Ceremony** **IPGSA Business Meeting** Nicollet ABC	

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Welcome to the 15th IPGSA Conference

On behalf of the Organizing Committee and the IPGSA we welcome you to the 15th International Conference on Plant Growth Substances.

Since the 14th Conference in Amsterdam in 1991, progress in plant hormone research and developmental plant biology has been truly astonishing. The five "classical" plant hormones, auxin, gibberellin, cytokinin, ethylene, and abscisic acid, have been joined by a number of new signal molecules, e.g. systemin, jasmonic acid, salicylic acid, whose biosynthesis and functions are being understood in ever greater detail. Molecular genetics has opened new vistas in our understanding of transduction pathways that regulate developmental processes in response to hormonal and environmental signals. The program of the 15th Conference includes accounts of this progress and brings together scientists whose work focuses on physiological, biochemical, and chemical aspects of plant growth regulation. While molecular genetics has moved the field further and faster than could have been imagined even a few years ago, natural products chemistry, biochemistry, and physiology will remain an integral part of our attempts to understand hormonally regulated processes in plants. At this Conference we will integrate research approaches at the chemical, biochemical, molecular, cellular, and physiological levels. Sessions are also organized to summarize progress in fields related to hormone action, such as the regulation of developmental plant processes by light.

For the first time, IPGSA is meeting in conjunction with the Plant Growth Regulator Society of America. Joint sessions and events are planned for Tuesday, July 18, and the PGRSA will continue its meeting on July 19 and 20. These sessions will focus on applied aspects of plant growth regulation.

Minnesota is at its most beautiful in July, and the weather should be ideal. Welcome to the United States and Minnesota. We are looking forward to your participation in an exciting and stimulating Conference.

Gary Gardner and Bernard O. Phinney
Co-Chairs, Organizing Committee

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Program

FRIDAY, JULY 14

Opening Reception — **Nicollet Ballroom**

6:00 pm Enjoy the opportunity to visit with old friends and meet new colleagues

SATURDAY, JULY 15

Opening Ceremony — **Nicollet ABC**

8:00 am **Gary Gardner,** Co-chair, Organizing Committee (University of Minnesota, St. Paul)
Mark L. Brenner, Vice President for Research and Dean of the Graduate School, University of Minnesota, Minneapolis; Secretary-Treasurer, IPGSA
Bernard O. Phinney, Co-chair, Organizing Committee and President, IPGSA (University of California, Los Angeles, USA)

Plenary Session 1
Signal Transduction I — **Nicollet ABC**

Chair: **Terri L. Lomax** (Oregon State University, Corvallis, USA)

8:30 am **Terri L. Lomax:** Introduction
8:40 **Ira Herskowitz** (University of California, San Francisco, USA)
Signal transduction and its role in regulating cell division and cell polarity in budding yeast
9:20 **Joseph R. Ecker** (University of Pennsylvania, Philadelphia, USA)
The ethylene signal transduction pathway in *Arabidopsis*
10:00 **Coffee break**
10:30 **Russell L. Jones** (University of California, Berkeley, USA)
Gibberellic acid, abscisic acid, and the calcium-calmodulin signal transduction pathway in barley aleurone cells
11:10 **Günther Neuhaus** (ETH, Zurich, Switzerland)
Analysis of signal transduction in plants

12:00 pm **Lunch**

CONCURRENT SESSIONS:

Symposium 1
Signal Transduction II — **Nicollet A**

Chair: **Anthony B. Bleecker** (University of Wisconsin, Madison, USA)

1:20 pm **Anthony B. Bleecker:** Introduction
1:30 **Anthony B. Bleecker**
The *ETR1* gene from *Arabidopsis* codes for an ethylene receptor

2:00 **Jeffrey Leung** (CNRS, Gif-sur-Yvette, France)
Genetic and molecular approaches to abscisic acid signaling

2:30 **Neil E. Olszewski** (University of Minnesota, St. Paul, USA)
Molecular characterization of *SPY*: a gene involved in response to gibberellins

3:00 **Coffee break**

3:30 **Malcolm J. Bennett** (University of Warwick, Coventry, UK)
Molecular characterization of the *AUX1* gene from *Arabidopsis thaliana*

4:00 **Catherine Perrot-Rechenmann** (CNRS, Gif-sur-Yvette, France)
Molecular cloning and characterization of auxin-binding protein in *Nicotiana tabacum*

4:15 **Claudia Oecking** (Ruhr Universität, Bochum, Germany)
Towards cloning the fusicoccin-binding protein

4:30 **Alison Lovegrove** (Long Ashton Research Station, Bristol, UK)
Gibberellin-binding proteins in aleurone and leaf plasma membrane

4:45 **Olga Kulaeva** (Timiryasev Institute, Moscow, Russia)
Zeatin-binding protein involved in transcription regulation

Nicollet BC

Symposium 6
Hormone Biosynthesis I

Chair: **Jake MacMillan** (Long Ashton Research Station, Bristol, UK)

1:20 pm **Jake MacMillan:** Introduction

1:30 **Clive R. Spray** (University of California, Los Angeles, USA)
Metabolic studies of gibberellin pathways in vegetative tissues of plants

2:00 **Yuji Kamiya** (RIKEN, Saitama, Japan)
Cloning of *ent*-kaurene synthase A and B genes from plants

2:30 **Rod Winkler** (University of Arizona, Tucson, USA)
Cloning and analysis of the *DWARF3* gene of maize

3:00 **Coffee break**

3:30 **Peter Hedden** (Long Ashton Research Station, Bristol, UK)
Dioxygenases of the gibberellin biosynthetic pathway

4:00 **Hui-Hwa Chiang** (Massachusetts General Hospital, Boston, USA)
Cloning of the *Arabidopsis GA4* locus by T-DNA insertion mutagenesis

4:20 **A. Keith Cowan** (University of Natal, Pietermaritzburg, South Africa)
Abscisic acid biosynthesis in a cell-free system prepared from *Citrus sinensis* exocarp

4:40 **Elena Marin** (INRA, Versailles, France)
Cloning of an ABA biosynthetic gene of *Nicotiana plumbaginifolia* by transposon tagging

Nicollet D1/D2

Symposium 12
Regulation of Flowering

Chair: **Maarten Koornneef** (Agricultural University, Wageningen, The Netherlands)

1:20 pm **Maarten Koornneef:** Introduction

1:30 **Maarten Koornneef** (CIT-INIA, Madrid, Spain)
The genetics of flowering time in *Arabidopsis*

2:00 **Elizabeth Dennis** (CSIRO, Canberra, Australia)
Genes modulating the time of flowering in *Arabidopsis*

2:30 **K. Diane Jofuku** (University of California, Santa Cruz, USA)
Control of *Arabidopsis* floral meristem identity and development by photoperiod and gibberellins

3:00	**Coffee break**
3:30	**Richard M. Amasino** (University of Wisconsin, Madison, USA) Regulation of flowering time in *Arabidopsis*
4:00	**James L. Weller** (University of Tasmania, Hobart, Australia) New pea mutants with altered sensitivity to photoperiod
4:20	**Page W. Morgan** (Texas A&M University, College Station, USA) Regulation of flowering and gibberellin metabolism in phytochrome mutants of the short-day monocot *Sorghum bicolor*
4:40	**Arthur W. Galston** (Yale University, New Haven, USA) Floral transition in the long-day plant *Sinapis alba* in relation to putrescine fluxes

Symposium 13 **Greenway FG**
Hormones and Cell Wall Metabolism

Chair: **Daniel Cosgrove** (Pennsylvania State University, University Park, USA)

1:20 pm	**Daniel Cosgrove:** Introduction
1:30	**Maureen McCann** (John Innes Institute, Norwich, UK) Changes in wall architecture during plant cell growth and differentiation
2:00	**Stephen Fry** (University of Edinburgh, UK) Xyloglucan endotransglycosylases (XETs): their action on wall polymers and role in the hormonal control of cell expansion
2:30	**Donald J. Nevins** (University of California, Davis, USA) Wall hydrolysis as a growth mechanism in the mediation of hormone responses
3:00	**Coffee break**
3:30	**Jeffrey P. Woessner** (Washington University, St. Louis, USA) Cell wall assembly and disassembly in *Chlamydomonas reinhardtii*
4:00	**Imad N. Saab** (University of Illinois, Urbana, USA) A novel flooding-responsive gene homologous to the putative cell-wall loosening xyloglucan *endo*-transglycosylase.
4:20	**Gerhard Leubner-Metzger** (Friedrich Miescher Institut, Basel, Switzerland) Evidence for a role of ß-1,3-glucanase in dicot seed germination
4:40	**General Discussion**

Workshop 1 **Greenway HIJ**
Auxin-Binding Proteins

1:20 pm Organizer: **Alan Jones** (University of North Carolina, Chapel Hill, USA)

Poster Presentations 1 **Exhibit Hall**

5:00 pm- **Odd-Numbered Posters**
7:00 pm

SUNDAY, JULY 16

CONCURRENT SESSIONS:

Symposium 3 **Nicollet D1/D2**
Signaling Molecules I: Elicitors, Jasmonate, Systemin, etc.

Chair: **Thomas Boller** (University of Basel, Switzerland)

8:00 am **Thomas Boller:** Introduction

8:10 **Benno Parthier** (Institute of Plant Biochemistry, Halle, Germany)
Jasmonates and their role in stress-related signaling to stress responses

8:40 **Andreas Schaller** (Washington State University, Pullman, Washington)
Systemin: analyses of its signaling pathway

9:10 **Herman P. Spaink** (University of Leiden, The Netherlands)
Signal molecules involved in the formation of root nodules

9:40 **Coffee break**

10:10 **Thomas Boller**
Parallels between elicitor signaling and hormone signaling

10:40 **Suzanne R. Abrams** (NRC, Saskatoon, Canada)
Importance of the chiral centres of jasmonic acid in biological responses in plants

11:00 **Robert A. Creelman** (Texas A&M University, College Station, USA)
Jasmonic acid levels and wound gene expression in linolenic acid deficient and LOX cosuppressed *Arabidopsis*

11:20 **Youngsook Lee** (Pohang University, Korea)
Systemic activation of phospholipases in wounded tomato and broad bean plants

Symposium 5 **Greenway FG**
Hormonal Regulation of Membrane Functions

Chair: **Karen Schumaker** (University of Arizona, Tucson, USA)

8:00 am **Karen Schumaker:** Introduction

8:10 **Michael R. Blatt** (Wye College, UK)
Paradigms for hormonal control of K^+ channels in stomatal function

8:40 **Hélène Barbier-Brygoo** (CNRS, Gif-sur-Yvette, France)
Auxin signaling and action at the plasma membrane of plant cells

9:10 **Rainer Hedrich** (University of Hannover, Germany)
Anions modify the response of guard cell anion channels to auxin

9:40 **Coffee break**

10:10 **Marianne Sommarin** (University of Lund, Sweden)
Mechanism of regulation of the plasma membrane H^+-ATPase

10:40 **Julian I. Schroeder** (University of California-San Diego, La Jolla, USA)
Ion channel-mediated ABA signal transduction in guard cells

11:10 **Robert E. Cleland** (University of Washington, Seattle, USA)
Auxin-induced acidification of the cytoplasm is a light-dependent process in *Avena* coleoptile cells

11:25 **Robert C. Schuurink** (University of California, Berkeley, USA)
Calmodulin levels and a putative CaM- and cyclic-nucleotide-regulated ion channel in barley aleurone

Symposium 8 **Nicollet A**
Hormone Metabolism

Chair: **Gernot Schneider** (Institute of Plant Biochemistry, Halle, Germany)

8:00 am **Gernot Schneider:** Introduction
8:10 **Robert S. Bandurski** (Michigan State University, East Lansing, USA)
Metabolism of indole-3-acetic acid (IAA)
8:40 **David W.S. Mok** (Oregon State University, Corvallis, USA)
Cytokinin metabolic enzymes in *Phaseolus*
9:10 **Takao Yokota** (Teikyo University, Utsunomiya, Japan)
Biosynthesis and metabolism of brassinosteroids
9:40 **Coffee break**
10:10 **Gernot Schneider**
Gibberellin conjugation and seed development
10:40 **Bonnie Bartel** (Whitehead Institute, Cambridge, USA)
Indole-3-acetic acid deconjugation in *Arabidopsis thaliana*
10:55 **Björn Nicander** (Swedish University of Agricultural Sciences, Uppsala, Sweden)
Potato tuber shoots contain an N-glucoside of *cis*-zeatin
11:10 **Melinda N. Martin** (USDA-ARS, Beltsville, USA)
Conjugation of ACC in tomato
11:25 **Nobuhiro Hirai** (Kyoto University, Japan)
8'-Modified abscisic acids as highly potent, long-lasting analogues of abscisic acid

Symposium 11 **Nicollet BC**
Hormones and Stress

Chair: **Elizabeth A. Bray** (University of California, Riverside, USA)

8:00 am **Elizabeth A. Bray:** Introduction
8:10 **Robert E. Sharp** (University of Missouri, Columbia, USA)
Role of ABA in root and shoot growth under water stress: involvement of ethylene
8:40 **Elizabeth A. Bray**
Regulation of gene expression by abscisic acid during drought stress
9:10 **Dorothea Bartels** (Max-Planck-Institut für Züchtungsforschung, Köln, Germany)
Molecular approaches to dissect the ABA signal transduction chain
9:40 **Coffee Break**
10:10 **Salomé Prat** (CID-CSIC, Barcelona, Spain)
Jasmonates as signal molecules in systemic plant responses to wound stress
10:40 **Abul Mandal** (Swedish University of Agricultural Sciences, Uppsala, Sweden)
Identification of abscisic acid-induced genes of *Arabidopsis thaliana* by T-DNA tagging and *in vivo* gene fusion
10:55 **Gábor Galiba** (Hungarian Academy of Sciences, Martonvásár, Hungary)
RFLP mapping of a gene regulating cold- and drought-induced abscisic acid production on chromosome 5A of wheat
11:10 **Jaakko Kangasjärvi** (University of Kuopio, Finland)
Ozone activation of ethylene biosynthesis in tomato proceeds via differential accumulation of transcripts for 1-aminocyclopropane-1-carboxylate synthase genes
11:25 **Jan G.H.M. Rijnders** (University of Nijmegen, The Netherlands)
Growing out of stress: submergence, ethylene and gibberellin in *Rumex* species with contrasting flooding resistance

Workshop 2 **Greenway HIJ**
The Role of Hormones in Root Growth and Development

8:00 am Organizer: **Ann M. Hirsch** (University of California, Los Angeles, USA)

11:45 am **Lunch**

Plenary Session 2 **Nicollet ABC**
Integration of Growth Processes: Regulation of Cell Division and Cell Elongation

Chair: **Bernard O. Phinney** (University of California, Los Angeles, USA)

1:00 pm **Thomas Jacobs** (University of Illinois, Urbana, USA)
Control of cell division in plants

1:40 **Daniel Cosgrove** (Pennsylvania State University, University Park, USA)
Expansins - their properties and functions in plant cell enlargement

2:20 **Hans Kende** (Michigan State University, East Lansing, USA)
Integration of growth processes in deepwater rice

Conference Banquet and Excursion **Minnesota Landscape Arboretum**

3:15 pm **Board Buses in Front of the Hyatt Regency for Tour of the University of Minnesota Landscape Arboretum, Conference Banquet and Gospel Concert**

MONDAY, JULY 17

Plenary Session 3 **Nicollet ABC**
Hormonally Regulated Gene Expression I

Chair: **Gretchen Hagen** (University of Missouri, Columbia, USA)

8:30 am **Gretchen Hagen:** Introduction

8:40 **Keith R. Yamamoto** (University of California, San Francisco, USA)
Signals and surfaces: making one transcriptional regulator play multiple roles

9:20 **Steffen Abel** (Plant Gene Expression Center, Albany, USA)
Early auxin-induced gene expression

10:00 **Coffee break**

10:30 **Thomas J. Guilfoyle** (University of Missouri, Columbia, USA)
Modular structure of minimal auxin response elements

11:10 **John V. Jacobsen** (CSIRO, Canberra, Australia)
How does a GA drive gene expression?

12:00 pm **Lunch**

CONCURRENT SESSIONS:

Symposium 2 **Nicollet A**

Hormonally Regulated Gene Expression II

Chair: **John V. Jacobsen** (CSIRO, Canberra, Australia)

1:20 pm **John V. Jacobsen:** Introduction

1:30 **Kazuo Shinozaki** (RIKEN, Tskuba, Japan)
Analysis of ABA-inducible genes and their gene expression in *Arabidopsis thaliana* under drought conditions

2:00 **William R. Woodson** (Purdue University, West Lafayette, USA)
Ethylene-responsive gene expression during flower senescence

2:30 **Jan M. Kooter** (Vrije Universiteit, Amsterdam, The Netherlands)
Hormonal control of anthocyanin pigmentation genes in developing petunia flowers

3:00 **Coffee break**

3:30 **John C. Rogers** (University of Missouri, Columbia, USA)
Biochemical and molecular dissection of hormone regulated transcription from barley α-amylase gene promoters

4:00 **Frank Gubler** (Co-operative Research Centre for Plant Science, Canberra, Australia)
GA and ABA response pathways in barley aleurone cells: GAMYM is a transcriptional activator of a high-pI α-amylase gene promoter

4:20 **Jill Deikman** (Pennsylvania State University, University Park, USA)
Regulation of gene transcription by ethylene during tomato fruit ripening

4:40 **General Discussion**

Symposium 7 **Nicollet BC**

Hormone Biosynthesis II, The Sandoz Agro, Inc. Symposium on Hormone Biosynthesis

Chair: **Jerry D. Cohen** (USDA/ARS, Beltsville, USA)

1:20 pm **Jerry D. Cohen:** Introduction

1:30 **Sharman D. O'Neill** (University of California, Davis, USA)
Interorgan coordination of postpollination development in flowers by spatial regulation of ethylene biosynthetic genes

2:00 **Jennifer Normanly** (University of Massachusetts, Amherst, USA)
Use of transgenics to examine indole-3-acetic acid biosynthesis in *Arabidopsis thaliana*

2:30 **Janet P. Slovin** (USDA/ARS, Beltsville, USA)
Measurement of IAA turnover and what it can tell us

3:00 **Coffee break**

3:30 **Youji Sakagami** (Nagoya University, Japan)
Biosynthetic studies on 4-chloroindole acetic acid

4:00 **Mark L. Brenner** (University of Minnesota, St. Paul, USA)
4-Cl-IAA, a potent substitute for seeds to promote pea pericarp growth

4:20 **Jutta Ludwig-Müller** (University of Frankfurt, Germany)
Regulation of indole-3-butyric acid biosynthesis in maize

4:40 **Kathleen B. Evensen** (Pennsylvania State University, University Park, USA)
Ethylene in post-pollination responses in a non-climacteric flower

Symposium 14 **Greenway FG**
Tropisms

Chair: **Roger Hangarter** (Indiana University, Bloomington, USA)

1:20 pm **Roger Hangarter:** Introduction
1:30 **Peter Nick** (University of Freiburg, Germany)
Tropisms and the cytoskeleton
2:00 **Terri L. Lomax** (Oregon State University, Corvallis, USA)
Lateral auxin redistribution in two tomato gravitropism mutants supports the Cholodny-Went hypothesis
2:30 **Emmanuel Liscum** (Carnegie Institution of Washington, Stanford, USA)
Genetic dissection of phototropism in *Arabidopsis*
3:00 **Coffee break**
3:30 **Carl R. Simmons** (Yale University, New Haven, USA)
Physiological and molecular analysis of *rgr1*, an *Arabidopsis thaliana* auxin physiology mutant affecting root gravitropism and lateral formation
4:00 **John Digby** (University of York, UK)
Gravitropic set-point angle – a new concept in plant development

Symposium 16 **Nicollet D1/D2**
Interactions of Hormones with other Signaling Systems

Chair: **Russell L. Jones** (University of California, Berkeley, USA)

1:20 pm **Russell L. Jones:** Introduction
1:30 **Simon Gilroy** (Pennsylvania State University, University Park, USA)
Calcium-dependent and -independent signaling pathways in barley aleurone
2:00 **Daniel F. Klessig** (Rutgers University, Piscataway, USA)
Salicylic acid and reactive oxygen species in plant disease resistance
2:30 **M. Kay Walker-Simmons** (Washington State University, Pullman, USA)
Characterization of ABA-responsive protein kinases upregulated during environmental stress
3:00 **Coffee break**
3:30 **Karen S. Schumaker** (University of Arizona, Tucson, USA)
The regulation and role of calcium in cytokinin-induced bud formation in moss
4:00 **Youngsook Lee** (Pohang University, Pohang, South Korea)
Structure and function of microfilaments in guard cells
4:30 **Angela J. Russell** (John Innes Centre, Norwich, UK)
Probing cytokinin action – I
4:45 **Steve Neill** (UWE, Bristol, UK)
Monoclonal antibodies generated against pea guard cells that modulate ABA responses in barley aleurone protoplasts

Workshop 3 **Greenway HIJ**
The Acid-Growth Hypothesis: How to Reach a Consensus

1:20 pm Organizer: **Rainer Hertel** (University of Freiburg, Germany)

Poster Presentations 2 **Exhibit Hall**

5:00 pm-7:00 pm **Even-Numbered Posters**

General Meeting of the IPGSA **Nicollet ABC**

8:00 pm	**Silver Medal Lecture** **Jan A.D. Zeevaart** (Michigan State University, East Lansing, USA)
8:40	**Award Ceremony**
9:00	**IPGSA Business Meeting**

TUESDAY, JULY 18

Plenary Session 4 **Nicollet ABC**
(Joint Session with the Plant Growth Regulator Society of America)
Generating Fundamental Knowledge and Applications Using Transgenic Plants

Chair: **Harry J. Klee** (University of Florida, Gainesville, USA)

8:30 am	**Harry Klee:** Introduction
8:40	**Richard Walden** (Max-Planck-Institut für Züchtungsforschung, Köln, Germany) Using T-DNA tagging to dissect the molecular basis of auxin action
9:20	**Harry J. Klee** Transgenic plants and hormone biology: an overview of the potential and limitations
10:00	**Coffee break**
10:30	**Donald Grierson** (Nottingham University, Loughborough, UK) Controlling growth, ripening and senescence by manipulating hormone levels in transgenic tomato plants
11:10	**Göran Sandberg** (Swedish University of Agricultural Sciences, Umeå, Sweden) Regulation of auxin and cytokinin turnover in plants
12:00 pm	**Lunch**

CONCURRENT SESSIONS:

Symposium 4 **Greenway FG**
Signaling Molecules II: Phenolic Derivatives

Chair: **David G. Lynn** (University of Chicago, USA)

1:20 pm	**David Lynn:** Introduction
1:30	**Andrew N. Binns** (University of Pennsylvania, Philadelphia, USA) Phenolic perception by *Agrobacterium tumefaciens*: a genetic analysis
2:00	**Ann M. Hirsch** (University of California, Los Angeles, USA) The role of plant hormones and hormone inhibitors in the early stages of nodule development in legumes
2:30	**Jean-Pierre Métraux** (University of Fribourg, Switzerland) The phenomenon of systemic acquired resistance and the role of the plant growth regulator salicylic acid
3:00	**Coffee break**
3:30	**Hartmut Förster** (Pädagogische Hochschule, Mühlhausen, Germany) Seasonal activity of uridine 5'-diphosphoglucose:conyferyl alcohol glucosyl transferase activity linked to cambial growth in jackpine
3:50	**Loverine P. Taylor** (Washington State University, Pullman, USA) Flavonols in reproductive organs: effects on plant fertility and fecundity

4:20 **David G. Lynn**
Phenolic signals in pathogenesis

Symposium 9 **Nicollet A**
Hormone Perception and Sensitivity

Chair: **Göran Sandberg** (Swedish Agrigultural University, Umeå, Sweden)

1:20 pm **Göran Sandberg:** Introduction
1:30 **Thomas Schmülling** (University of Tübingen, Germany)
Chemically induced transgenic expression of cytokinin-related *Agrobacterium* T-DNA genes: hormonal aspects of gene product activity
2:00 **Olof Olsson** (Swedish Agricultural University, Umeå, Sweden)
Expression of the *Agrobacterium rolC* gene in transgenic poplar; effects of the RolC protein on hormone homeostasis and seasonal variation in *rolC* promoter activity
2:30 **Hélène Barbier-Brygoo** (CNRS, Gif-sur-Yvette, France)
Alterations of auxin perception in tobacco protoplasts transformed by the *rolB* gene from *Agrobacterium rhizogenes*
3:00 **Coffee break**
3:30 **Paolo Costantino** (University of Rome, Italy)
Regulation and function of *rolB* in meristem formation
4:00 **Yi Li** (Kansas State University, Manhattan, USA)
Effects of clinorotation and microgravity on expression of *SAUR* and *GH3* genes in transgenic plants
4:15 **Peter J. Davies** (Cornell University, Ithaca, USA)
Genetic dissection of the relative roles of auxin and gibberellin in the regulation of stem elongation in intact light-grown pea plants
4:30 **Catherina Coenen** (Oregon State University, Corvallis, USA)
The tomato gene *diageotropica* affects responses both to auxin and cytokinin
4:45 **Volker Magnus** (Ruder Bošković Institute, Zagreb, Croatia)
Structure-activity correlations for ring-fluorinated indole-3-acetic acid

Symposium 10 **Greenway HIJ**
Hormones and the Cytoskeleton

Chair: **Hiroh Shibaoka** (Osaka University, Japan)

1:20 pm **Hiroh Shibaoka:** Introduction
1:30 **Anne L. Hitt** (Worchester Foundation for Experimental Biology, Shrewsbury, USA)
Ponticulin, the major high-affinity link between the plasma membrane and the cortical actin network
2:00 **Clive W. Lloyd** (John Innes Centre, Norwich, UK)
Dynamic reorientation of cortical microtubules and the effects of gibberellic acid
2:30 **Peter Schopfer** (University of Freiburg, Germany)
Microtubule reorientation by mechanical stress
3:00 **Coffee break**
3:30 **Karl H. Hasenstein** (University of Southwest Louisiana, Lafayette, USA)
Probing microtubule function in root gravitropism using cytoskeletal inhibitors
3:50 **Gloria K. Munday** (Wake Forest University, Winston-Salem, USA)
NPA binding activity is associated with the cytoskeleton *in vitro*

4:10 **Hiroh Shibaoka**
Hormone-induced reorientation of microtubules in cells with the wall of a crossed polylamellate structure

Symposium 15 **Nicollet D1/D2**
Regulation of Growth and Development by Light

Chair: **Joanne Chory** (The Salk Institute, La Jolla, CA, USA)

1:20 pm **Joanne Chory:** Introduction
1:30 **Lon S. Kaufman** (University of Illinois, Chicago, USA)
Blue light regulation of gene expression
2:00 **Roger P. Hangarter** (Indiana University, Bloomington, USA)
Interaction between the phytochrome and gravity sensory/response systems in *Arabidopsis*
2:30 **Joanne Chory**
Signal transduction pathways controlling light-regulated development in *Arabidopsis*
3:00 **Coffee break**
3:30 **Richard E. Kendrick** (Agricultural University, Wageningen, The Netherlands and RIKEN, Saitama, Japan)
Analysis of the photocontrol of anthocyanin biosynthesis in tomato seedlings using photomorphogenic mutants
4:00 **James L. Weller** (University of Tasmania, Hobart, Australia)
Recent advances in the genetic control of photomorphogenesis in pea
4:15 **Elizabeth Van Volkenburgh** (University of Washington, Seattle, USA)
Light-stimulated growth of leaf cells
4:30 **Colin G.N. Turnbull** (University of Queensland, Australia)
Gibberellins, photoperiod and petiole growth in strawberry
4:45 **General Discussion**

Symposium 17 **Nicollet BC**
(Joint Session with the Plant Growth Regulator Society of America)
Biologically Active Natural Products from Plants and Microorganisms

Chair: **Horace G. Cutler** (USDA, Athens, USA)

1:20 pm **Horace G. Cutler:** Introduction
1:30 **Stephen O. Duke** (USDA, Stoneville, USA)
Novel mechanisms of action of microbial phytotoxins with potential as herbicides
2:00 **Steven F. Vaughn** (USDA, Peoria, USA)
Natural products from plants that influence microorganisms
2:30 **Horace G. Cutler**
Natural products from microorganisms that influence growth and development in plants
3:00 **Coffee break**
3:30 **Y. Fujii** (Shikoku National Agricultural Experiment Station, Kagawa, Japan)
The use of allelopathic chemicals in sustainable agriculture
4:00 **General Discussion**

Closing Reception and Dinner **Minnesota Zoo**
(Joint Event with the Plant Growth Regulator Society of America)

5:30 pm **Board Buses in Front of the Hyatt Regency for the Minnesota Zoo**

Special Events

1. Opening Reception, Nicollet Ballroom, Hyatt Regency Minneapolis
Friday, July 14, 1995, 6:00 pm to 11:00 pm

This event will welcome you to the Twin Cities and give you the opportunity to visit with old friends and meet new colleagues. A buffet will be provided featuring delicacies from several international cuisines. This event is included in Registration Fee.

2. The Minnesota Landscape Arboretum, Conference Banquet, and Gospel Concert
Sunday, July 16, 1995, 3:15 pm to 10:00 pm

At the Minnesota Landscape Arboretum, a unit of the University of Minnesota Department of Horticultural Science, you'll discover the beauty that this unique public garden offers: spectacular annual and perennial display gardens; extensive horticultural collections of northern climate plants; a conservatory with tropical plants, houseplants, cactus and succulents; and six miles of hiking trails through 905 acres of rolling hills, woodlands, prairie, and marsh. Free walking tours of the gardens and free narrated tram tours aboard the "Trumpet Creeper" will be available for Conference participants, .

Following your extended tour of the gardens, the official Conference Banquet will be held under a tent on the Arboretum grounds. The gala dinner, complete with beverages, will be prepared by one of the Twin Cities' leading caterers.

After dinner, you will be treated to a concert of African-American gospel music featuring Robert (Eddie) Robinson. Mr. Robinson, a native of Minnesota, is called the "Pavorotti of Gospel" in the Twin Cities; he is known for his powerful delivery and extraordinary range. He will be accompanied this evening by a group of four vocalists and four instrumentalists. This event will be a unique combination of outstanding experiences that you will never forget.

Buses will depart from the Hyatt at 3:15 pm, and they will begin the return journey from the Arboretum to Downtown at approximately 9:30 pm. Included in the cost of this event are bus transportation, admission to the Arboretum, a full course dinner, beverages, and the gospel concert. A separate ticket is required.

3. Closing Reception and Dinner, Minnesota Zoo
Tuesday, July 18, 1995, 5:30 pm to 10:00 pm
(Joint with the Plant Growth Regulator Society of America)

The Minnesota Zoo, located in suburban Apple Valley, is the site of the closing event of the 15th International Conference on Plant Growth Substances as well as the opening reception for the Plant Growth Substances of America Annual Meeting. Our reception will be held in the Tropics atrium, adjacent to the Tropics Trail which provides lush sights and sounds of a Southeast Asian rain forest. A buffet dinner will feature specialties of Minnesota.

This event, part of the Registration Fee, includes bus transportation, admission to the Zoo, and a buffet dinner. Buses will depart from the Hyatt at 5:30 pm, and they will leave from the Zoo at approximately 9:00 pm to return to the Hyatt.

Welcome to the Twin Cities

The Twin Cities of Minneapolis and St. Paul are situated on the banks of the Mississippi River and are the cultural and artistic center for the Upper Midwest. They are renowned for their diverse cultural attractions in a clean, friendly urban setting. With more than 100 lakes within the boundaries of the two cities, one is never far from water. Although the lakes are beautiful year-round, in the summertime they are popular sites for walking, running, roller-blading, biking, skateboarding, sailing, and windsurfing.

THEATER. The Twin Cities metropolitan area is home to more than 90 theaters. Most famous is the Guthrie, one of the world's best regional theaters, whose repertory company produces outstanding theater year round. The Guthrie is housed in a spectacular building, designed by architect Ralph Rapson. The State, Fitzgerald, and Orpheum theaters present national touring companies of major productions. Local companies such as Penumbra, Illusion, and Mixed Blood present top-notch seasons that include productions throughout the summer months.The Twin Cities are also home to a variety of specialty theaters. The award-winning Penumbra Theater, in St. Paul, is one of only twelve professional black theater companies in the United States. The Heart of the Beast Puppet Theater, in Minneapolis, works with life-size banraku puppets. The Chanhassen Dinner Theater, located in a western suburb, is the largest theater complex of its kind in the nation.

MUSIC. As home to two major state-of-the-art recording studios, the Twin Cities supports an extraordinarily high level of local musical talent. Jazz, gospel, blues, country, folk, and ethnic musicians perform nightly in clubs throughout Minneapolis' Riverbank, West Bank, and downtown Warehouse districts, in St. Paul's Bandana Square and Warehouse District, and elsewhere throughout the metro area. Watch for the Steele Family, Robert "Eddie" Robinson, Moore by Four, Dennis Spear, and others. The St. Paul Chamber Orchestra and the Minnesota Orchestra (housed in Minneapolis' acoustically perfect Orchestra Hall) produce first-rate classical music throughout the year.

ART. The Minneapolis Institute of Arts is famous for its Chinese Jade Collection. Minneapolis' Walker Art Center is internationally known for its inventive exhibits of contemporary art, its Sculpture Garden and Conservatory, and its graceful foot bridge over Hennepin Ave. The Walker shares a beautiful building with the Guthrie Theater. St. Paul's Landmark Center presents travelling exhibits. Minneapolis' newest art museum, the Weisman, houses a contemporary collection. Perched high on the bluffs overlooking the Mississippi River, on the University of Minnesota's East Bank Campus, the building won a design award for architect Frank O. Gehry. In the late afternoon its gleaming facets reflect the sunset in a glorious display for pedestrians who cross the river from west to east.

SCIENCE. The Science Museum of Minnesota and its Omni Theater cater to children with many "hands on" exhibits. The Minnesota Zoo, located in a suburb, prides itself on its huge outdoor exhibits where many different kinds of animals co-exist. It also has an indoor tropical exhibit, and a manmade coral reef. The University of Minnesota Landscape Arboretum runs a major horticultural education center for children throughout the metropolitan area. Located west of Minneapolis and across the street from the Horticultural Research Center, the Arboretum provides a favorite outing with beautiful vistas, hiking trails, indoor tropical collections, and outdoor annual and perennial gardens. Inside the Snyder building, the Arboretum offers a pleasant tea room, and an excellent horticultural library and reading room, appointed with one of the world's largest collection of furniture by Japanese-American designer George Nakashima.

ETHNICITY. Throughout the Twin Cities, restaurants, bakeries, delicatessens, and craft shops reflect the population's ethnic diversity. Scandinavian, Slavic, German, Thai, Hmong, Cambodian, Latino, "Soul," specialties -- and more -- are all available. Ethnic festivals are held throughout the summer and are open to the public. Each week's special celebrations are listed in local newspapers.

HISTORY. St. Paul's famous Summit Avenue has one of the nation's most extensive collection of turn of the century mansions, capped off by the James J. Hill Mansion. St. Paul's new Historical Museum depicts local history in a way that excites children. The beautiful State Capitol building, was built by the same architect who designed the United States Capitol. The Capitol and the domed St.Paul Cathedral sit on adjoining hills in downtown St. Paul. Minneapolis offers the Fort Snelling Living History Museum. Here the docents wear costumes from the early 1800's as they bake bread, work in the blacksmith's forge, hold military drills, etc. West of Minneapolis, in Shakopee, a working village has been collected of houses that were built when the prairie was first settled, in the last century. These two living museums bring to life the early frontier days in the Upper Midwest of the United States.

SHOPPING. Downtown Minneapolis and St. Paul shopping districts feature the famous skyway systems that connect department stores, shopping centers, eateries, and businesses. Heated in winter and air conditioned in summer, these skyways allow 20th-century business and commerce to proceed, uninterrupted by Minnesota's always harsh winters and sometimes uncomfortable summers. Minneapolis' Uptown district and St. Paul's Grand Avenue (near Victoria) offer a large variety of small shops that range from fashionable to funky. Of course the new Mall of America, located south of Minneapolis, near the Minneapolis Airport, is the largest indoor shopping mall in the United States.

FESTIVALS. During your stay in the Twin Cities there will be two major festivals, the Aquatennial and the Viennese Sommerfest. The Aquatennial features water sports including the famous, and hilarious, milk-carton boat race and a major parade through downtown Minneapolis. The Viennese Sommerfest is located in downtown Minneapolis at Orchestra Hall. The outdoor Peavey Plaza is transformed into a Viennese marketplace, and music lovers divide their attention among concerts, bratwurst, and colorful folk dancers.

FURTHER AFIELD. For those with extra time, the North Shore along Lake Superior and the Boundary Waters Wilderness Area of northern Minnesota offer sharp contrasts to life in the Twin Cities metro area. The terrain becomes more rugged as you move further north. The North Shore offers numerous state parks, campgrounds, hiking trails, fishing lakes, and more. The pristine lakes, magnificent forests, exciting rapids, and wildlife make a canoe trip through the Boundary Waters the experience of a lifetime.

CLIMATE. The weather in the Twin Cities in July is generally quite pleasant, with an average high temperature of 83°F (28°C). Although the average monthly rainfall for July is 3.33" (8.5 cm), the probability of a sunny day is 72%. The days are long -- over 15 hours of daylight in mid-July -- and the average relative humidity is a comfortable 50-55%. A light sweater or jacket is sometimes necessary in the evenings.

General Information

Registration and information: The Registration/Information desk is located on the ground floor of the Hyatt hotel next to the Nicollet ballroom. Please contact this desk for information about registration, hotel accommodations, special events, city bus schedules, the optional tour programs, and any other questions. A message board will be located next to the desk.

The Registration/Information desk will be open:

Friday,	14 July	2:00 pm - 8:00 pm
Saturday,	15 July	8:00 am - 5:00 pm
Sunday,	16 July	8:00 am - 4:00 pm
Monday,	17 July	8:00 am - 5:00 pm
Tuesday,	18 July	8:00 am - 5:00 pm
Wednesday	19 July	8:00 am - 5:00 pm

Banking facilities: There are several banks within walking distance of the Hyatt hotel. They are open from 9:00 am until 5:00 pm Monday through Friday. Information on which banks will exchange foreign currency is available at the front desk of the Hyatt hotel and the Registration/Information desk. Currency may also be exchanged at the front desk of the Hyatt, although supplies may be limited.

Name badges: Name badges are required for all conference events. Only registered participants who are wearing their name badge will be admitted to the lecture halls. All participants and accompanying persons are kindly requested to wear their name badge during all conference events. Please note that accompanying persons are not entitled to attend sessions. For the Arboretum banquet and tours, tickets are required in addition to your name badge.

Business facilities: The Hyatt hotel has a business center equipped with a copy machine, computer, printer and fax. Please contact the front desk of the hotel for location and the cost of using the center. Kinko's (IDS Center, Suite 180, 343-8000) and Beckwith (100 Pillsbury Center, 332-2606) are two economical, commercial business centers located close to the Hyatt hotel. They have extensive equipment and will assist you immediately.

Emergency Services: If a medical emergency arises, please go to the nearest hotel phone and dial the operator. If you are near a public telephone, please dial 911 for police, fire, and medical emergencies. There is no charge for this call.

Messages: Messages for participants may be placed on the message board next to the Registration/Information desk. Please check it regularly.

Car parking: There is a parking ramp next to the Hyatt hotel and one across the street next to the Park/Regal hotel. Both charge for parking by the hour.

Rental cars: Cars may be rented at the Hyatt hotel. Please contact the Registration/Information desk for special conference rates.

Public transportation: Taxis will be summoned by the doorman at the Hyatt hotel. You do not need to book them in advance. City busses stop next to the Hyatt hotel. Bus schedules may be obtained at the Registration/Information desk or by calling the Metropolitan Transit Commission at 349-7000. They go all over the cities including to the Mall of America. If you intend to take the van on your return to the airport, please call them 24 hours in advance to reserve a place.

Shopping: Most stores are open from 10:00 am until 6:00 pm Monday through Saturday. On Sunday most stores are open from 12:00 noon until 6:00 pm. The main shopping in downtown Minneapolis is located five blocks north of the Hyatt hotel on the Nicollet Mall. The largest shopping mall in the United States, the Mall of America, can be reached by taxi or by a 30-minute bus ride from the Hyatt hotel.

Tipping: Tips are expected for all services. In a restaurant 15% of the total is customary. Bellhops should be tipped $1 for each bag. Taxi drivers should be tipped 10%.

Insurance: Registration for this congress implies that the participant agrees that the organizers do not assume any liability whatsoever. Participants are requested to make their own arrangements in respect to medical, travel, and personal insurance.

Liability: The organizers cannot assume liability for changes in the program due to external or unforeseeable circumstances.

Instructions for Poster Presentations

The Exhibit Hall will be open for poster viewing for the entire Conference. On Saturday, July 15, Sunday, July 16, and Monday, July 17, the Hall will be open from 8:00 am to 11:00 pm, and on Tuesday, July 18, the Hall will be open from 8:00 am until noon.

1. Bring long point push pins to mount posters. Posters should be mounted and remain up as follows:

   Posters may be mounted after 1:00 pm Friday, July 14, and should remain up until 12:00 Noon Tuesday, July 18. Authors of odd-numbered posters must be present to discuss their work from 5:00 - 7:00 pm, Saturday, July 15, and authors of even-numbered posters must be present from 5:00 - 7:00 pm, Monday, July 17. Posters must be taken down by midnight, July 18. The Conference cannot be responsible for posters not removed by that time.

2. All illustrations should be made prior to the meeting and brought to the assigned room where large (4 ft high x 8 ft long or 1.2 m high x 2.4 m long) bulletin boards will be set up. Each poster is assigned one half (4 ft x 4 ft or 1.2 m x 1.2 m) of a board. The authors will mount their posters on the assigned space prior to the Opening Ceremony, 8:00 am, July 15.

3. If appropriate, hand-out material may be supplied via a pocket or envelope on the board. Do NOT leave papers, cartons, etc. on the floor near your poster; they will be removed.

4. Authors should remain with their posters for the allotted time and take an active role in the presentation of data and answering questions.

Slide Preview Room

The Regency Room, located on the second floor near the Greenway Ballroom, will be available as a slide preview room. The area will be equipped with Carousel slide viewers and a limited number of Carousel trays.

Room Hours:

Friday, July 14	6:00 pm to 9:00 pm
Saturday, July 15	7:30 am to 3:30 pm
Sunday, July 16	7:30 am to 3:30 pm
Monday, July 17	7:30 am to 3:30 pm
Tuesday, July 18	7:30 am to 3:30 pm

Key to Abstracts of Invited Presentations

	Abstract No.
P1: Signal Transduction I	001-004
P2: Integration of Growth Processes	005-007
P3: Gene Expression I	008-011
P4: Transgenic Plants	012-015
S1: Signal Transduction II	016-019
S2: Gene Expression II	020-024
S3: Signaling Molecules I	025-028
S4: Signaling Molecules II	029-033
S5: Membrane functions	034-038
S6: Biosynthesis I	039-043
S7: Biosynthesis II	044-048
S8: Metabolism	049-052
S9: Perception/Sensitivity	053-055
S10: Cytoskeleton	056-059
S11: Stress	060-063
S12: Flowering	064-067
S13: Cell Wall Metabolism	068-071
S14: Tropisms	072-076
S15: Regulation by Light	077-080
S16: Interactions with Other Signaling Systems	081-085
S17: Active Natural Products	086-089
W1: Auxin-binding Proteins	090-092

Key to Abstracts of Posters

(Some of these have been selected for oral presentations in Symposia)

	Abstract No.
S6: Biosynthesis I	093-129
S7: Biosynthesis II	130-153
S8: Metabolism	154-183
S1: Signal Transduction II	184-211
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S5: Regulation of Membrane Functions	226-236
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S4: Signaling Molecules II	279-284
S2: Gene Expression II	285-316
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S14: Tropisms	410-412
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001

SIGNAL TRANSDUCTION AND ITS ROLE IN REGULATING CELL DIVISION AND CELL POLARITY IN BUDDING YEAST

Ira Herskowitz. Dept. of Biochemistry & Biophysics, Univ. California, San Francisco, CA 94143-0448 USA FAX 415 476-0943

Yeast has two haploid cell types, **a** and α, which mate to form the third cell type, the **a**/α cell. Mating is coordinated by peptide pheromones, **a**-factor and α-factor, produced by **a** and α cells respectively. They evoke transcriptional induction of numerous genes, morphological changes, and arrest of the cell cycle in G1 in target cells [1]. The pathway begins with a serpentine receptor and a heterotrimeric G protein, whose beta-gamma subunit activates a MAP kinase cascade containing three protein kinases: STE11 (MEKK), STE7 (MEK), and FUS3/KSS1 (MAPK). The MAPK activates STE12, a transcription factor, which stimulates transcription of genes involved in mating. STE12 and FUS3 activate FAR1 protein, which causes cell-cycle arrest by binding to the cyclin-dependent kinase CDC28-CLN1(CLN2) and inhibiting its activity [2]. Vegetative yeast cells are highly polarized and choose their axis of polarity in a genetically programmed manner. A morphogenetic hierarchy has been established that is responsible for choosing the site for polarity and for organizing cellular components towards it [3]. Cells that mate with each other exhibit a tropism towards their partner, reorienting their cytoskeleton towards the partner. We have recently found that FAR1 is necessary for identifying this new trophic landmark [Valtz, Peter & I.H., in prep.].

[1] Herskowitz, I. (1995). *Cell* **80**:187-197.
[2] Chant, J. (1994). *Trends in Genetics* **10**:328-333.
[3] Peter, M. & I. Herskowitz (1994). *Cell* **79**:181-184.

002

THE ETHYLENE SIGNAL TRANSDUCTION PATHWAY IN *ARABIDOPSIS*

Joseph R. Ecker, Gregg Roman, Madge Rothenberg, Anne Lehman, Barry Lubarsky, David Hall, Qimin Chao, Vered Raz, Jose M. Alonso. & Saeid D. Nourizadeh. Dept. of Biol., Univ. Pennsylvania, Philadelphia, PA E-mail: jecker@atgenome.bio.upenn.edu

Ethylene has a profound influence on plant growth and development. Molecular genetic studies in *Arabidopsis* are beginning to unravel the biochemical processes controlling ethylene perception and signal transduction. The response of etiolated seedlings to ethylene gas, known as the "triple response," has been used to identify mutations that affect hormone production and signal transduction pathways. Several components of the pathway have now been identified by cloning of the *Arabidopsis* mutant genes, and results emerging from these early studies are beginning to paint a tantalizing picture of an evolutionarily conserved signaling system. CTR1 encodes a protein that is most closely related to the Raf (MAPKKK) family of protein kinases while ETR1 is related to "bacterial" two-component histidine kinases (Chang *et al.* 1993). These results, along with genetic epistasis studies, are consistent with a model in which CTR1 and ETR1 act in a multi-step protein phosphorylation cascade. More recently, two positive regulatory genes in the pathway, *ETHYLENE INSENSITIVE 2 (EIN2)* and *ETHYLENE INSENSITIVE 3 (EIN3)* have been isolated by positional cloning and insertional mutagenesis, respectively. *EIN2* encodes an integral membrane protein that shows significant sequence similarity to the mouse Nramp family of "12-pass" membrane proteins. EIN3 is a member of a family of highly similar acidic/basic proteins and is localized in the nucleus. Biochemical studies of these proteins coupled with the identification and cloning of additional *Arabidopsis* genes will allow further dissection of the ethylene gas signaling pathway in plants.

003

GIBBERELLIC ACID, ABSCISIC ACID AND THE CALCIUM-CALMODULIN SIGNAL TRANSDUCTION PATHWAY IN BARLEY ALEURONE CELLS

Robert Schuurink, Willie Lin, Stacey Shartzer and Russell L. Jones. Department of Plant Biology, Univ California, Berkeley, CA 94720-3102. E-mail: rljones@garnet.berkeley.edu

Cytosolic calcium $[Ca^{2+}]_i$ and calmodulin (CaM) are implicated in the response of the cereal aleurone to gibberellic acid (GA) and abscisic acid (ABA). CaM is the principle sensor of $[Ca^{2+}]_i$ in eukaryotic cells, and signalling via the CaM and $[Ca^{2+}]_i$ pathway is mediated by the activated Ca-CaM complex. We have used a molecular approach based on protein-protein interactions to identify targets of the Ca-CaM signalling pathway in the aleurone cell. Recombinant *Arabidopsis thaliana* CaM-2 covalently coupled to horseradish peroxidase (HRP) was used to screen barley aleurone cDNA expression libraries. We isolated seven cDNAs encoding proteins that interacted with the CaM-HRP probe in a Ca^{2+}-dependent manner and two have been studied in detail. One (clone C-13) encodes a protein of 700 amino acids that shows high homology to the *KAT1* potassium channel from *Arabidopsis* and to olfactory channels. Hydropathy plots indicate six transmembrane spanning regions in the deduced sequence of C-13 and CaM and cyclic nucleotide binding domains at the carboxy terminus. The activity of C-13 is being studied by expression of C-13 mRNA in oocytes of *Xenopus laevis*. Another clone encoding a Ca-CaM-binding protein (D-2) encodes an arginine-rich protein showing high homology to DNA binding proteins. Northern blotting shows that the levels of RNA hybridizing to D-2 decline dramatically within one hour of treatment of aleurone layers with GA. The roles of these Ca-CaM binding proteins in signal transduction in the aleurone cell will be discussed.

This research is supported by grants form the National Science Foundation and the National Research Initiative of the USDA.

004

ANALYSIS OF SIGNAL TRANSDUCTION IN PLANTS

Gunther Neuhaus[1], Chris Bolwer[2], & Nam-Hai Chua[3]. [1]Institute of Plant Sciences, ETH-Zurich, CH-8092 Zurich,Switzerland; [2]Stazione Zoologica, Neaples, Italy; [3]Lab. of Plant Molecular Biology, Rockefeller University, New York , USA. E-mail: ipmb3@aeolus.ethz.ch

Light is perceived in plants by four major classes of photoreceptors: the phytochromes, the blue light receptors, the UVA receptors, and the UVB receptors. Of these, the most intensively studied are the phytochromes, which can behave as binary molecular switches due to their ability to exist in two photoreversible forms (Pr and Pfr). Responses mediate by Pfr can be reversed by far-red light, which converts Pfr back to Pr. The plant photoreceptor phytochrome utilises three signal transduction pathways, depending upon calcium and/or cyclic GMP, to stimulate light induced gene expression as well as photomorphogenetic responses (chloroplast formation, anthocyanin production). Using a similar approach we have studied the regulation by phytochrome of a gene that is downregulated by light, asparagine synthetase (AS1). We show that AS1 is repressed in the light and constitutively expressed in the dark, and that the repression in the light is controlled by the same calcium/cGMP-dependent pathway, that is used to activate other light responses. The use of the same signal transduction pathway for both activating and repressing different responses provides a mechanism for phytochrome to act as a molecular switch, one of its well known properties. We also have identified a cis/acting element within the AS! promoter that is both necessary and sufficient for this regulation.

005

CONTROL OF CELL DIVISION IN PLANTS

Thomas Jacobs & Christine Roush, Department of Plant Biology, University of Illinois, Urbana, IL 61801 USA. E-mail: t-jacobs@uiuc.edu.

The conventional view that mitosis merely provides cellular modules for tissue and organ construction in animals does not apply to the plant kingdom, where cell division serves to subdivide three-dimensional space in the expanding organism so as to limit, in proliferating tissues, the cellular volume serviced by a single nucleus. All cells have mechanisms that sense and respond to conditions by passing into or out of the proliferative state. These passages are most likely controlled by plant growth regulators. The MAP kinase pathway has emerged as a biochemical switching module that controls a variety of cellular events in animal, and, we hypothesize, plant cells, including passages into and out of the mitotic state.

We have cloned a MAP kinase-encoding cDNA, MsERK1, from alfalfa, and have used immunoprecipitation and protein kinase assays to investigate stimuli that activate the encoded enzyme. Alfalfa roots respond to auxin treatment (20 μM NAA) with a rapid, biphasic induction in MsERK1 activity. An initial peak at 5 minutes is followed by a slow rise over the following 1.5 hours. MsERK1 activity is induced by cyclohexamide, but not by actinomycin-D. But auxins can induce both expansion and division in plant cells. Fusicoccin (Fc) acidifies cell walls and thus mimics the cell expansion -- but not the cell division -- aspect of auxin action. Fc treatment induces a rapid, biphasic MsERK1 enzyme activation, essentially indistinguishable from that elicited by auxin. This result invites a variety of testable hypotheses regarding potential connections between Fc action and mitotic induction in plants via the MAP kinase pathway.

006

EXPANSINS - THEIR PROPERTIES & FUNCTIONS IN PLANT CELL ENLARGEMENT

D. Cosgrove*, T. Shcherban, J. Shi, Z.-C. Li, M. Guiltinan, Mark Shieh, S. Hoffmann, Department of Biology, Penn State University, University Park, PA 16802

Expansins constitute a family of novel wall proteins that mediate the "acid-growth" responses of tissues and walls by catalyzing the extension of walls when pH is less than 5.5. Cucumber hypocotyls contain at least two forms of expansins, which have slightly different biochemical characteristics. We have evidence for expansins bound to the walls of many growing tissues, including oat coleoptiles, tomato leaves, celery stalks, and cucumber tendrils tendrils. These proteins lack detectable hydrolytic activity; rather, they appear to act by disrupting the adhesion between matrix polysaccharides and cellulose microfibrils. With immuno-gold labeling and electron microscopy, the protein is found specifically and uniformly in the walls, with little or no stratification to particular wall regions. Golgi-derived vesicles are also sometimes found to be heavily labeled, indicating that the protein is packaged and processed in the Golgi before secretion to the wall. Peptide sequences of the purified proteins were used to design oligonucleotide primers for PCR amplification of cDNA from growing cucumber hypocotyls. The specific PCR product was used to isolate expansin clones in a cDNA library. The expansin cDNA codes for a novel protein without sequence motifs previously discovered for catalytic sites or sugar binding domains. We have identified expansin homologs in rice and *Arabidopsis*. Sequence comparisons show that expansins are highly conserved. The results to date indicate that expansins mRNAs are selectively expressed in growing tissues, consistent with their proposed role in plant cell enlargement.

007

INTEGRATION OF GROWTH PROCESSES IN DEEPWATER RICE

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Survival of deepwater rice (*Oryza sativa* L.) depends on its capacity to elongate rapidly when it becomes partially submerged and to keep part of its foliage above rising flood waters. We have reconstructed the chain of events that leads from submergence to accelerated growth. The initial signal for elongation is the altered gas atmosphere in the submerged internode, *i.e.* the reduced level of O_2, and the increased levels of CO_2 and ethylene. Three plant hormones – ethylene, abscisic acid (ABA), and gibberellin (GA) – participate in regulating growth. Reduced O_2 tensions promote ethylene synthesis; ethylene causes a reduction in the level of ABA, a growth inhibitor; and GA is the immediate growth-promoting hormone. The rate of growth may be determined by the balance of endogenous ABA and GA. Internodal elongation is based on increased production of new cells in the intercalary meristem and on enhanced elongation of these newly formed cells. The primary site of GA action is the intercalary meristem. Cells in this meristem first elongate in response to GA; subsequently, cells in the G2 phase of the cell cycle go through mitosis; DNA synthesis is induced only later. Cells that have moved out of the intercalary meristem before GA treatment do not elongate in response to the hormone because the deposition of cellulose microfibrils is reoriented from transverse to oblique with respect to the direction growth. GA causes a delay in this reorientation and an expansion of the internodal elongation zone. Using gene-specific probes for transcripts encoding the cell cycle regulatory proteins $p34^{cdc2}$ protein kinase and cyclin, we showed that GA increases the transcription of one of the two *cdc2* genes and of two cyclin genes. Activation of these genes parallels the transition of the meristematic cells from the G2 to the M phase. We propose that GA acts on cells of the intercalary meristem that have transverse cellulose microfibrils and that GA-induced cell elongation precedes cell division.

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008

SIGNALS AND SURFACES: MAKING ONE TRANSCRIPTIONAL REGULATOR PLAY MULTIPLE ROLES. Keith R. Yamamoto, Department of Cellular and Molecular Pharmacology, UCSF, San Francisco, CA 94143-0450. yamamoto@socrates.ucsf.edu

The glucocorticoid receptor (GR) is a transcriptional regulator that enhances or represses different genes in different cells or physiologic conditions. Hormone binding is an established determinant of its activity. By examining the interaction of GR and its ligands with cellular components, we identified four points at which other signaling pathways might affect GR activity. First, we isolated in yeast a mutant, *lem1*, with altered ligand responsiveness; sequencing and characterization of *LEM1* suggest that it is an ABC transporter that exports particular steroids. This provides a potential mechanism for regulating hormone availability, and thereby signaling, in a cell-specific manner. Second, we isolated point mutants in a yeast Hsp90 homolog that are defective in GR function, and defined distinct phenotypic classes with respect to effects on GR binding and release, and on cellular stress responses. This suggests that Hsp90 can selectively affect its target functions, and conceivably might itself be a signaling target that results in selective modulation of GR activity. Third, we isolated two classes of mutations in the GR zinc-binding region that suggest that the GR-GRE interaction triggers an allosteric change in GR structure that affects its regulatory activity, and that different GRE sequences may induce distinct changes. Thus, GREs may serve as allosteric ligands that signal different GR conformations. Fourth, we identified and mutated GR Ser and Thr residues that are phosphorylated, and showed in yeast that CDK and MAP kinases appear to modulate selectively its transcriptional regulatory activities. This indicates that GR is the recipient of additional signaling inputs that trigger changes in the activities of those enzymes. We conclude that GR may integrate several forms of cellular signaling information, and that these signals therefore are key determinants of the cell- and gene-specificity of GR action in different contexts.

009

EARLY AUXIN-INDUCED GENE EXPRESSION

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The plant hormone auxin transcriptionally activates early genes which provide powerful molecular probes to elucidate auxin signal transduction. We have characterized two primary auxin-responsive genes in pea, *PS-IAA4/5* and *PS-IAA6*, and a large family of related genes in *Arabidopsis thaliana*, *IAA1-IAA15*. The *IAA* genes respond in a highly differential and hierarchical fashion to the hormone. The auxin-responsive *cis*-element of *PS-IAA4/5* has been functionally identified, providing a platform to explore steps between early gene activation and auxin perception. PS-IAA4 and PS-IAA6 are short-lived nuclear proteins. All related IAA-proteins contain functional nuclear localization signals and a putative βαα-fold reminiscent of the β-ribbon DNA recognition motif of prokaryotic repressor polypeptides. These properties suggest a regulatory function of PS-IAA4-related polypeptides in auxin-induced events responsible for plant cell growth.

010

MODULAR STRUCTURE OF MINIMAL AUXIN RESPONSE ELEMENTS

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We have previously reported on the multiplicity of auxin-response elements (AuxREs) in the soybean GH3 promoter and the identification of two independent 25 and 32 bp AuxREs in this promoter [Liu et al. (1994) Plant Cell 6: 645-657] . Related elements are found in the DUE/NDE fragment of soybean SAUR promoters and in other auxin responsive gene promoters [Li et al. (1994) Plant Physiol. 106: 37-43]. We have recently determined that an 11 bp element is sufficient for conferring auxin inducibility to a minimal -46 CaMV 35S promoter. Site specific mutations within the native 11 bp AuxRE have resulted in the identification of a synthetic AuxRE that is more strongly induced by auxin than the natural AuxRE. Other site directed mutations have identified nucleotides critical for auxin induction and revealed a modular structure for AuxREs. We have found that a part of the native 11 bp AuxRE can confer auxin inducibility to a promoter construct containing yeast GAL4 DNA binding sites in the presence of a GAL4-*c*REL activator. We have screened cDNA expression libraries with elements in the GH3 promoter that bind nuclear proteins, and we have cloned two different types of transcription factors that bind to these elements. Our results provide insight into the molecular mechanisms for auxin-induced gene expression and provide strategies for designing novel auxin-inducible genes.

011

HOW DOES GA DRIVE GENE EXPRESSION?

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Elements required for up-regulation by GA have been identified in the promoters of α-amylase genes expressed in aleurone cells of barley and similar elements are present in other GA regulated genes in monocots. There are also dicot genes which have similar promoter elements although evidence that they are associated with GA-regulation is not as abundant as for monocot genes. Systems for studying GA-regulated gene expression will be discussed.

There are several possible mechanisms for α-amylase gene regulation. Factors which bind to the GA response elements have been detected and some are GA-regulated. A direct role for GA in α-amylase gene regulation has not been proven. The nature of the signal transduction pathway is unclear but recent information on the location of the GA receptor and the likely mechanism involved in α-amylase gene regulation is beginning to define the limits of the pathway.

012

USING T-DNA TAGGING TO DISSECT THE MOLECULAR BASIS OF AUXIN ACTION.

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We are interested in the molecular basis of auxin signal transduction. We have used T-DNA tagging to create tobacco mutants selected by the ability of their protoplasts to grow in the absence of auxin *in vitro*. The tag used contains multiple transcriptional enhancers located near the T-DNA border so that following insertion into the genome expression of flanking sequences becomes deregulated creating dominant mutations. Tagged genes which upon overexpression produce auxin independent growth are, in effect, cellular proto-oncogenes and likely play a role in auxin signal transduction. Regenerated plants contain single T-DNA inserts genetically co-segregating with the selected phenotype. Several mutant lines have been studied in detail. In *axi 159,* the tagging event results in isolated protoplasts being able to grow in the absence and in supra-optimal concentrations of both auxin and cytokinin suggesting the mutation uncouples the cell from the normal effects of these hormones. *Axi 159* plants appear morphologically normal. The gene tagged in *axi 159, axi 1,* is a member of a small gene family, expressed principly in roots with no homology to previously characterised genes. However, results suggest that *axi 1* is an auxin inducible factor playing a role in transcription. Another mutant line, *axi 4,* while being auxin independent, requires cytokinin for protoplast growth. *axi 4* encodes a protein involved with ion transport. Molecular analysis of these and other tagged genes allows us to construct a preliminary model of how auxin triggers protoplast division.

013

TRANSGENIC PLANTS AND HORMONE BIOLOGY: AN OVERVIEW OF THE POTENTIAL AND LIMITATIONS

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The ability to produce transgenic plants that are altered in their hormonal content presents many opportunities to answer fundamental questions and to create novel improved commodities. Genes that affect all of the hormones are available and transgenic plants altered in auxin, cytokinin and ethylene content have been produced. Analysis of transgenic plants with altered hormonal content has advanced our understanding of the roles of hormones in developmental processes such as apical dominance, senescence and fruit ripening. While transgenic technology offers significant advantages over exogenous application of hormones, there are limitations to the practical use of transgenes. An overview of hormone manipulation including available genes, transformation systems, advantages and disadvantages of the technology will be provided. Some practical applications of hormone manipulation in plants will also be discussed.

014

CONTROLLING GROWTH, RIPENING AND SENESCENCE BY MANIPULATING HORMONE LEVELS IN TRANSGENIC TOMATO PLANTS

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Several novel genes have been cloned as part of a programme to identify and characterise tomato ripening genes (Gray *et al*, Plant Molecular Biology **19**, 69-87, 1992; Gray *et al*, Plant, Cell and Environment, 1994). These include genes involved in cell wall metabolism and biosynthesis of carotenoids and ethylene. Manipulation of the expression of these genes in transgenic plants, using gene silencing methods, has provided information about gene function and also generated transgenic plants with altered physiology and biochemistry and improved characteristics (Grierson and Schuch, Phil Trans R Soc Lond B342, 241-250, 1993; Grierson and Fray, Euphytica **79**, 251-263, 1994). Antisense technology identified the ACC oxidase gene (*Aco*1), which catalyses the terminal step in the biosynthesis of ethylene, and a phytoene synthase gene, which is required for carotenoid production. Inhibiting expression of *Aco*1 inhibited ethylene synthesis and reduced the rate of fruit ripening, prevented overripening, and delayed leaf senescence (Grierson and Fray, Euphytica **79**, 251-263, 1994; John *et al*, Plant Journal **7**, 483-490, 1995). Over-expression of phytoene synthase (*Psy*1) caused unscheduled carotenoid production in transgenic plants and, surprisingly, caused dwarfism. The dwarf plants were deficient in GA1. This is due to the competition between the *ent*-kaurene synthetase and phytoene synthase for geranyl geranyl diphosphate in the transgenic plants. The dwarf phenotype is inherited, is related to over-expression of *Psy*1 and can be partially reversed by supplying GA. Sense-suppression studies indicate there is a second gene, *Psy*2 which is normally involved in carotenoid production in green organs. Three *Aco* genes have now been cloned and sequenced. Ribonuclease-protection assays, using specific probes for each *Aco* gene have shown they are differentially expressed during fruit ripening, leaf senescence, in response to wounding and during flower development and senescence. Major accumulation of *Aco* mRNA occurs during a number of developmental stages and this had been visualised using *Aco* promoter-GUS fusion experiments in transgenic plants. This suggests that *Aco* is not constitutive and that ethylene synthesis levels are determined, at least in part, by the regulation of *Aco*.

015

REGULATION OF AUXIN AND CYTOKININ TURNOVER IN PLANTS

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It has long been known that the differentiation of plant tissues can be dramatically affected by applying different ratios of cytokinin to auxin, and a number of developmental traits are also believed to be regulated by the endogenous balance of these hormones. However, little information is available on the regulatory systems controlling the levels of active auxin and cytokinin in the cell. We have, therefore, studied the synthetic, conjugative and catabolic processes involved, using transgenic tobacco with altered rates of auxin and cytokinin production. Auxin precursors, conjugates and catabolites labelled with stable isotopes were synthesised, and used for turnover studies. We found that IAA-conjugation, transport and degradation systems were induced in the IAA overproducers, but there was no effect on the tryptophan dependent pathway of IAA synthesis. The increased IAA synthesis in the transgenic plants were associated with a number of phenotypic alterations. This has enabled us to establish correlation's between IAA content and various phenotypic responses. These data show that several of the well known 'auxin' traits, such as root formation, increased apical dominance and epinasty are associated with surprisingly low levels of over production. Finally, evidence for cross-communication between the metabolic systems regulating endogenous auxin and cytokinin levels is presented. This suggests that refinements are needed for the hypothesis, developed from the pioneering work of Miller and Skoog, that key developmental events are controlled by auxin and cytokinin ratios. Our data suggests that variations in the cytokinin levels is relatively more important than changes in the auxin level.

016

THE *ETR1* GENE FROM *ARABIDOPSIS* CODES FOR AN ETHYLENE RECEPTOR

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The *ETR1* locus of *Arabidopsis* was first identified through the isolation of a mutant, designated *etr1-1*, which rendered the plant insensitive to the plant hormone ethylene. Genetic studies have indicated that additional ethylene-associated loci are epistatic to *ETR1*. As a result, current models of ethylene signal transduction postulate a central role for *ETR1* in the initial perception and/or processing of the ethylene signal. The recent cloning of the *ETR1* gene provided some information on the possible mechanism of action of the ETR1 gene product. Sequence analysis indicated a protein with a hydrophobic N-terminal domain and a C-terminal domain with homology to signal-transducing protein kinases from bacteria. While all of these studies are consistent with a central role for *ETR1* in ethylene signal transduction, the interpretation of the genetic evidence has been complicated by the fact that only dominant mutant alleles have been identified at the *ETR1* locus. To investigate more directly the role of the ETR1 protein in ethylene perception we have expressed the protein in yeast. Biochemical analysis indicates that the protein is expressed as a membrane-associated dimer in both yeast and *Arabidopsis*. Using an in-vivo binding assay with ^{14}C-ethylene, we have found that the ETR1 protein expressed in yeast is capable of binding ethylene with high affinity (K_d = 0.5 uL L^{-1} ethylene). Expression of altered forms of the ETR1 protein in yeast indicate that ethylene binding is associated with the hydrophobic domain and that the etr1-1 mutant protein lacks detectable ethylene binding. These results provide compelling evidence that the ETR1 protein acts as a receptor for ethylene in *Arabidopsis*.

017

GENETIC AND MOLECULAR APPROACHES TO ABSCISIC ACID SIGNALING

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Abscisic acid (ABA) plays a prominent role in regulating seed dormancy and in signaling conditions of water stress in vegetative tissues. To establish the mechanistic basis of some of these processes, we have cloned and characterized the corresponding genes for two of the mutants, *abi3* and *abi1*, in *Arabidopsis* that show reduced sensitivity to ABA (Koornneef *et al.*, Physiol. Plant. 61: 377, 1984). The *ABI3* gene codes for a putative transcriptional activator; the mutations of which lead to altered seed-specific developmental processes. We have defined these processes further by monitoring the accumulation kinetics of marker silique mRNAs in the mutant *abi3-4*. Our results indicate that the wild-type ABI3 protein is required during several developmental programs and that the protein appears to regulate several of the seed-specific cascades in both ABA-dependent and independent manners. Mutations in *ABI1* also reduce seed dormancy slightly, but the major phenotype is the mutant plants' tendency to wilt due to stomatal dysfunctioning. Biochemical and yeast complementation studies suggest that the ABI1 protein functions as a phosphatase and thus implicates protein phosphorylation/dephosphorylation in ABA-mediated control of guard cell membrane transport. To establish further the relationship between *ABI1*, ABA-evoked signaling and control of the ion channels, we have introduced the dominant *abi1-1* allele into the diploid tobacco *N. benthamiana*. Voltage clamp analyses of the tobacco guard cells showed that the mutant transgene reduces the activity of $I_{K,out}$, and that it effects a loss of ABA response in both K_{in} and K_{out} channels. Furthermore, K^+ channel response to the hormone was recovered with protein kinase inhibitors. These observations are consistent with ABI1 functioning *in vivo* as a phosphatase and infer its activity to sensitivity modulation of guard cell K^+ channels in ABA signaling.

018

MOLECULAR CHARACTERIZATION OF *SPY*: A GENE INVOLVED IN RESPONSE TO GIBBERELLINS

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The *SPINDLY* (*SPY*) locus of *Arabidopsis thaliana* is believed to be involved in gibberellin (GA) signal transduction. Mutations at this locus cause a phenotype that is consistent with constitutive activation of the GA signal transduction pathway. Screening of the *Agrobacterium* seed transformation pools, available from the Arabidopsis Biological Resource Center, identified a new allele, *spy-4*. Genetic analysis of this allele suggested that it was caused by the insertion of T-DNA. Using the T-DNA as a probe, we have cloned the *SPY* gene. Successful cloning of the *SPY* gene was confirmed by sequencing the available mutant alleles and demonstrating the presence of a mutation in each allele, and also by demonstrating that introduction of the cloned *SPY* gene into the *spy-1* mutant partially reverts its mutant phenotype. The *SPY* gene contains 17 introns and encodes a 3.5 kb transcript. The deduced SPY protein is approximately 100 kDa and contains 10 TPR (tetratricopeptide repeat) repeats. The TPR and non-TPR regions exhibit significant homology to a putative gene of unknown function from *C. elegans*. This homology suggests that SPY is a member of a family of regulatory proteins that may be present in all eucaryotes. This work was supported, in part, by National Science Foundation grant IBN-9317524.

019

MOLECULAR CHARACTERISATION OF THE *AUX1* GENE FROM *ARABIDOPSIS THALIANA*

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Although many developmentally important signalling molecules, such as auxin, have been identified in plants, little is known about their mode of action. The recent advances in plant molecular genetics, particularly in the plant model *Arabidopsis*, has allowed the isolation of mutants specifically affecting phytohormone-related processes. We have isolated a T-DNA-induced *aux1* mutant from the Arizona *Arabidopsis* T-DNA population. The *aux1* mutant exhibits an agravitropic root phenotype, which is probably a consequence of its reduced root growth sensitivity to auxin and ethylene. We have subsequently cloned the *AUX1* gene, which encodes a novel highly hydrophobic polypeptide that is expected to play a key role during phytohormone-mediated signalling. *In situ* hybridisation experiments have identified that *AUX1* mRNA is spatially expressed within all root apical tissues except the Columella cells. This spatial expression pattern is suggestive of a role during gravi-transduction/response, rather than gravi-perception. In conclusion, we have isolated a novel phytohormone signalling component which regulated auxin-dependent root growth. Further studies aim to elucidate the biochemical mode of action of the *AUX1* polypeptide.

020

ANALYSIS OF ABA-INDUCIBLE GENES AND THEIR GENE EXPRESSION IN ARABIDOPSIS THALIANA UNDER DROUGHT CONDITIONS

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Under drought conditions, various genes are induced whose products are thought to function in protecting cells from dehydration. To understand the molecular process of signal transduction from water stress to gene expression in Arabidopsis thaliana, we cloned 9 independent cDNAs (named RD), that are induced by dehydration stress. In the present study, we report structure and expression of RD genes which are induced by exogenous ABA. The GUS reporter gene driven by these rd gene promoters was induced at significant level by dehydration in both transgenic Arabidopsis and tobacco. Different cis-acting elements seem to function in the dehydration responsive expression in rd29A, rd29B and rd22 genes. One is ABRE and the other is a novel cis-acting element, DRE (TACCGACAT: Dehydration Responsive Element). Atmyb2, a gene encoding a transcription factor myb homolog, was cloned from Arabidopsis. Northern analysis showed that Atmyb2 is induced under dehydration or high salt conditions or by exogenous ABA treatment. An ATMYB2 protein expressed in Escherichia coli cells could specifically bind to the myb recognition sequence (PyAACPyPu). These results suggest that Atmyb2 is involved in the ABA responsive expression in Arabidopsis under drought.

021

ETHYLENE-RESPONSIVE GENE EXPRESSION DURING FLOWER SENESCENCE

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In carnations, the developmentally programmed senescence of flower petals is regulated by the increased production of ethylene. The senescence of carnation flowers is an active process involving programmed cell death as evidenced by the necessity for gene transcription and protein synthesis. This led us to clone several senescence-related (SR) genes in an attempt to understand their functional significance to the processes of senescence and the role ethylene plays in their transcriptional regulation. One of these ethylene-responsive SR genes was recently found to encode a glutathione *S*-transferase. We have investigated the molecular basis for this ethylene-responsive transcription by examining the cis elements and trans-acting factors involved in the expression of the *GST1* gene. A single positive regulatory element was localized to 126 bp between -596 and -470 of the *GST1* 5' flanking sequences and this element was found to confer ethylene-regulated expression upon a minimal CaMV 35S TATA-box promoter in an orientation-independent manner. DNase I footprinting defined a sequence between -510 and -488 that was protected by bound protein and an oligonucleotide representing this region was used to screen a cDNA expression library. Two identical cDNA clones were obtained and found to represent a 1.3 kb transcript expressed in all floral organs tested. The predicted amino acid sequence of the ERE binding protein (ERE-BP1) contains a ribonucleoprotein consensus sequence consisting of two highly conserved motifs, RNP-1 and RNP-2. This domain has been identified in a variety of proteins believed to interact with RNA and/or DNA. A highly acidic region present in ERE-BP1 may provide a site for protein-protein interactions. Post-translational modification or protein complex formation with ERE-BP1 is likely required for the ethylene-responsive activation of *GST1*.

022

HORMONAL CONTROL OF ANTHOCYANIN PIGMENTATION GENES IN DEVELOPING PETUNIA FLOWERS

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In many flowering plants, anthers play a crucial role in the expansion and pigmentation of corollas. However, little is known about the signals involved. Since GA_3 can substitute for the anthers in emasculated flowers, endogenous GAs could be one of these signals. A role for GAs is further supported by the transitory increase of the GA level in corollas during their expansion. We are interested in the role of gibberellins and other hormones in the regulation of genes encoding enzymes of the anthocyanin biosynthesis pathway in flowers of *Petunia hybrida*. To study this we use an *in vitro* culture system in which detached corollas are incubated in sucrose medium. It was observed that only in the presence of GA_3, GA_1, GA_4, GA_9, or the cytokinin benzyladenine (BA), the corollas continue to develop and accumulate anthocyanins. As anthocyanin synthesis involves many enzymatic steps, the expression of all the biosynthetic genes must be co-ordinated in which GA and/or BA may play a role. Although the early biosynthetic genes (EBG), such as chalcone synthase (*chs*) and chalcone flavanone isomerase (*chi*), and the late biosynthetic genes (LBG), such as dihydroflavonol 4-reductase (*dfr*) and anthocyanin synthase (*ant17* or *as*) are controlled by different regulators, the hormones activate these genes co-ordinately. By using a transgene construct consisting of the *chs* promoter linked to the *uidA* reporter gene, we could show that the hormones activate transcription. Given the kinetics of this activation and the inhibition by cycloheximide, we infer that activation occurs indirectly in that the hormones first induce the production of a transcriptional regulator of the pigmentation genes. We have isolated a number of cDNAs that encode proteins with homology to the DNA-binding domain of the animal *c-myb* proto-oncogene transcription factor. Some of these *myb* genes may indeed be involved in the control of pigmentation genes or in that of other genes that are required for corolla maturation, as their expression depends on the presence of GA_3. Reference: Weiss et al. (1995) Plant Phys. 107 (3).

023

BIOCHEMICAL AND MOLECULAR DISSECTION OF HORMONE REGULATED TRANSCRIPTION FROM BARLEY α-AMYLASE GENE PROMOTERS. John C. Rogers[1], Sally W. Rogers[1], and John Mundy[2]. [1]Biochemistry Department, University of Missouri, Columbia MO 65203 USA; [2]Institute of Molecular Biology, Copenhagen University, 1353 Copenhagen K, Denmark. E-mail: bcjroger@muccmail.missouri.edu

Promoters of both low-pI and high-pI α-amylase genes contain a Gibberellin Response Element (GARE); this 16 bp DNA sequence is responsible for increased transcription in the presence of GA and suppression of transcription by ABA. Alone, however, a GARE permits only very low levels of transcription from a promoter, probably because it offers a low affinity binding site for GARE-binding protein(s) (GABPs). Other DNA sequence elements in those promoters have very specific effects on the GARE that allow high levels of hormone-regulated transcription. We hypothesize that proteins interacting with these other "coupling" elements also interact with the GABPs to form a more stable transcription-activating complex. Our work is directed towards identifying both GABPs as well as proteins that specifically bind to other functionally important *cis*-acting DNA elements. Using a particle-bombardment transient expression system in intact aleurone layers, we find that introduction of additional GARE elements on a plasmid separate from the amylase promoter-GUS reporter construct substantially diminishes the level of its GA-induced transcription. Thus, GABPs are present in limiting amounts in aleurone cells. A reporter gene under control of a minimal promoter augmented by the presence upstream of three tandemly arranged GARE elements is properly GA regulated, albeit at low levels. Overexpression of a protein, cloned from barley aleurone, that interacts with the GARE sequence increases the no hormone level of expression from this construct to that obtained with GA; this result indicates that the cloned protein may have a role *in vivo* in GA-regulated transcription. Lastly, we have purified >2000-fold from wheat germ nuclei a ~45 kDa protein that interacts with the O2S coupling element in low-pI promoters. DNA sequence mutations that abolish binding of this protein also abolish function of the element. This biochemical approach may permit identification of other proteins that are functionally important in the system.

024

GA AND ABA RESPONSE PATHWAYS IN BARLEY ALEURONE CELLS: GAMYB IS A TRANSCRIPTIONAL ACTIVATOR OF A HIGH-pI α-AMYLASE GENE PROMOTER. Frank Gubler[1], Roger Kalla[2], James Roberts[1], John V. Jacobsen[2]. [1]Co-operative Research Centre for Plant Science, PO Box 475, Canberra City, ACT, Australia; [2]Division of Plant Industry, CSIRO, GPO Box 1600, Canberra City, ACT, Australia. E-mail: frank@pi.csiro.au

Functional analysis of a high-pI α–amylase gene promoter has identified a gibberellin (GA) response complex in the region between -174 and -108. The sequence of the central element, TAACAAA is very similar to the c- and v-MYB consensus binding site. To investigate the possibility that a GA-regulated MYB transactivates α–amylase gene expression in barley aleurone cells, we screened a barley aleurone cDNA library for MYB-like transcription factors. A cDNA clone, GAMyb, which encoded a novel MYB was isolated and sequenced. Northern analysis revealed that GAMyb expression in isolated aleurone layers is GA up-regulated and the GA effect is partially inhibited by ABA. The kinetics of GAMyb expression indicates that it is an early event in GA-regulated gene expression and precedes α–amylase gene expression. Gel retardation experiments with recombinant GAMYB showed that GAMYB binds specifically to the TAACAAA box in vitro. We demonstrated in transient expression experiments that GAMYB activates transcription of a high-pI α–amylase promoter fused to a GUS reporter gene in the absence of GA. Our results indicate that the GAMYB is the sole GA-regulated trancription factor required for transcriptional activation of the high-pI α–amylase promoter. Studies are underway to investigate mechanisms of GA and ABA action on GAMyb gene expression.

025

PARALLELS BETWEEN ELICITOR SIGNALLING AND HORMONE SIGNALLING

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Plants have exquisitely sensitive chemoperception systems for specific exogenous signal substances derived from micro-organisms. For example, suspension-cultured tomato cells perceive characteristic fungal molecules such as chitin fragments, ergosterol, and *N*-linked glycopeptides with fungal-type mannosyl linkages at threshold concentrations of ~10^{-12} - 10^{-10} M. Interestingly, the perception system for chitin fragments present in tomato cells also recognizes Nod factors secreted by rhizobia. Some of these perception systems for "non-self" molecules show rapid desensitization, leading to a "refractory state" reminiscent of adaptation phenomena.

Systemin, an endogenous signal substance, produces very similar reactions to the exogenous "non-self" signals in cells of a wild tomato species (*Lycopersicon peruvianum*), inducing alkalinization and ethylene production at a threshold of ~10^{-11} M.

The fact that plants recognize specific substances produced by plant-associated microbes in a similar way as hormones has been taken as an indication for a co-evolutionary adaptation, in which pre-existing hormone signalling pathways are exploited in plant-pathogen and plant-symbiont-interactions. Alternatively, certain endogenous signals, such as systemin, may have been "invented" by the plant according to the blueprint of elicitors.

026

JASMONATES AND THEIR ROLE IN STRESS-RELATED SIGNALING TO STRESS RESPONSES

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Jasmonates (JA) represent bioactive cyclopentanone substances which show many of the characteristics of well-studied "classic" phytohormones: ubiquitous occurrence in the plant kingdom; multiplicity of physiological and cytological modes of action; molecular involvement in signaling steps leading to the expression of specific genes. The biosynthesis is accelerated or induced under stress conditions, in particular by osmotic stress, desiccation, wounding. JA actions show similarities to those of ABA (the "stress hormone"), however, ABA cannot replace jasmonate function in various plants (species specificity). Stress responses in terms of JA-induced expression of JIP genes is species- and organ-specific and takes place in different cell compartments. Most of the JA-induced proteins (JIP) are still unknown in function (known: thionin, lipoxygenase, protease inhibitors, ribosome inactivating protein). Besides a positive control of JIP gene expression at the transcription level JA exert likewise a negative control at the translation level of normal proteins, e.g. chloroplast proteins. Thus, JA is involved in cellular and molecular stress responses in stressed plants, where it induces the expression of specific genes by a closely related mechanism of its biosynthesis and signal transducing action.

027

SIGNAL MOLECULES INVOLVED IN THE FORMATION OF ROOT NODULES

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Rhizobia, bacterial symbionts of leguminous plants, produce lipo-chitin oligosaccharide (LCO) signal molecules after induction of their nod genes by the plant host. These LCO molecules have various effects on the roots of the host plants. One of these effects is dedifferentiation of groups of cells located in the cortex which leads to the formation of a (nodule) primordium. An important feature is that the LCO signals are host specific in their activity: only the signals produced by compatible bacteria are able to induce the nodule primordia. I will present recent data on the molecular mechanism of the host specificity of the rhizobial factors and on plant signal molecules which are involved in the nodulation process.

028

SYSTEMIN: ANALYSES OF ITS SIGNALLING PATHWAY

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A model for the signalling pathway that activates defensive genes in response to pathogen and herbivore attacks includes oligosaccharins as localized signals, released from infection sites by pathogens, and an 18 amino acid polypeptide systemic signal, released from wound sites by herbivores. Systemin is derived from a 200 amino acid precursor protein and is active in plants at fmole/plant. It is readily mobile and is transported through the phloem at a rate near that of sucrose, supporting a role for systemin as systemic wound signal. Oligosaccharins and systemin, when supplied to young tomato plants, induce a large increase in intracellular levels of jasmonic acid, indicating that they all utilize the proposed octadecanoid signalling pathway. Tomato plants transformed with an antisense prosystemin gene exhibited severely impaired systemic signalling, whereas plants transformed with a CaMV-prosystemin gene (called sense plants) constitutively expressed proteinase inhibitor genes in leaves throughout the plants at extraordinarily high levels. Wild type plants grafted onto the sense plants strongly expressed the proteinase inhibitor genes in leaves, confirming the systemic effects of systemin. Using biotinylated systemin, a strongly binding plasma membrane protein was identified that is a candidate for a transporter or receptor of systemin. The protein has the properties of a proteinase of the furin family. The current model of the signalling pathway and of the systemin binding protein will be presented. The cumulative evidence continues to support a role for systemin as a mobile signal that activates proteinase inhibitor synthesis in plants in response to wounding by predators. Supported by an EMBO Post-doctoral Fellowship (to AS) and grants from the National Science Foundation (to CAR).

029

THE ROLE OF PLANT HORMONES AND HORMONE INHIBITORS IN THE EARLY STAGES OF NODULE DEVELOPMENT IN LEGUMES.

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Alfalfa (*Medicago sativa* L.) nodules, like other cylindrical nodules, develop from the *de novo* initiation of cell divisions in the already differentiated cells of the inner root cortex. These cell divisions are initiated soon after inoculation with *Rhizobium meliloti* or after treatment with Nod factor, a substituted chitooligosaccharide. In cylindrical nodules, a nodule primordium develops within the inner root cortex and a nodule meristem is secondarily established along its periphery. The nodule meristem serves as the source of the various tissues in the nodule and may serve as a source of endogenous plant hormones. However, the rhizobia themselves are known to produce cytokinins and auxins as well as a compound that competes with radioactive *N*-1-(napthyl)phthalamic acid (NPA) for its binding site on microsomal membranes. We have shown that several of the genes expressed early in alfalfa nodule formation are induced by altering the root's endogenous hormone balance. Cell divisions are triggered by treating the roots with auxin transport inhibitors (ATIs); several early nodulin genes, including *enod2* and *enod40*, are expressed. We now show that physiological concentrations of cytokinin induce the expression of these early nodulin genes as well as cytological changes in the treated roots. Treatment of a non-nodulating genotype of alfalfa with cytokinin also leads to these responses suggesting that a change in the plant's hormone balance is autonomous and downstream of Nod factor perception.

030

PHENOLIC PERCEPTION BY *AGROBACTERIUM TUMEFACIENS*: A GENETIC ANALYSIS

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Agrobacterium tumefaciens provides a model system to study mechanisms of phenolic signal transduction. Transcriptional activation of the virulence (*vir*) genes necessary for pathogenesis is regulated by phenolics through the activities of VirA and VirG, members of the conserved two component regulatory family. VirA is a membrane bound protein that is necessary for signal input, and, ultimately, for the phosphorylation of VirG which then activates transcription of the various *vir* genes. The mechanism by which phenolics are perceived, however, is not clear. While genetic evidence demonstrates VirA is necessary for phenolic signal transduction, physical evidence suggests the involvement of other proteins as phenolic binding elements. Experiments designed to identify the phenolic receptor will be presented. In particular, progress in selecting mutants with altered phenolic specificity will be described. Finally, genetic and biochemical analysis of VirA and its role in the phenolic signal transduction pathway will be described.

031

FLAVONOLS IN REPRODUCTIVE ORGANS: EFFECTS ON PLANT FERTILITY AND FECUNDITY

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Flavonols are required for successful fertilization in maize and petunia. Although viable, flavonol-deficient pollen is unable to germinate and produce a functional pollen tube. The phenotype is termed conditional male fertility (CMF) because germination can be "restored" by the timely application of flavonols to the pollen. Feeding studies with a radioactive tracer indicate that flavonols are uniquely metabolized in the pollen grain. Two glycosyltransferase activities are responsible for producing a pollen-specific class of flavonols. Wild type stigmas can also provide the requisite flavonols to CMF pollen. Wounding adjacent tissues such as the corolla causes high levels of the germination-inducing flavonol aglycone, kaempferol, to accumulate in the stigma exudate. This phenomenon was exploited to enhance fecundity in petunia: slitting the corolla 24 h before pollination increased seed set in all crosses with either flavonol-deficient or wild type pollen. Our objective is to characterize elements of the signal transduction pathway initiated by wounding the corolla and terminating in the responding stigma. This work was supported by grants from the National Science Foundation and the U.S. Department of Agriculture National Research Initiative Competitive Grants Program.

032

THE PHENOMENON OF SYSTEMIC ACQUIRED RESISTANCE AND THE ROLE OF THE PLANT GROWTH REGULATOR SALICYLIC ACID.

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In many plants, necrotizing infections can result in protection of the uninfected parts against subsequent infections. The resistance manifests itself around the site of initial attack but also at sites remotely located from the primary infection. This type of resistance is defined as systemic acquired resistance, and is directed against a broad spectrum of pathogens including fungi, bacteria and viruses. The separation in time and space of the initial necroses from systemic acquired resistance implies the translocation of an endogenous signal from the infection site to other parts of the plant. Salicylic acid was proposed as a putative signaling molecule for the induction of systemic acquired resistance. This molecule is produced endogenously upon infection, and when applied to plants, can induce resistance as well as the symptoms which typically appear during systemic acquired resistance such as the production of so-called pathogenesis-related proteins. Salicylic acid has been implied in a number of physiological processes such as flowering or thermogenesis. The involvement of salicylic acid in the defence reactions of plants will be discussed with a special emphasis on its synthesis, transport and mode of action.

033

PHENOLIC SIGNALS IN PATHOGENESIS.

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My discussion as organizer and initial speaker of this session will focus on two themes. First, an overview of some of the signaling events uncovered over the past several years that are initiated by this class of compound will be presented as an introduction to the subsequent papers. This entire session should provide some perspective into the kinds of signaling roles that phenolics can serve and where we might expect to find them. The second and concurrent theme will focus on the mechanism of signal transmission through the phenolic signals. This is an area in which very little in known but is critical if we hope to understand these signals and how to use them. Two pathogenic systems will be discussed, the parasitic plant *Striga asiatica* and the bacterial plant pathogen, *Agrobacterium tumefaciens*. Models for the mechanism of signal transmission are beginning to emerge and it should be of value to consider these models in light of the other signaling events that will be discussed.

034

PARADIGMS FOR HORMONAL CONTROL OF K^+ CHANNELS IN STOMATAL FUNCTION

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Our current understanding of plasma membrane ion transport in higher-plant cells has benefitted tremendously from the past decade of work on stomatal guard cells. Knowledge of H^+-ATPase, K^+ and anion channel characteristics have drawn heavily on electrophysiological studies and form the basis for current models of stomatal function. It is now clear that these transporters are modulated in concert to effect solute flux for stomatal movement and some common paradigms are emerging, founded on studies with ABA and auxin. Efforts to identify the signalling events evoked by these hormones have focused on cytoplasmic-free Ca^{2+} ($[Ca^{2+}]_i$) -- including its relation to inositol 1,4,5-trisphosphate metabolism and G-proteins -- and, more recently, on cytoplasmic pH (pH_i). Notably, the pH_i signal is common to ABA and auxin signal cascades and regulates, among others, the activities of both the major K^+ channels at the guard cell plasma membrane. Evidence from work with plants carrying the *abi1* mutant phosphatase gene now points also to interaction between protein phosphorylation and pH_i in modulating the K^+ channels. These studies add a further dimension to our understanding of guard cell transport, and may yield a clue to hormonal "sensitivity control" in stomatal functioning.

035

AUXIN SIGNALLING AND ACTION AT THE PLASMA MEMBRANE OF PLANT CELLS

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Auxin has been shown to induce early modifications of the electrical potential of tobacco mesophyll protoplasts. To characterize the basis of this response, ion channels at the plasma membrane of protoplasts from tobacco cell suspensions were studied by the patch-clamp technique. An anion current was described which showed a phosphorylation-dependent voltage regulation. The active auxin 1-NAA caused a time- and concentration-dependent shift of the activation potential of the anion channel. In addition, the channel reacted to the auxin agonist antibody D16, raised to the putative auxin-binding domain of ZmER-abp1, the major auxin-binding protein from maize coleoptile. This provided evidence for the recognition of the auxin signal at the outer face of the plasma membrane. These results and other data from our group supported a model where a soluble auxin-binding protein would interact with a docking transmembrane protein to constitute functional auxin receptors at the plasma membrane. We recently isolated two tobacco cDNAs encoding proteins homologous to ZmER-abp1 and monoclonal antibodies were raised to one of these Nt-abps. Some of the antibodies showed antagonist activity on the auxin-induced hyperpolarization of mesophyll protoplasts, whereas one exhibited agonist activity. These data confirmed that Nt-abp was present and active at the plasma membrane, as previously showed for ZmER-abp1. The C-terminal region of ZmER-abp1 has been postulated to be involved in the interaction of abp1 with the plasma membrane (Thiel *et al*, 1993, Proc Natl Acad Sci USA, 90:11493). We showed that this maize peptide hyperpolarized tobacco mesophyll protoplasts with a monophasic dose-response curve, reproducing partly the effects of auxin. Similar results were obtained using a C-terminus peptide from Nt-abp. Work is in progress to identify the plasma membrane protein interacting with Nt-abp. Present investigations also aim at establishing if this plasma membrane receptor involving Nt-abp and triggering early membrane responses to auxin participate in other hormonal responses such as regulation of gene expression.

036

ANIONS MODIFY THE RESPONSE OF GUARD CELL ANION CHANNELS TO AUXIN

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GCAC1, an anion channel in the guard cell plasma membrane of *Vicia faba*, possesses recognition sites for the plant growth hormone auxin at the extracellular mouth of the pore[1]. Using the patch clamp technique we could demonstrate that auxins induce a shift of the voltage dependence of the anion channel to hyperpolarized potentials which is increased during a decrease in the extracellular chloride concentration[2], indicating an interaction between the binding sites for the anion and the hormone. The auxin-induced shift was dose-dependent, characterized by a Michaelis-Menten type of behaviour with a half saturation constant for 1-NAA ($K_m^{1\text{-NAA}}$) of about 10 μM. In the presence of malate, another gating modulator of GCAC1[3, 4], auxins were less effective, suggesting that both ligands compete for common binding sites.

[1] Marten I., Lohse G. & Hedrich R. (1991) Plant growth hormones control voltage-dependent activity of anion channels in plasma membrane of guard cells. Nature **353**, 759-762

[2] Lohse G. & Hedrich R. (1995) Anions modify the response of guard cell anion channels to auxins. Planta, submitted

[3] Hedrich R. & Marten I. (1993) Malate-induced feedback regulation of plasma membrane anion channels could provide a CO_2 sensor to guard cells. EMBO J. **12**, 897-901

[4] Hedrich R., Marten I., Lohse G., Dietrich P., Winter H., Lohaus G. & Heldt H.W. (1994) Malate-sensitive anion channels enable guard cells to send changes in the ambient CO_2 concentration. Plant J. **6**, 741-748

037

MECHANISM OF REGULATION OF THE PLASMA MEMBRANE H^+-ATPase

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The fungal toxin fusicoccin and the plant hormone auxin have in numerous experiments in different laboratories been demonstrated to promote proton extrusion from plant tissues. This increased rate of proton extrusion has usually been ascribed to an increased rate of proton pumping by the plasma membrane H+-ATPase. Activation of the H+-ATPase by fusicoccin *in vivo* proceeds by a mechanism involving the C-terminal autoinhibitory domain of the enzyme and results in an enzyme with a higher Vmax, lower Km for ATP, and a changed pH dependence resulting in increased activity at physiological pH. The same type of activation can be obtained *in vitro* by treating isolated plasma membrane vesicles with either lysophosphatidylcholine or trypsin; the latter resulting in the removal of a ca 10 kDa fragment from the C-terminal end of the enzyme including the autoinhibitory domain. To determine steps in the signal transduction chain from the fusicoccin receptor to the H+-ATPase we have looked at the effect of a number of membrane permeable inhibitors on fusicoccin activation. We have also compared the effects of auxin and fusicoccin on the maize coleoptile plasma membrane H+-ATPase in an attempt to reveal the mechanism of auxin action. In contrast to fusicoccin, auxin had no effects on Vmax, Km for ATP or pH dependency. Neither fusicoccin nor auxin had any effect on the abundancy of H+-ATPase molecules in the plasma membrane, as determined by immunoblotting using 4 different antibodies, and protein staining.

038

ION CHANNEL-MEDIATED ABA SIGNAL TRANSDUCTION IN GUARD CELLS,

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The plant hormone abscisic acid (ABA) triggers stomatal closing in response to drought stress. Stomatal closing is mediated by efflux of K^+ and anions from guard cells. Recent research has suggested that slow anion channels are a central rate-limiting control mechanism for stomatal closure. Potent blockers of slow anion channels completely abolish ABA-induced stomatal closing. Patch clamp studies will be presented showing that phosphorylation and dephosphorylation events strongly activate and completely inactivate slow anion channels. *In vivo* studies of ABA-induced stomatal closing suggest that the presented slow anion channel modulation mechanisms play key roles in ABA signaling.

Stomatal closing requires K^+ efflux from guard cell vacuoles. An increase in the cytosolic Ca^{2+} concentration activates vacuolar SV channels and a novel type of K^+ selective channel (VK) in the guard cell vacuolar membrane (1). VK channels may provide a mechanism for K^+ efflux from vacuoles during stomatal closing. Furthermore we have found that the broadly distributed slow vacuolar (SV) channels are Ca^{2+} permeable in guard cell vacuoles suggesting that SV channels may provide a mechanism for Ca^{2+}-induced Ca^{2+} release in higher plants. Recent data on the proposed Ca^{2+}-induced Ca^{2+} and K^+ release function of SV channels in guard cells will be presented.

1) Ward, J.M. and Schroeder, J.I. *The Plant Cell*, 6:669-683 (1994).

039

METABOLIC STUDIES OF GIBBERELLIN PATHWAYS IN VEGETATIVE TISSUES OF PLANTS

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Gibberellins (GAs) control a variety of growth responses in higher plants including shoot elongation. The use of single gene dwarf GA-mutants, which block steps in the GA-biosynthetic pathway and which contain reduced levels of GAs has provided critical data relating endogenous GA levels with growth. However, understanding the role of these dwarfing genes in the control of shoot growth requires detailed knowledge of the GA-biosynthetic pathways. Pathway knowledge is also necessary for an understanding of the molecular basis of GA-dependent gene expression. The present status of the GA-biosynthetic pathways will be reviewed with special emphasis on vegetative shoots of maize, rice and *Arabidopsis*. For both maize and rice, results documenting the early 13-hydroxylation pathway from GA_{12} will be presented together with information on the metabolism of GA_{20} and the control of this metabolism by the maize *D1* gene and the rice *Dy* gene. For *Arabidopsis*, identification of the endogenous GAs (Talon, Koornneef and Zeevaart, 1990, PNAS **87**:7983-7) suggests the presence of three hypothetical biosynthetic pathways. Information on the current status of metabolic studies and on the role of the *GA4* gene in 3β-hydroxylation will be presented. Supported by grants from the Department of Energy, and the National Science Foundation.

040

CLONING OF *ENT*-KAURENE SYNTHASE A AND B GENES FROM PLANTS

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The first important step in gibberellin (GA) biosynthesis is the conversion of geranylgeranyl pyrophosphate, via copalyl pyrophosphate, to *ent*-kaurene. These reactions are catalyzed by *ent*-kaurene synthase A (KSA) and B (KSB), respectively. Recently KSA has been cloned from Arabidopsis (*GA1* gene, Sun & Kamiya, '94) and a putative KSA has been cloned from maize (*An1* gene, Bensen et al '95). Using a *GA1* cDNA fragment as a probe or RT-PCR, we have isolated putative KSA cDNA fragments from rice, pea and pumpkin. Isolation of full length cDNA clones is in progress. Analysis of pumpkin cDNA fragments suggests the presence of KSA isoenzymes. KSA isoenzymes can explain the production of low levels of kaurene and GAs in a 5 kB deletion mutant of the Arabidopsis *GA1* locus (*ga1-3*) and a maize mutant which completely lacks the *An1* locus. We purified KSB from pumpkin endosperm by several steps of HPLC. KSB showed an apparent molecular weight of 81 kDa on SDS-PAGE. We have isolated a nearly full-length cDNA from a cDNA library prepared from immature cotyledons. The deduced amino acid sequence showed high homology with other terpene cyclases such as limonen, casbene and *epi*-aristolochene synthase. Pumpkin KSB has a putative polyprenyl pyrophosphate binding site, DDXXD. By contrast, GA1, An1 and putative rice and pumpkin KSA have a similar sequence, DXDDTA, located earlier in the coding sequence. Unfortunately the cDNA clone of KSB was missing the N-terminal part and did not give information about the signal peptide sequence of KSB. Isolation of the full-length cDNA is in progress. Both the Arabidopsis KSA and the putative maize KSA have a signal peptide for chloroplast localization but their signal peptide sequences are quite different. Further characterization of KSB may reveal how *ent*-kaurene produced in the chloroplast is transported into the endoplasmic reticulum in the cell.

041

CLONING AND ANALYSIS OF THE *DWARF3* GENE OF MAIZE

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The maize *dwarf3* mutation were isolated by early maize geneticists (Demerec, 1926) and shown to be normalizable by GA (Phinney 1956). Biochemical analysis of *d3* plants (Fujioka et al., 1988) and bioassay experiments (Phinney and Spray, 1982) suggest that *D3* gene encodes an early enzymatic step of the GA-biosynthesis pathway before the biosynthesis of GA53 and after the biosynthesis of *ent*-kaurene, most likely the C-13-hydroxylation step. We have transposon-tagged the *D3* locus using Robertson's Mutator. The mutant allele *d3-2* (Mu) had a linked Mu8 element. The flanking DNA was cloned and shown to be tightly linked to the *D3* locus (< 0.2 cM) by high resolution genetic mapping. To identify the nature of the *D3* gene product cDNAs were isolated. Database comparisons revealed that the predicted *D3* protein had significant sequence similarity to cytochrome P450s. It has the highly conserved cytochrome P450 signature sequence (FXXGXXXCXG). The cytsteine of the signature sequence is involved in binding heme Fe. A second mutant allele of *d3* (*d3-4*) was cloned and shown to have a novel 327 bp insertion element, named *Sleepy* in an exon which is flanked by five bp direct repeats. The isolation of two independent mutant alleles establishes the identity of the cloned gene as *D3*. Analysis of *D3* mRNA expression showed that the *D3* transcript is expressed in roots, developing leaves and the vegetative meristem. An alternatively spliced form of the mRNA is observed to be expressed in a tissue specific manner; its role is being analyzed. To determine the enzymatic function of the *Dwarf3* protein, *D3* is being expressed in yeast under the control of an inducible promoter and analyzed for enzymatic activity. The results of these experiments will be discussed.

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DIOXYGENASES OF THE GIBBERELLIN BIOSYNTHETIC PATHWAY

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The final steps of the gibberellin (GA)-biosynthetic pathway are catalyzed by soluble oxidases for which 2-oxoglutarate is a co-substrate and which require Fe^{2+} and ascorbate for maximum activity. The reactions catalyzed by these 2-oxoglutarate-dependent dioxygenases include oxidation at C-20 and 3β-hydroxylation, leading to the formation of the biologically active forms. The 2β-hydroxylases, which inactivate GAs, and the enzyme(s) that convert GA_{20} to GA_3 also belong to this group. There is evidence that one or more of the GA, 2-oxoglutarate dioxygenases are regulated by environmental factors, such as photoperiod, or by GA action in a type of feedback regulation. The recent isolation of cDNAs encoding GA 20-oxidases and other GA, 2-oxoglutarate dioxygenases is enabling the regulation of these enzymes to be examined at the level of gene expression. Furthermore, heterologous expression of cDNAs in *E. coli* has produced sufficient quantities of pure enzyme for structural and mechanistic studies. It has, for example, been shown that the GA 20-oxidases are multi-functional enzymes that catalyze the sequential oxidation of C-20 from a methyl group to an aldehyde, with the subsequent loss of C-20 as CO_2 that results in the formation of the C_{19}-GA skeleton. The availability of these cDNAs also allows expression of the dioxygenase genes to be altered in transgenic plants with resulting changes in GA biosynthesis. It should thus be possible to manipulate plant development for agronomic benefit.

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CLONING OF THE *ARABIDOPSIS GA4* LOCUS BY T-DNA INSERTION MUTAGENESIS

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The *Arabidopsis ga4* mutant is impaired in the 3β-hydroxylation step in the GA biosynthesis pathway. Its semidwarf phenotype results from a decrease in the 3β-hydroxy-GAs such as GA_1 and GA_4. Progeny of a transgenic *Arabidopsis* plant generated by *Agrobacterium* transformation were found to segregate for a GA-responsive semidwarf phenotype. Complementation analysis with genetically characterized GA-responsive mutants revealed that the mutation is an allele of the *GA4* locus. The semidwarf phenotype is inherited as a recessive mutation that cosegregates with both the T-DNA insert and the kanamycin resistance trait. DNA gel blot analysis indicates that the insertion site contains a complex T-DNA unit. A genomic library was constructed with DNA from the T-DNA tagged *ga4* mutant (*ga4-2*) and a DNA clone was isolated from the library that flanks the T-DNA insert. The plant sequence from this clone was used to isolate the corresponding full length genomic and cDNA clones from wild type libraries. The *ga4* cDNA is 1077 nucleotides with an open reading frame of 359 amino acids. Sequence comparison suggests that it encodes a protein with a high degree of sequence similarity to other hydroxylase genes from plants. This conclusion agrees with a biochemical study that indicated the *ga4* mutant is deficient in the protein 3β-hydroxylase in the GA biosynthetic pathway of *Arabidopsis*. RNA gel blot analysis shows that the message is ubiquitously expressed in different tissues of *Arabidopsis* but most abundantly in the silique. Unexpectedly, a higher level of transcription is detected in the EMS-induced *ga4-1* mutant and this over-expression can be feedback regulated by treatment with exogenous GA. The results of these studies demonstrate the importance of molecular regulatory mechanisms in the control of GA biosynthesis.

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INTERORGAN COORDINATION OF POSTPOLLINATION DEVELOPMENT IN FLOWERS BY SPATIAL REGULATION OF ETHYLENE BIOSYNTHETIC GENES

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Pollination of orchid flowers induces a syndrome of developmental events including perianth senescence, pigmentation changes and the initiation of ovary growth and ovule development. Each of these developmental processes occurs in distinct floral organs, suggesting that the primary pollination signal is transduced and amplified by mechanisms that also coordinate interorgan developmental processes. We determined the role of pollination-associated factors in initiating and amplifying the postpollination response. These studies indicated that ethylene and auxin jointly regulate the syndrome of postpollination development. Because we have identified the presence of auxin in orchid pollen, we speculate that auxin may represent the primary pollination signal that is amplified by stimulating the autocatalytic production of ethylene. We have identified an ACC synthase gene that is expressed specifically in stigma tissue, the site of primary pollen signaling, and that is regulated by auxin rather than by ethylene or ACC. In addition, characterization of the temporal and spatial regulation of expression of two genes encoding a distinct ACC synthase and an ACC oxidase suggest that interorgan amplification of the pollination signal occurs by spatial separation of ACC synthesis and of ACC oxidation to ethylene and by the interorgan translocation of ACC. This research was supported by grants from the USDA/NRICGP and BARD to S. O'N.

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USE OF TRANSGENICS TO EXAMINE INDOLE-3-ACETIC ACID BIOSYNTHESIS IN *ARABIDOPSIS THALIANA*

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A. thaliana tryptophan auxotrophs defective in the conversion of indole or indole glycerol phosphate to tryptophan accumulate IAA, suggesting that IAA biosynthesis is tryptophan-independent (1). One of the proposed IAA biosynthetic pathways is referred to as the indole acetonitrile (IAN) pathway and involves conversion of tryptophan to indoleacetaldoxime, followed by formation of IAN which is converted to IAA by a nitrilase. Several members of the Brassicacae have been shown to have these intermediates and the enzyme activities that catalyze their interconversion. Since IAN is postulated to come from tryptophan, we expected that IAN levels should be less than normal in the *A. thaliana* tryptophan auxotrophs; however, isotope dilution GC-SIM-MS revealed an accumulation of IAN (1). This is unexpected in light of the hypothesis that tryptophan is the precursor to IAN and suggests that IAN could be derived from an intermediate in the tryptophan biosynthetic pathway. Recently 4 *A. thaliana* nitrilase genes have been isolated and shown to convert IAN to IAA *in vitro* (2). We have generated transgenic *A. thaliana* lines carrying each of the 4 nitrilase cDNAs in sense and antisense configuration under control of a strong, constitutive promoter and are currently quantifying the level and half life of IAA in these plants.

1. Normanly et al., (1993) PNAS 90: 10355
2. Bartel and Fink, (1994) PNAS 91: 6649; Bartling et al., (1994) PNAS 91: 6021

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MEASUREMENT OF IAA TURNOVER AND WHAT IT CAN TELL US

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Hormone turnover in plants has been a relatively unexplored facet of how hormone level and activity is modulated during plant growth, development and response to environmental conditions. Turnover is a measure of the dynamic nature of hormone metabolism, and represents the balance between biosynthesis and catabolism. This balance is critical both to hormone levels and to the time allotted to signal stability. We have recently measured IAA turnover in an inbred line of the aquatic monocot *Lemna gibba* G-3 (3F7-11) and in a 3F7-11 variant line (MTR-1). MTR-1 was obtained following EMS mutagenesis and selection for resistance to the tryptophan analog, α-methyl tryptophan. MTR-1 has enhanced anthranilate synthase activity which results in high production of anthranilate and increased levels of tryptophan and its precursors. Our previous work (Baldi *et al.*, 1991), showed that in *Lemna*, IAA is synthesized predominantly by the tryptophan independent pathway. In our usual growing conditions, MTR-1 has a ten fold higher rate of IAA turnover than 3F7-11. These measurements were made by pulse-labeling with 10^{-7}M $^{13}C_6$ IAA and GC-SIM-MS analysis at two time points following treatment. This type of analysis has now been done using plants that have been exposed to high or low temperature, or to various light conditions including UV-B. Clear differences in IAA turnover rates have been observed in some cases where static measurements of IAA levels showed little or no change.

References: Baldi, Maher, Slovin and Cohen, 1991, Plant Physiol. 95:1203-1208.

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BIOSYNTHETIC STUDIES ON 4-CHLOROINDOLE ACETIC ACID

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4-Chloroindole-3-acetic acid (4-Cl-IAA) had been isolated in 1968 from immature seeds of pea, *Pisum sativum*, as a second natural auxin. Its possible biosynthetic precursor, 4-chlorotryptophan (4-Cl-Trp) had also isolated from the same seeds as malonyl derivatives and then a free form. The absolute stereochemistry of the 4-Cl-Trp was determined to be L-form and D-form have never been detected. Radioisotope labeled 4-Cl-Trp was synthesized and resolved to D- and L-form by HPLC. Each compound was used for the incorporation experiments to 4-Cl-IAA. First, the experiments were carried out using immature pea pods containing seeds or homogenate of immature seeds. Although L-4-Cl-Trp was incorporated to 4-Cl-IAA more than D-form, the incorporation ratio was very low. Next, broad bean, *Vicia faba*, which is the only plant containing 4-Cl-IAA and 4-Cl-Trp not only in seeds but also in leaves, was used for the experiments. L-4-Cl-Trp absorbed to young plants of broad bean was metabolized to 4-Cl-IAA more than D-4-Cl-Trp. Since it was observed that only D-4-Cl-Trp was racemized to L-isomer in the plant, D-4-Cl-Trp might be racemized and then metabolized to 4-Cl-IAA. 6-Chloroanthranilic acid and 4-chloroindole were also examined their possibility to be the precursor of 4-Cl-IAA, however, it seemed to be negative. Thus, L-4-Cl-Trp is considered to be the biosynthetic precurser of 4-Cl-IAA.

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4-Cl-IAA, A POTENT SUBSTITUTE FOR SEEDS TO PROMOTE PEA PERICARP GROWTH.

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Deseeding pea (*Pisum sativum* L.) pericarps arrests pericarp growth and subsequently leads to abscission of the pericarp. Application of either auxins or gibberellins partially reverses the effect of seed removal. 4-Cl-IAA, an endogenous auxin of peas, is uniquely active. One of the consequences of seed removal is suppression of the oxidation of GA_{19} to GA_{20}. Application of 4-Cl-IAA reverses this effect. Much of the work previously done in our laboratory used 4-Cl-IAA at 1 μg/pericarp. We now have determined that 50 ng of 4-Cl-IAA is a much more appropriate treatment. By following the metabolism of [^{14}C]-GA_{12} - ald in deseeded pericarp, we observed that treatment with 1 μg 4-Cl-IAA promotes the formation of a compound that chromatographs on C18 HPLC like [^{14}C]-GA_{53}. However, treatment of similar pericarp with 50 ng 4Cl-IAA, which is adequate to stimulate pod growth, has no detectible effect on the formation of the compound that chromatographs like [^{14}C]-GA_{53}. Five ng of 4-Cl-IAA applied to a pericarp-half elicits a significantly positive growth response. Wounding developing pericarp tissue also suppresses the oxidation of GA_{19} to GA_{20}. Treatment with 4-Cl-IAA overcomes the suppressed formation of GA_{20}. Compared to other halogenated analogues of IAA, 4-Cl-IAA is uniquely active in the promotion of growth of deseeded pericarps.

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METABOLISM OF INDOLE-3-ACETIC ACID (IAA)

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Work of the past few decades has provided knowledge of the chemical structures of the products of IAA metabolism. In addition two of the enzymes catalyzing IAA metabolism in Zea mays, have been extensively purified. Now, we can begin to attempt to manipulate endogenous IAA amounts by genetic manipulation of the enzymes of IAA metabolism. The first, and possibly the best candidate, is IAA-glucose synthetase, the enzyme catalyzing the reaction: IAA + UDPG <===> 1-0-IAGlu + UDP [1]. The gene for this enzyme, iaglu, has been cloned and sequenced and the first studies using sense and anti-sense constructs of the gene are in progress. The equilibrium of reaction [1] is towards free IAA and thus to greatly decrease endogenous free IAA levels it is necessary to couple reaction [1] with reaction [2] 1-0-IAGlu + myo-inositol ===> IAInos + Glu. The sum of the free energy changes of [1] + [2] is a large negative number and, at equilibrium, some 95% of the IAA will occur as IAA-inositol. Our current objective is to genetically manipulate reactions [1] and [2] so as to increase, or decrease, free IAA levels. A longer range objective would be to manipulate IAA synthesis and IAA oxidation, since synthesis would be the major input to the IAA pool, in non-seedling plants, and oxidation would possibly be a major output from the IAA pool.

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CYTOKININ METABOLIC ENZYMES IN *PHASEOLUS*

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Zeatin specific metabolic enzymes have been isolated from immature seeds of *Phaseolus* . These include zeatin O-xylosyltransferase, O-glucosyltransferase, reductase and *cis-trans* isomerase. The products of the enzymic reaction are active in bioassays and the enzymes appear to be tissue specific. Selective expression of such enzymes may represent important mechanisms regulating cytokinin activity. Localization and gene cloning experiments began with the zeatin O-glycosylation enzymes. Tissue printing studies using mono-specific antibodies indicated that zeatin O-xylosyltransferase is primarily localized in the endosperm. Immunolocalization studies revealed that the enzyme is associated with the endosperm cytoplasm and nuclei. In vegetative tissues, enzyme activity is not detectable in the cytoplasm while an antigenic protein occurrs in the nucleus. Screening of *P. vulgaris* expression libraries led to the identification of immuno-positive clones. Results of sequence analyses, processing of cDNA products and transgenic experiments will be discussed.

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BIOSYNTHESIS AND METABOLISM OF BRASSINOSTEROIDS

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It was found using Catharanthus roseus crown gall and normal cells that brassinolide is synthesized through a sequential pathway of teasterone → 3-dehydroteasterone → typhasterol → castasterone → brassinolide. The presence of this pathway in some plant tissues was also confirmed. The significance of this pathway in morphological processes of higher plants was investigated in relation to seed germination and dwarfism. Quantitative changes of these brassinosteroids during germination of mung bean seed were investigated by GC-MS using deuterated internal standards, revealing that brassinolide decreased rapidly while the others significantly increased. On the other hand, endogenous brassinosteroids in shoots of gibberellin-response pea mutants *lka* and *lkb* whose dwarfism has remained to be clarified were likewise quantified, revealing that both *lka* and *lkb* have lower level of brassinolide compared with the wild-type. On the basis of endogenous levels of biosynthetic precursors of brassinolide, it was suspected that *lka* and *lkb* have a lesion in the late and early biosynthetic pathways of brassinolide, respectively. Further, a finding is also discussed that 6-deoxocastasterone was accumulated to large extents in both mung bean seed and dwarf pea shoot.

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GIBBERELLIN CONJUGATION AND SEED DEVELOPMENT

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Within the spectrum of plant hormone metabolites, the group of conjugates exhibits a special feature because of the reversibility of their formation. From the known occurrence of gibberellin glucosyl conjugates within immature seeds, a storage function of these metabolites can be derived and a key function during the germination of seeds has been postulated[1].

In order to analyze the proposed function of gibberellin glucosides in course of seed development, endogenous gibberellin glucosides were identified in mature barley grains using GC-MS. The subsequent quantification of the ratio of free gibberellins (GAs) and GA-O-glucosides during seed ripening of barley caryopses was performed by GC-MS of permethylated derivatives. As internal standards a series of deuterium-labelled GA-O-glucosides has been synthesized. From the measured amounts of GA_{20} / GA_{20}-13-O-glucoside and GA_8 / GA_8-2-O-glucoside could be shown that the levels of free GAs decrease, whereas the content of GA_{20}-13-O-glucoside, a potential conjugate pool for reconversion to active free GAs, remains unaffected during the maturing period. The amounts of GA_8-2-O-glucoside, however, increase permanently. This demonstrates the active GA metabolism within seeds in general and reflects the total GA turnover. Attempts to include GA glucosyl esters by using LC-MS for the analysis will be discussed.

[1] G. Sembdner, R. Atzorn, & G. Schneider: *Plant Molecular Biology* **26**,1459 (1994)

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EXPRESSION OF THE *AGROBACTERIUM ROLC* GENE IN TRANSGENIC POPLAR; EFFECTS OF THE ROLC PROTEIN ON HORMONE HOMEOSTASIS AND SEASONAL VARIATION IN *ROLC* PROMOTER ACTIVITY

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We have transferred and expressed a *35S::rolC* construct in hybrid aspen (*Populus tremula x tremuloides*) trees. Regenerated plants showed severe morphological alterations compared to wildtype, including a dwarf-like phenotype with reduced stem elongation, but with an increased number of internodes and very small leaves. Several individuals produced a flat twisted stem by multiple divisions of the apical meristem in one plane as a reult of fasciation. The RolC phenotype was maintained in plants that reactivated after overwintering. The plants were analyzed for differences in their content of plant hormones like indole-3-acetic acid (IAA), gibberellins and cytokinins. The hormones were quantified by gas chromatography-mass spectrometry or by liquid chromatography-mass spectrometry. The content of IAA conjugates in both the leaves and the apex was similar in wildtype and *rolC*-expressing plants, while the levels of free IAA and some gibberellins were decreased and cytokinins of the zeatin family increased in the *rolC* plants. In addition, we monitored the activity of a *rolC::GUS* fusion in hybrid aspen throughout the annual cycle of meristem activity and dormancy. In stems of rapidly growing shoots, the tissue-specific *rolC* promoter was expressed in the phloem companion cells. However, unexpectedly, during dormancy induction, the promoter did not retain this tissue-specific expression but shifted laterally from the phloem, to include most stem tissue types. During subsequent exposure to chilling temperatures the activity practically disappeared in these tissues, but shifted back to the original phloem specificity after the meristems reactivated. The seasonal variation in the cellular location of *rolC* promoter activity was correlated with an *in planta* redistribution of sucrose.The implications of these data will be discussed in relation to both the possible functions of the RolC protein and the phenotype of the transgenic plants.

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REGULATION AND FUNCTION OF *rolB* IN MERISTEM FORMATION

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We have shown that, in thin cell layers (TCL), the oncogene *rolB* strongly promotes the formation of meristems which will then give rise to different organs according to cell competence and/or hormonal conditions in the culture medium. As *rolB in vitro* enhances and accelerates the hormone-mediated organization of new meristems in all differentiative programs (root as well as shoot and flower formation), it is possible that the gene enhances the stimuli of different hormones. We are exploiting the *rolB*-transformed TCL system to identify plant genes involved in meristem formation and/or maintenance. The expression of *rolB* is induced by auxin and is most conspicuous in root meristems. We identified a regulatory domain in the *rolB* promoter (domain B), which is necessary for gene expression in (root) meristems and for auxin inducibility. We have now isolated, from an expression library of tobacco cells, the gene (*NTBBF1*) encoding a plant regulatory factor which binds to a specific sequence in domain B. The protein encoded by *NTBBF1* contains a single zinc finger of a novel type. We identified an identical zinc finger in other tobacco genes with otherwise different sequences, and also in maize and *Arabidopsis* genes. We have thus identified a new family of plant regulatory factors acting *via* a zinc finger conserved throughout the plant kingdom. We are characterizing the regulatory role of NTBBF1 in *rolB* expression in meristems and its role in regulating endogenous plant genes. These latter may well be relevant for meristem organization and/or maintenance.

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CHEMICALLY INDUCED TRANSGENIC EXPRESSION OF CYTOKININ-RELATED *AGROBACTERIUM* T-DNA GENES: HORMONAL ASPECTS OF GENE PRODUCT ACTIVITY

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At present the *ipt* and *rolC* genes of *Agrobacterium* T-DNA are the only genes identified, whose products can interfere with cytokinin metabolism. We have investigated the influence on cytokinins and other hormones in plants expressing these genes under the transcriptional control of constitutive or inducible promoters. The latter approach should avoid the interference with secondary effects or plant homeostatic mechanism which could mask primary *in planta* events. Examples demonstrate that secondary effects might play a so far underestimated role in establishment of altered morphogenesis in transgenic plants. Results obtained with respect to the extend of repression of the the tetracycline inducible system, the kinetic of gene induction and product formation, consequences of local application of the inducer, examples for sink-source experiments and first considerations for the stability of different cytokinin pools and the activation of homeostatic mechanisms will be presented. We have investigated the influence of the *rolC* ß-glucosidase on cytokinin-O- and -N-glucoside levels, when expressed *in planta*. Only minor changes in concentrations of free cytokinins were observed in *rolC* transgenic tissues. HPLC radiocounting assays of feeded ^{3}H-labelled cytokinin glucosides showed that only cytokinin-N3- and O-glucosides, but not -N7- and -N9-glucosides were more rapidly hydrolyzed in *rolC* transgenic tissues than in control tissues. However, endogenous concentrations of N-glucosides were found to be extremely low in tobacco. The identification of tissues particularly rich in O-glucosides makes it feasible to test their accessibility as *rolC* substrates.

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PONTICULIN, THE MAJOR HIGH-AFFINITY LINK BETWEEN THE PLASMA MEMBRANE AND THE CORTICAL ACTIN NETWORK.

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Ponticulin, a well characterized integral membrane protein that binds F-actin and nucleates actin assembly in vitro, is a major component of the *Dictyostelium* membrane skeleton. We have previously cloned the gene for ponticulin from *Dictyostelium discoideum* (Hitt *et al. J. Cell Biol.* 1994, *126*:1421-1431). Ponticulin is an unusual membrane protein, possessing both a glycosyl anchor and transmembrane peptide domains. To study the function of ponticulin *in vivo*, ponticulin-minus (*pon*$^{-}$) cells were generated by homologous recombination. Two independent isolates of *pon*$^{-}$ cells have been characterized. These cells grow at the same rate as the parental strain (AX3K). In growth media, log-phase *pon*$^{-}$ cells do not differ significantly from AX3K cells in uptake rates of either fluid (pinocytosis) or particles (phagocytosis). In addition, the rate and extent of capping of fluorescently labeled Con A is similar to that seen in AX3K cells. Immunoblots of various actin-binding proteins show no change between the *pon*$^{-}$ and AX3K cells. However, crude membranes from *pon*$^{-}$ cells have greatly reduced amounts of associated actin and myosin by SDS-PAGE, as compared to AX3K plasma membranes. Highly purified membranes from the transformants do not nucleate actin assembly in vitro, nor do they bind actin in co-sedimentation assays. Also, *pon*$^{-}$ plasma membranes have at least 70% less associated F-actin than do AX3K plasma membranes. After initiation of development, *pon*$^{-}$ cells aggregate faster than AX3K cells. Subsequent morphogenesis of the *pon*$^{-}$ cells proceeds asynchronously, but viable spores can form. Current investigations show that pseudopod dynamics are aberrant in the *pon*$^{-}$ cells, suggesting that ponticulin may act as a pseudopod "anchor". Thus, ponticulin, the major high-affinity link between the plasma membrane and the actin ctyoskeleton in *Dictyostelium*, is involved in pseudopod stability and pattern formation. (Supported by NIH grant GM33048)

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DYNAMIC REORIENTATION OF CORTICAL MICROTUBULES AND THE EFFECTS OF GIBBERELLIC ACID

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The orientation of cortical microtubules usually reflects the alignment of cellulose microfibrils and the direction of cell expansion. A wide variety of agonists, including ethylene, light and gibberellic acid, are known to re-orientate MTs and thereby modulate the direction of expansion. To study how MTs switch their alignment through 90^0 we have microinjected rhodamine-conjugated tubulin into pea epidermal cells and observed reorientation from transverse to longitudinal in living cells, using the confocal laser scanning microscope. We have also seen the reverse reorientation when GA3 is added to cells pre-injected with rho-tubulin. In both cases, the realignment is indicated by the appearance of "discordant" MTs which do not share the pre-existing orientation and come to dominate the pattern on the outer epidermal wall. By taking multiple optical sections and rotating the projections by computer methods we are also able to image MTs down the anticlinal cell walls and find that the major MT shifts are confined to the outer periclinal cell wall. This tends to emphasize the role of the outer epidermal wall in controlling cell expansion. Addition of GA3 to stem segments of dwarf peas induces both elongation and reorientation of MTs from longitudinal to transverse within 2 hours. By probing 2-D Western blots with anti-tubulin antibodies we find that GA3 causes one of the isotypes to become unrecognisable by an antibody specific for tyrosinated α-tubulin. The possibility that there is a de/tyrosination cycle, which reflects MT stability and perhaps alignment, will be discussed.

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HORMONE-INDUCED REORIENTATION OF MICROTUBULES IN CELLS WITH THE WALL OF A CROSSED POLYLAMELLATE STRUCTURE

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Epidermal cells of azuki bean epicotyls have the wall of a crossed polylamellate structure. It means that the cyclic reorientation of cortical microtubules (MTs) from transverse to longitudinal and longitudinal to transverse is occurring in the cells. Gibberellins (GAs) increase the percentage of cells with transverse MTs. This does not mean that GAs cause the reorientation of MTs from longitudinal to transverse. Since GAs prolong the period in which transverse microfibrils are being deposited on the walls, GAs seem to inhibit the reorientation of MTs from transverse to longitudinal. Auxin does not increase the percentage of cells with transverse MTs in freshly prepared epicotyl segments, but it does increase the percentage when it is applied to the segments pretreated with an auxin-free solution which causes the depletion of auxin and the increase in the percentage of cells with longitudinal MTs. Auxin seems to be required for the reorientation of MTs from longitudinal to transverse and the temporal depletion of auxin seems to cause the synchronization of the reorientation cycle and, thereby, causes a temporal increase in the percentage of cells with transverse MTs. The occurrence in cells with the crossed polylamellate wall of the cyclic reorientation of MTs should be taken into consideration in discussing the mechanism of reorientation of MTs in such cells.

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Microtubule reorientation by mechanical stress

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The cortical microtubules (MT) of elongating plant cells in axial organs (hypocotyl, coleoptile) have been shown to respond to growth-stimulating agents (e.g. gibberellin, auxin) by assuming a transverse orientation (perpendicular to the long cell axis) whereas growth-inhibiting agents (e.g. abscisic acid,ethylen, Ca^{2+}) reorient the microtubules in a longitudinal direction (parallel to the long cell axis). Similar effects can be produced by red and blue light, respectively. In maize coleoptiles, these reorientations can be elicited within 30 to 60 min after inducing an increase or a decrease in growth rate or a tropistic curvature. Only MT underneath the outer epidermal wall of the coleoptile participate in these directional changes. In the past, hormone-mediated MT reorientation was often regarded as a part of the mechanism by which gibberellin, auxin etc. control elongation growth although this hypothesis rests only on correlative evidence. We have now investigated the converse, equally possible hypothesis, namely that MT reorientations are consequences rather than causes of alterations in growth rate. It will be shown that MT reorientations can be produced by replacing growth-promoting or -inhibiting hormones by mechanical stress applied either by bending coleoptiles or subjecting them to longitudinal tension or compression. Mechanical stress can mimick and completely overcome the effects of auxin, light or tropistic stimulation on MT orientation. These results suggest that the mobile MT of the epidermis are involved in sensing vectorial forces and provide vectorial information to the cell which can be utilized in the orientation of coleoptile growth.

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ROLE OF ABA IN ROOT AND SHOOT GROWTH UNDER WATER STRESS: INVOLVEMENT OF ETHYLENE

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Root growth is generally less inhibited than shoot growth at low water potentials (ψ_W). Previous work with maize seedlings showed that the maintenance of primary root elongation at low ψ_W requires the accumulation of ABA. This was achieved by inhibiting carotenoid (and ABA) synthesis using fluridone (FLU) or the *vp5* mutant. Root elongation at low ψ_W was severely inhibited in both cases, but recovered when internal ABA levels in the growth zone were restored with exogenous ABA. In contrast, shoot elongation at low ψ_W was enhanced by ABA-deficiency, indicating that ABA also plays a role in shoot growth inhibition. The root tips of both FLU-treated and *vp5* seedlings at low ψ_W exhibit a pattern of radial swelling similar to the known effect of ethylene treatment. Therefore, the role of ABA in root growth maintenance was hypothesized to involve the action of ethylene. This has been confirmed by demonstrating that each of several inhibitors of ethylene synthesis or binding can partially to fully restore elongation of ABA-deficient roots at low ψ_W, while having little effect on ABA-sufficient controls. To distinguish between ABA effects on ethylene synthesis and sensitivity, studies of ethylene evolution are in progress. Initial results using a flow-through system showed that whole seedling ethylene evolution was enhanced several-fold by ABA-deficiency at low ψ_W; this could be prevented with the same exogenous ABA concentration that causes recovery of root elongation. At the ψ_W used for these experiments (-1.6 MPa) shoot development was almost completely inhibited in all treatments, so the restriction of ethylene synthesis by ABA probably occurred in the roots. To confirm this, a compartmented system is being used to separate root ethylene evolution from that of the shoot and kernel. The ethylene-sensitivity of root elongation will be determined in the same system by applying a range of ethylene concentrations to ABA-deficient or -sufficient roots. The possibility that an interaction of ABA with ethylene may also be involved in the regulation of shoot growth at low ψ_W is being addressed.

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REGULATION OF GENE EXPRESSION BY ABSCISIC ACID DURING STRESS

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One of the important signaling molecules during stress is the plant hormone abscisic acid (ABA). One role of ABA during stress is the induction of a number of genes. In tomato and sorghum, we are studying ABA-induced genes encoding proteins which fall into several different classes, including nucleus-located, and proline-rich. An ABA-induced H1 histone, *le20*, from tomato was found to be located in the nucleus. It is divergent from another tomato H1 histone that is not stress induced, and therefore may play a special role in the organization of the genome during stress. A proline-rich protein from sorghum is only stress induced in the root; it is expressed in the shoot in the absence of stress. The protein is predominantly located in the cell wall and is found in the endodermis during salt stress. It is also ABA-induced in the root, but the protein appears to be located in the cytoplasm as well as the cell wall. It is proposed that this protein is involved in the regulation of solute accumulation. A better understanding of the function and regulation of these genes improves our understanding of the stress response and the role of ABA in plant growth and development.

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MOLECULAR APPROACHES TO DISSECT THE ABA SIGNAL TRANSDUCTION CHAIN

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Results from previous work studying gene regulation during drought stress suggest that ABA is a central molecule in the signal transduction from stress perception to gene expression. The work presented here is directed towards the isolation of genes mediating the ABA response. For this project the observation is utilized that ABA-treated callus from the resurrection plant *Craterostigma plantagineum* is tolerant to desiccation and expresses relevant genes. Leaf discs are transformed with a T-DNA tagging vector (Walden et al., The Plant Journal 1, 1991) to target genes involved in the ABA response. Transgenic calli are selected for the ability to resist to a severe dehydration without undergoing an exogenous ABA treatment. One candidate callus was obtained and molecularly characterized. The targeted sequence was isolated, corresponding cDNA clones were obtained and the features of this gene will be described.

063

JASMONATES AS SIGNAL MOLECULES IN SYSTEMIC PLANT RESPONSES TO WOUND STRESS.

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Mechanical wounding of potato and tomato plants results in a rapid accumulation of several proteinase inhibitors in all the aerial parts of the plant, also in non-damaged tissues distal from the initial wound site. This systemic activation apparently is mediated by a hormone-like inducing factor transported out of the leaf via the vascular system. In addition to polysaccharide fragments derived from the plant cell wall, the peptide *systemin*, and the plant growth regulators abscisic acid (ABA), and jasmonic acid (JA), have been consistently found to induce proteinase inhibitor accumulation in various plant species. Increased levels of both JA and ABA are detected in response to mechanical wounding. Proteinase inhibitor mRNA accumulation does not occur in ABA deficient plants upon mechanical damage or *systemin* treatment, though methyl jasmonate is still capable of inducing high levels of transcript accumulation in these plants. These results suggest that wound-inducible activation of all these genes is regulated by a common signaling pathway, in which abscisic acid and jasmonates would function as intermediate inductive compounds.

Jasmonic acid is synthesized in plants through a octadecanoid biosynthetic pathway which parallels eicosanoid signaling in animals. Eicosanoid biosynthetic enzymes are constitutively present in the animal cell, precursor fatty acid release being the rate-limiting step for prostaglandin synthesis. Similarly, all of the enzymes responsible for jasmonate synthesis from linolenic acid appear to be present in leaves before wounding. Thus activation of a membrane-associated lipase seems to be the key event triggering the wound signaling pathway. Studies aimed to the characterization of this lipase activity will be discussed. Likewise, deletion analysis experiments addressed to identify the promoter cis-elements that mediate response to JA of these defense related genes will be presented.

064

ANALYSIS OF THE ROLE OF ABA AND GIBBERELLINS ON THE REGULATION OF FLORAL TRANSITION IN ARABIDOPSIS.

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More than 20 loci which mutations affect flowering time have currently been identified in Arabidopsis. Moreover, mutations that produce abscisic acid or gibberellins deficiencies or insensitivities can have profound effects on the flowering time and the vegetative development of this species, under certain environmental conditions. We are using treatments with hormones and hormone inhibitors in combination with hormone mutants and mutations that accelerate or delay the floral transition in Arabidopsis to understand the role of those hormones in the control of flowering time. Our results indicate that the Arabidopsis responses to two environmental conditions that delay or accelerate the floral transition, namely short day photoperiods and vernalization, seem to be specially sensitive to the levels of abscisic acid and gibberellins. Gibberellin biosynthesis seems to be required for the promotion of flowering under both enviromental conditions, while abscisic acid seems to play an inhibitor role under short days. On the other hand, these hormones do not seem to be required for the acceleration of flowering under inductive photoperiods (long days) or for the process of dark flowering, when plants complete their vegetative and reproductive development under complete darkness on a medium supplemented with sucrose as a carbon source.

065

GENES MODULATING THE TIME OF FLOWERING IN *ARABIDOPSIS*

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Flowering in *Arabidopsis* can be hastened by environmental or developmental stimuli. We proposed that vernalization promote flowering via demethylation of genes critical for flowering. Treatment with the demethylating agent 5 Azacytidine resulted in early flowering, mimicking vernalization. Demethylation was also induced by expression of the *Arabidopsis* DNA methyltransferase gene in antisense orientation. Plants with reduced levels of methylation flower considerably earlier than wild type supporting our hypothesis. We propose that demethylation of an apex specific GA biosynthesis gene, kaurenoic acid hydroxylase (KAH) occurs during vernalization. Our progress in isolating this gene will be reported.

We have cloned a gene (FLF) with a quantitative effect on flowering. The level of FLF product is correlated with the time to flowering.

066

CONTROL OF ARABIDOPSIS FLORAL MERISTEM IDENTITY AND DEVELOPMENT BY PHOTOPERIOD AND GIBBERELLINS

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A wealth of information has accumulated over the past several decades that implicate light and hormones in the regulation of plant reproductive processes. However, little is known about how these factors act at the cell and molecular levels to affect floral development. Genetic and molecular studies have revealed a small network of genes that control floral meristem and floral organ identity and development in many diverse plant species. In the model plant system *Arabidopsis thaliana*, floral meristem identity is established by the cooperative interactions of at least four genes *APETALA1 (AP1)*, *APETALA2 (AP2)*, *CAULIFLOWER (CAL)*, and *LEAFY (LFY)*. By contrast, floral organ identity is specified by a system of at least six genes that include *AP1*, *AP2*, and *AGAMOUS (AG)*. Although many of these genes have been isolated and characterized, it is not yet known how these genes promote flower development at the molecular level. Nor is it known whether these genes are controlled by environmental or intrinsic factors such as light and plant growth regulators. We have developed a novel system utilizing *ag*, *ap1*, *ap2*, and *lfy* mutants to study the photo- and hormonal control of floral meristem identity in Arabidopsis. This system is based on our recent observation that short-day photoperiod dramatically alters floral meristem identity and development in these mutant plants. The most striking changes occur in *ag (-/-)* and *lfy (+/-)*. Under short-day photoperiod, *ag (-/-)* and *lfy (+/-)* flowers undergo a reversion from flower to shoot development, a process called *floral reversion*. We found that these photoperiod-dependent changes in meristem identity can be suppressed by mutations in phytochrome biosynthesis (*hy* mutants) or by treating short-day grown plants with gibberellin (GA_3). Taken together, these results suggest a role for both photoperiod and gibberellins as potential regulators of Arabidopsis floral meristem identity and development through the activities of *AG*, *AP1*, *AP2*, and *LFY*. We will discuss new data regarding the involvement of the floral homeotic genes and in particular, the role of *APETALA2* in the photo- and hormonal control of flower development.

067

REGULATION OF FLOWERING TIME IN *ARABIDOPSIS*

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In many plant species, the timing of flowering is controlled by the sensing of environmental variables such as daylength and temperature and internal developmental programs such as the transition from a juvenile to adult phase. We are studying the regulation of flowering time in *Arabidopsis thaliana.* We have isolated a gene, *LUMINIDEPENDENS (LD)* , that, when mutated, renders Arabidopsis late flowering. The LD protein is located in the nucleus and contains a glutamine-rich region similar to certain transcription factors in animals. The *LD* promoter is active in all tissues of the plant, but is expressed to the highest level in the apical region of the shoot and in root meristems. We have also studied the effects of the *FRIGIDA (FRI)* gene. A dominant, naturally occurring *FRI* allele is the primary determinant of the difference in flowering time between early- and late-flowering ecotypes of Arabidopsis. The late-flowering phenotype of dominant *FRI* alleles can be suppressed by vernalization. Therefore, the wild-type role of *FRI* is to delay flowering until the plant is exposed to cold, i.e., to create a requirement for vernalization. The phenotype of *ld* mutations is also suppressed by vernalization indicating that *ld* and *FRI* act in the same flowering pathway. We have identified another locus, *FLC*, certain alleles of which completely suppress the effect of *ld* mutations and partially suppress the late-flowering effect of *FRI.* We speculate that *FLC* suppressor alleles result from a loss of *FLC* function, that the role of *FRI* and *FLC* is to inhibit flowering, and the role of *LD* is to counteract the *FLC*-mediated inhibition.

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CHANGES IN WALL ARCHITECTURE DURING PLANT CELL GROWTH AND DIFFERENTIATION

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Given the spatial diversity of architectures that we observe between species, between tissues, and between domains within a single wall, we have chosen to look at changes in wall architecture in suspension-cultured cells, since these are relatively homogeneous and easily manipulated in culture. We have mapped changes in composition and architecture of matrix polymers in carrot and tobacco suspension cultures during cell elongation, and *Zinnia* mesophyll cells during differentiation to tracheary elements at the single cell wall level. In elongating cells, orientation of matrix polymers in addition to cellulose microfibrils transverse to the long axis of the cell occurs. The original pectic network may be replaced by a more highly esterified network with altered rheological properties. Some constraints operate; wall thickness and microfibril spacing are maintained. The *Zinnia* mesophyll cell system consists of isolated leaf mesophyll cells in culture that can be induced, by auxin and cytokinin, to reproducibly trans-differentiate into tracheary elements (TEs). We have used this system to establish the precise time point at which the TE cell fate becomes determined, and then looked for changes in cell-wall composition and architecture that are associated with the establishment of competence, determination, and differentiation with the transition from primary to secondary cell wall formation. We have found changes in the repertoire of both protein and polysaccharide cell-wall components, both in the cell wall and secreted into the culture medium, at very early stages in this time-course.

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XYLOGLUCAN ENDOTRANSGLYCOSYLASES (XETs): THEIR ACTION ON WALL POLYMERS AND ROLE IN THE HORMONAL CONTROL OF CELL EXPANSION

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Primary cell walls contain several enzyme activities whose substrate-specificity suggests that they act on structural components to cause wall "loosening". We have measured changes in the activity of a putative wall-loosening activity, xyloglucan endotransglycosylase (XET). Positive correlations were frequently observed between hormone-induced wall-loosening and extractable XET activity, e.g. in GA_3-treated dwarf pea seedlings, GA_3-treated lettuce hypocotyls, and in IAA-treated cucumber hypocotyls. The transglycosylation reaction catalysed by XET could cause wall-loosening by reversibly breaking and re-annealing xyloglucan chains. In some systems, e.g. 2,4-D-induced elongation of segments of etiolated pea stems and dark-induced elongation of intact pea stems, negative correlations were observed. It thus appeared possible that XETs may also be involved in wall assembly (rather than loosening). An XET could participate mainly in wall assembly if it tended to cleave its donor substrate close to the reducing terminus rather than in mid-chain. We show that XETs from certain tissues do cleave near to the reducing terminus, while others do not. Thus, XET isoenzymes may differ in physiological role. Central to the idea that XETs modify wall properties *in vivo* is the assumption that they can utilise polymeric xyloglucan as both donor and acceptor substrate. This is difficult to demonstrate *in vivo* because the starting materials of the reaction would be chemically identical to the products. We have developed a novel method, based on *in vivo* feeding of ^{13}C- and ^{3}H-labelled sugars, by which polysaccharide-to-polysaccharide transglycosylation can be looked for. This method, with preliminary results, will be reported.

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WALL HYDROLYSIS AS A GROWTH MECHANISM IN THE MEDIATION OF HORMONE RESPONSES

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It has generally been believed that wall integrity is metabolically compromised to facilitate cell extension in response to auxin. Elongating cereal coleoptiles undergo a predominate alteration in the wall structure manifest in a decrease in both the amount and molecular size of a specific *B*- D glucan. Moreover the enzymes responsible for metabolism of glucan are active in the wall. One approach to probe the role of the enzymes has been the use of specific antibodies generated in response to both proteins and polysaccharides. In contrast to putative wall metabolism during later stages of elongation, during early phases of growth the autolytic processes are directed toward polysaccharides that yield pentoses. Antibodies capable of suppressing growth during the intervals of the maximum growth phase are ineffective during initial stages of coleoptile development.

071

CELL WALL ASSEMBLY AND DISASSEMBLY IN CHLAMYDOMONAS REINHARDTII

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Cell wall assembly and disassembly is an integral facet of the mating reaction in the green alga Chlamydomonas reinhardtii. As in higher plants, the Chlamydomonas cell wall defines the shape, function and developmental stage of the cell it encompasses; however, the walls in Chlamydomonas are comprised almost exclusively of proteins and lack the matrix polysaccharides that account for 80% of the higher plant cell wall. Careful analysis of the Chlamydomonas cell wall by EM and extensive biochemical manipulations both in vitro and in vivo have led to a better understanding of the cell wall proteins and their interactions during assembly. Clonal populations of vegetative cells enzymatically stripped of their cell walls will begin to synchronously assemble a new matrix. This assembly requires de novo transciption and translation of new wall proteins allowing us to isolate cDNAs for several wall genes. The derived amino acid sequences revealed peptide domains that are responsible for the characteristic rod and knob structure of the wall proteins and also demonstrated that these molecules are homologs of the cell wall proteins found in higher plants. A tyrosine-rich peptide domain in one of the wall genes led to the demonstration that oxidative cross-linking of proteins via tyrosine residues is involved in insolubilizing the cell wall. During the course of these studies, we discovered that another cross-linking activity, mediated by transglutaminase, is also essential to wall assembly. There are some interesting parallels of Chlamydomonas wall assembly with fertilization membrane formation in sea urchin and the hypersensitive response of infected plants.

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TROPISMS AND THE CYTOSKELETON

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Cortical microtubules can guide the directional deposition of cellulose microfibrils in the cell wall. The orientation of cortical microtubules is subject to environmental control by stimuli such as red light, blue light, gravity and various hormones. This provides a mechanism for signal-dependent morphogenesis on the cellular level.

Tropistic curvature results in a gradient of microtubule orientation accross the stimulated tissue. This gradient is correlated with the bending response as well as with the auxin displacement caused by the tropistic stimulation. The originally assumed causal relationship between tropistic stimulation, microtubules, and asymmetric growth is critically assessed. Microtubule reorientation and fast tropistic responses are shown to be parallel, not causal phenomena. However, microtubules seem to mediate a transverse polarity induced by the tropistic stimulus. This polarity is very stable and can be rendered visible in conditions of symmetric gravity.

In gravitropism, the cytoskeleton fulfills an important function in the establishment of the gravity-dependent polarity of the perceiving cells. Based on measurements of gravitropically induced auxin lateral transport in rice coleoptiles, the role of microtubules and actin microfilaments is discussed.

The final part of the talk presents a mutant approach, by which the factors might be identified, that mediate the link between stimulus-triggered hormonal signalling and the cytoskeleton.

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LATERAL AUXIN REDISTRIBUTION IN TWO TOMATO GRAVITROPISM MUTANTS SUPPORTS THE CHOLODNY-WENT HYPOTHESIS

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The Cholodny-Went hypothesis proposes that lateral transport of auxin is responsible for stem curvature in response to gravity or light stimulus. In the gravitropic response of dicot stems, auxin acts as the transducing signal between the sites of gravity perception (the starch parenchyma cells surrounding the vascular tissue) and asymmetric growth (the epidermal cells). We have studied lateral auxin redistribution in response to gravity in two single gene, recessive tomato mutants, *lazy-2* (*lz-2*) and *diageotropica* (*dgt*). Double labeling studies with ^{3}H-IAA (indoleacetic acid, the major naturally-occurring auxin) and ^{14}C-BA (benzoic acid, an inactive analog) in intact seedlings were used to correlate lateral auxin redistribution with curvature. Shoots of *lz-2* exhibit normal negative gravitropism in the dark but grow downward toward the gravity vector in red light. ^{3}H-IAA accumulation was found to be greater in the lower halves of wild type and *lz-2* hypocotyls in the dark and in wild type hypocotyls under red light. Red light treatment of gravistimulated *lz-2* hypocotyls, however, resulted in greater ^{3}H-IAA accumulation in their upper halves. The kinetics of ^{3}H-IAA redistribution were similar to those observed for the gravitropic bending of wild type and *lz-2* hypocotyls, respectively. Similar studies demonstrated that the slow gravitropic bending of the *dgt* mutant correlates with slow auxin redistribution. The *dgt* mutant exhibits other phenotypic characteristics which indicate either increased cytokinin production or sensitivity. We have found that exogenously-applied cytokinin mimics both the slow gravicurvature response and the retarded lateral auxin transport in wild type plants. Taken together, these findings support the Cholodny-Went hypothesis and suggest that both red light and cytokinin may play a role in gravitropism through controlling lateral auxin transport.

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GENETIC DISSECTION OF PHOTOTROPISM IN *ARABIDOPSIS*

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Despite their obvious importance in seedling establishment, little is known about the mechanisms underlying the perception and transduction of the light signals that induce the phototropic responses of higher plants. We have isolated several mutants of *Arabidopsis thaliana* that lack or have severely impaired phototropic responses. These *nph*, or nonphototropic hypocotyl, mutants comprise multiple genetic loci, including mutants in light perception, signal transduction, and response. Characterization of the *nph1* allele series indicate that the *NPH1* locus may encode the apoprotein for a dual-chromophoric or multichromophoric holoprotein photoreceptor capable of absorbing UV-A, blue, and green light and that this photoreceptor regulates all the phototropic responses of *Arabidopsis*. It appears that the NPH1 protein (the putative photoreceptor) is most likely a 120-kD plasma membrane-associated phosphoprotein. Furthermore, the putative NPH1 photoreceptor protein is genetically and biochemically distinct from the HY4 photoreceptor protein, and these proteins are not functionally redundant. By using various *nph* mutants and phytochrome-deficient mutants we have determined that the primary result of phytochrome activation during irradiations with second positive fluences of UV-A and blue light is to induce a weakened negative gravitropic response in the hypocotyl, thus resulting in an enhancement of the phototropic response when unilateral irradiations are given at right angles to the gravity vector.

075

PHYSIOLOGICAL AND MOLECULAR ANALYSIS OF *RGR1*, AN *ARABIDOPSIS THALIANA* AUXIN PHYSIOLOGY MUTANT AFFECTING ROOT GRAVITROPISM AND LATERAL FORMATION

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The *rgr1* mutation was isolated from the DuPont T-DNA insertional mutagenesis collection. The phenotypes of *rgr1* are as follows: decreased root gravitropism, clockwise root coiling, decreased lateral root formation, slightly recurved rosette leaves, altered root length, and increased auxin resistance (by root elongation assay) to auxins (IAA, 2,4-D, NAA) and auxin transport inhibitors (NPA and TIBA). There is also auxin resistance in hypocotyl elongation and root hair proliferation in response to auxin. The hormone resistance appears to be auxin specific, although small changes in sensitivity to other hormones has not been ruled out. The *rgr1* mutation maps to one map unit of the *ch1* locus on chromosome 1, and the T-DNA and the *RGR1* locus cosegregate, indicating that *rgr1* was apparently caused by T-DNA insertion. For more details see Simmons *et al.*, 1995, *Physiologia Plantarum*, in press. The *rgr1* mutant is allelic to *axr4* (Hobbie and Estelle, 1995, *Plant Journal*, in press). We have cloned the mutant DNA flanking the T-DNA insert by plasmid rescue, and we have isolated a wild-type lambda genomic clone overlapping this site. We have identified 4 distinct cDNAs which map to this site (two immediately to the left and two to the right of the T-DNA). We have sequenced these four cDNAs and their genes (7 kb of contiguous sequence). We will present our findings regarding identification of the candidate *RGR1* gene. This work was supported by the NASA/NSF joint program in plant biology.

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GRAVITROPIC SET-POINT ANGLE - A NEW CONCEPT IN PLANT DEVELOPMENT

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The angle at which an organ is maintained by gravitropism is characteristic of that organ, its developmental state and the prevailing environmental conditions (Myers et al., 1994). We propose that this angle be called the *gravitropic set-point angle (GSA)* and it is the angle with respect to the gravity vector at which an organ is maintained as a consequence of gravitropism. Experiments with hanging or trailing plants such as *Tradescantia* and *Oplimenus* show that an internode changes its GSA from above horizontal (negative gravitropism) to below horizontal (positive gravitropism) as it becomes older. Light conditions also change the GSA of an internode. Studies where internodes have been re-orientated above or below the GSA show that the position of the organ is positively controlled, with the GSA determined by the development age of the internode and/or the light conditions. The GSA can be anywhere between 180° (vertically up) and 0° (vertically down). It is proposed that GSA is controlled by a single gravitropic mechanism. There is no evidence for previous suggestions that various gravitropic orientations (negative and positive orthogravitropism, diagravitropism and plagiotropism) are controlled by different gravitropic mechanisms.

A.B. Myers, R.D. Firn and J. Digby (1994) Gravitropic sign reversal - a fundamental feature of the gravitropic perception or response mechanism in some plant organs. J. Exptl. Botany 45 77-83.

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INTERACTION BETWEEN THE PHYTOCHROME AND GRAVITY SENSORY/RESPONSE SYSTEMS IN *ARABIDOPSIS*

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Hypocotyls of dark-grown *Arabidopsis* seedlings exhibit strong negative gravitropism. However, when grown in continuous red light, the growth direction is randomized with respect to the gravity vector. The response has a fluence rate threshold of about 0.1 μmol m^{-2} s^{-1} and saturates at around 10.0 μmol m^{-2} s^{-1} and the response has been shown to be mediated by phytochrome B. Far-red light was also found to cause a similar disruption of hypocotyl growth orientation with a threshold of less than 0.1 μmol m^{-2} s^{-1}. However, the far-red response is optimal around 1.0 μmol m^{-2} s^{-1} and at higher fluence rates, the hypocotyls have negative gravitropism approaching that of dark-grown seedlings. The far-red effect appears to be mediated by phytochrome A. When dark-grown seedlings are transferred to red or far-red light, their growth pattern shifts from negative gravitropic to plagiotropic. The light-induction of plagiotropism is also dependent on phytochromes A and B. These phytochrome-dependent effects on gravitropism appear to be restricted to the hypocotyl since the roots continue to show normal positive gravitropism in red or far-red light. Red or far-red light has not been observed to alter gravitropism in elongating inflorescences. The red light-induction of plagiotropism in wild-type *Arabidopsis* hypocotyls has similarities to the phenotype of the lazy mutants in tomato and corn and provides a system for dissecting the interaction of the phytochrome and gravity sensory/response systems.

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BLUE LIGHT REGULATION OF GENE EXPRESSION

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The rate of transcription for several nuclear coded genes can be altered by a single, short pulse of low intensity blue light. The process does not require protein synthesis and the response is immediate. We are examining the signal transduction mechanism responsible for this inductive response using the Cab (Lhcb) gene family in both peas and *Arabidopsis*. Specific members of the Cab gene family which are blue light regulated have been identified in both systems and are being used for stable transgenic studies. Separately, we have identified a blue-light-activated G-protein associated with the plasma membrane of etiolated pea buds. Activation of the G-protein has the same fluence response characteristics as activation of the Cab genes. Treatment of dark-grown pea or *Arabidopsis* seedlings with mastoparan, a known G-protein activator, induces the expression of the blue-light regulated members of the Cab Gene family in both plants. The coincidence in location and activation characteristics of the G-protein and the transcription of specific members of the Cab gene family together with the mastoparan studies indicates a causal relationship between the G-protein and transcription.

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ANALYSIS OF THE PHOTOCONTROL OF ANTHOCYANIN BIOSYNTHESIS IN TOMATO SEEDLINGS USING PHOTOMORPHOGENIC MUTANTS

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Anthocyanin biosynthesis has been studied in *ca.* 3.5-day-old dark-grown seedlings of tomato (*Lycopersicon esculentum* Mill.) wild types (WTs) and photomorphogenic mutants during a subsequent 24-h period of monochromatic irradiation at different fluence rates. In red light (R) the fluence-rate response relationships for induction of anthocyanin in the WTs are similar, yet complex, showing a response at very low (VL) fluence rates followed by a fluence rate dependent high irradianceresponse (HIR). The high-pigment-1 (*hp-1*) mutant exhibits a strong amplification of both response components. Another mutant, called *atroviolatia* (*atv*) shows strongest amplification of the HIR component. In contrast, a transgenic line over-expressing an oat phytochrome A gene (*PHYA3*) shows a most dramatic amplification of the VL fluence rate component. The *fri* mutant, deficient in phytochrome A (phyA), lacks the VL fluence component whilst retaining a normal HIR. The *tri* mutant, deficient in phytochrome B1 (phyB1) retains the VL fluence rate response, but lacks the HIR. The *fri,hp-1* and *tri,hp-1* double mutants exhibit amplified, yet qualitatively similar responses to the *fri* and *tri* mutants. The *fri,tri* double mutant lacks both response components. Experiments at other wavelengths demonstrate that while there is only a small response in the far-red spectral region (729 nm) in tomato, there is an appreciable HIR response at 704 nm, which is retained in the *tri* mutant. This suggests that the labile phyA pool participates in the HIR at this wavelength (the authors thank B.T. Boylan & P.H. Quail for provision of the *PHYA3* transgenic line).

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SIGNAL TRANSDUCTION PATHWAYS CONTROLLING LIGHT-REGULATED DEVELOPMENT IN ARABIDOPSIS

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We are taking a combined genetic and molecular biological approach to identify components of the light signal transduction pathways in plants. Our studies have identified a class of Arabidopsis mutants that show many characteristics of light-grown plants even when grown in complete darkness (*de-etiolated*). Because these mutations are both pleiotropic and recessive, we hypothesized that *DET* genes play a negative regulatory role in photoregulated gene expression and development in Arabidopsis. To determine the molecular mechanisms of light signal transduction, we are cloning the various *DET* genes by the method of chromosome walking. *DET1* encodes a nuclear-localized protein that is ubiquitously expressed during Arabidopsis development. We are testing the idea that DET1 acts to repress the expression of light-regulated promoters by interactions with other nuclear proteins. In a separate set of experiments, we have identified mutations in two of the 5 Arabidopsis phytochrome apoprotein genes. Our results suggest that phytochrome B plays a general role in cell elongation in response to light and also controls flowering time and chlorophyll accumulation. In contrast, phytochrome A appears to play a highly specialized role in Arabidopsis development. The phenotype of *phyA phyB* doubly null mutants indicates that for some processes PHYA and PHYB appear to have complementary functions. Lastly, we have utilized a genetic approach that focuses on one particular downstram light-regulated response, the transcription of the *CAB3* promoter. Using transgenic lines, we have identified mutations that define at least 5 complementation groups in which the *CAB3* promoter is expressed at aberrant high levels in the dark or low levels in the light. Some of the mutations also affect the levels of *RBCS* mRNAs, while others affect only *CAB*. We used the same transgenic lines to identify mutations in four genes defining an intracellular signal transduction pathway from the chloroplast to the nucleus. Presumably, this pathway functions to coordinate the expression of nuclear and plastid-encoded genes involved in photosynthesis.. The mutations also impede the early steps in etioplast to chloroplast transition. The results from epistasis studies suggest a hierarchical regulatory network among the various genes in the control of light-regulated responses.

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CALCIUM-DEPENDENT AND -INDEPENDENT SIGNALING PATHWAYS IN BARLEY ALEURONE

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The cells of the aleurone layer of the barley grain are well established as a model system for studying hormonal perception and signal transduction. The aleurone layer secretes hydrolazes (principally α-amylases) that mobilize endosperm reserves during germination. Gibberellin (GA) stimulates the synthesis and secretion of these hydrolazes and this stimulation is antagonized by abscisic acid (ABA). These hormones induce a complex set of changes in the aleurone cell ranging from altered gene transcription to ultrastructural changes in the secretory apparatus. Although GA and ABA are known to be perceived at the plasma membrane, the cellular events leading to the transduction of these signals and the coordination of cellular responses are poorly understood. Elements of a putative Ca^{2+}-calmodulin (CaM) based signal transduction system are present in these cells. However, the direct relationship between Ca^{2+}, CaM and coordination of responses in the aleurone has yet to be demonstrated. In this study, aleurone protoplasts have been microinjected with caged (photoactivateable) Ca^{2+}, caged Ca^{2+}-chelators or caged-CaM. The effect of photoactivating these compounds on hormone regulated gene transcription from the barley amylase promoter (GA and ABA responsive) and wheat EM promoter (ABA responsive), on exocytosis and on cell development were studied in individual aleurone cells. These experiments suggest Ca^{2+}/CaM-dependent events coordinate exocytosis whereas Ca^{2+}-independent pathways control gene transcription in response to the GA signal. Control of gene transcription in response to ABA operates by both Ca^{2+}-dependent and Ca^{2+}-independent mechanisms. Immunolocalization of CaM and microinjection of fluorescently labeled CaM also indicates that GA induces both an increase in the level and redistribution of this regulatory molecule. These results suggest Ca^{2+} and CaM interact with other signal transduction systems in coordinating aleurone cell responses to GA and ABA. This work was supported by USDA grant 94-37303-0955.

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SALICYLIC ACID AND REACTIVE OXYGEN SPECIES IN PLANT DISEASE RESISTANCE

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Salicylic acid (SA) is an endogenous inducer of several plant defense responses. We have purified and characterized a soluble SA-binding protein (SABP) from tobacco, whose binding specificity and affinity are consistent with a receptor function. SABP is a catalase whose ability to convert H_2O_2 to H_2O and O_2 is inhibited by SA binding. The resulting rise in H_2O_2 levels then appears to act as a signal to activate defense-related genes such as pathogenesis-related (PR)-1 genes. In support of this model, increasing H_2O_2 levels by treatment of tobacco leaves with SA or with the known catalase inhibitor 3-amino triazole induces PR-1 gene expression. Similarly, several different prooxidants induce PR-1 expression while antioxidants suppress PR-1 activation by SA. PR-1 genes are also induced in transgenic tobacco plants expressing an antisense copy of the catalase cDNA thereby inhibiting synthesis of endogenous catalases. In addition, INA, a synthetic inducer of PR gene expression and enhanced disease resistance, also binds to and inhibits catalase activity. It is likely that SA inhibits catalase by donating an electron to the enzyme intermediate compound I to form inactive compound II. This results in not only inhibition of catalase activity but formation of a free radical of SA. SA induces lipid peroxidation, which is probably mediated by the SA free radical and involved in PR gene activation.

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CHARACTERIZATION OF ABA-RESPONSIVE PROTEIN KINASES UPREGUATED DURING ENVIRONMENTAL STRESS

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ABA acting as a signal molecule may coordinate whole plant responses to dehydration and cold temperature, and protein phosphorylation mediated by protein kinases may link initial stress responses with accumulation of osmoprotectants. Recent experiments from our laboratory show that initial increases in ABA are followed by the upregulation of a unique protein kinase mRNA, called PKABA1 (protein kinase-ABA responsive). Two features of the kinase make it unique. First, in response to stress the protein kinase mRNA accumulates, which is unlike the signal responses usually reported in animal systems where signalling protein kinases are activated at the protein level but rarely at the mRNA level. In contrast when wheat seedlings are dehydrated and ABA levels increase, PKABA1 mRNA is upregulated within one to two hours, and when seedlings are placed at 2°C, both ABA and PKABA1 mRNA levels rise within 10 hours. PKABA1 mRNA also accumulate in response to osmotic and salt stress. The PKABA1 cDNA has been expressed in the pET28 and pTRX-FUS recombinant systems. Second, sequence analysis of PKABA1 protein kinases shows a unique sequence that has little homology to other types of protein kinases. A characteristic sequence feature of the PKABA1 subfamily is an acidic amino acid stretch outside of the catalytic domain and similar kinase sequences have been reported by other laboratories in Arabidopsis, soybean, rapeseed and ice plant. Sequence comparison of the PKABA gene subfamily shows the closest homology with the SNF1 yeast and plant protein kinases, involved in nutritional stress responses. Supported in part by USDA-NRICGP No. 94-37100-0313.

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THE REGULATION AND ROLE OF CALCIUM IN CYTOKININ-INDUCED BUD FORMATION IN MOSS

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In the moss, *Funaria hygrometrica*, cells of the proper physiological stage respond to cytokinin by producing vegetative buds. Cytokinin causes a rapid, transient increase in cytoplasmic calcium (Ca^{2+}) which stimulates the cascade of events leading to bud formation. The goal of our work is to understand Ca^{2+} regulation and action during moss vegetative development. We have characterized a dihydropyridine (DHP)-sensitive Ca^{2+} transport system in the moss plasma membrane and have shown that influx of $^{45}Ca^{2+}$ into moss protoplasts is voltage-dependent, DHP-sensitive and stimulated by cytokinin. We have characterized the binding of [^{3}H]azidopine (an azidolabeled DHP) to isolated plasma membranes to understand the interaction of the Ca^{2+} channel with DHPs and to identify the channel polypeptide. DHP-specific [^{3}H]azidopine binding (K_D= 5.1 nM, B_{max} = 412 pmol/mg protein) is stimulated by Ca^{2+} and cytokinin. To look at the temporal and spatial distribution of the Ca^{2+} channel during bud formation, we are isolating the gene encoding the channel protein. Degenerate oligonucleotide primers made to conserved regions of the DHP-sensitive Ca^{2+} channel from animal cells have been used to amplify a portion of the putative DHP-sensitive channel from moss. A number of cellular and morphological changes occur during bud formation, including: reorientation of the cell walls, cytoskeletal redistribution, movement of cellular organelles to and swelling at the presumptive bud site, and cell division leading to the formation of the bud initial. We have begun to determine if there are Ca^{2+}-mediated changes in enzyme activity and gene expression which regulate these cellular and morphological changes. We are using stage-specific cultures to examine the cytokinin-induced, Ca^{2+}-mediated changes in phosphorylation during bud formation. To look at the spatial and temporal regulation of phosphorylation, we have isolated the moss homologs of Ca^{2+}-dependent protein kinase, casein kinase IIα and calmodulin via amplification of stage-specific cDNA using degenerate primers. In addition, putative stage-specific cDNAs have been identified using differential display. We are using RNA blot analysis to monitor expression of these cDNAs through the developmental switch to vegetative bud formation.

085

STRUCTURE AND FUNCTION OF MICROFILAMENTS IN GUARD CELLS

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Since guard cells have served as a model system to study signal transduction pathways and cell shape determination in plants, and microfilaments (MFs) appear to be involved in both processes in many other cell types, we examined the structure and function of MFs in fully differentiated guard cells. We observed by immunolocalization and by microinjecting phalloidin-FITC into live guard cells that MFs in mature guard cells of *Commelina communis* L. are radially distributed fanning out from the stomatal pore site. This pattern of distribution closely resembles that of microtubules in developing guard cells, suggesting possible involvement of MFs in guard cell shape determination. The role of MFs in stomatal movements was tested using phalloidin (PH), an inhibitor of actin depolymerization, and cytochalasin B (CB) or D (CD), inhibitors of actin polymerization. Pretreatment of peeled epidermis of *C. communis* with PH inhibited the stomatal closing induced by ABA and inhibited the stomatal opening under light, while pretreatment with CB or CD induced the stomatal opening even in the dark condition, suggesting an important role of MFs in regulation of stomatal movements. The opening effect of CD is likely to be mediated by its effects on voltage-dependent K^+ channels in plasma membrane of guard cells; CD enhanced inward K^+ channel activity and decreased the outward K^+ channel activity, which would facilitate accumulation of K^+ in the cell and consequent stomatal opening. (Supported by a grant from Korea Ministry of Education)

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NOVEL MECHANISMS OF ACTION OF MICROBIAL PHYTOTOXINS WITH POTENTIAL AS HERBICIDES

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There is great incentive to discover effective and economically feasible herbicides that are toxicologically and environmentally benign. Furthermore, there is growing interest in introducing herbicides with new molecular sites of action. Almost all of the known molecular sites of action of highly active microbial phytotoxins are not molecular targets of commercial herbicides. Microbial phytotoxins have provided two herbicides with a new site of action, glutamine synthetase. They have also yielded valuable information on potential molecular sites of action that might be exploited in herbicide discovery research. This knowledge has also been extremely useful in probing the biochemistry of plants. The novel sites of action of tentoxin, cornexistin, hydantocidin, cyperine, and AAL-toxin and its analogues will be discussed in detail as examples of microbial toxins that are very effective herbicides with novel sites of action.

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NATURAL PRODUCTS FROM PLANTS THAT INFLUENCE MICROORGANISMS

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In their interactions with microorganisms, higher plants encounter both beneficial and deleterious relationships. The response of plants to deleterious interactions such as disease has been a major area of plant science research during the past several decades. In order to invade plant tissues, attacking microorganisms must first penetrate surface layers, most of which possess physical barriers such as waxy cuticles and lignified/suberized cells. If the microorganism(s) are able to overcome these physical barriers, however, only chemical barriers produced by the plant prevent further infection. Chemical barriers include both pre-infectional, constitutive compounds and post-infectional, pathogen-induced compounds, representing many different chemical classes. Both constitutive and induced compounds, their respective chemical classes, and their potential uses in agriculture will be discussed.

088

NATURAL PRODUCTS FROM MICROORGANISMS THAT INFLUENCE GROWTH AND DEVELOPMENT IN PLANTS

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During the past two decades, a number of microbial metabolites have been isolated and identified that characteristically influence the growth and development of plants. Some exhibit plant growth regulatory properties while others have herbicidal properties. In the former category are compounds like 2′, 5′-dihydroxy-3′-nitrophenylacetic acid methyl ester, rotihibin A, thienodolin, ampucillin, isoampucillin, and deoxyfunicone. The latter category, which is composed of phytotoxins and plant growth inhibitors include 4-hydroxy-3′-(3methyl-2′-butenyl) benzoic acid, the triticones and botcinolide. Examples of these and other compounds will be discussed.

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THE USE OF ALLELOPATHIC CHEMICALS IN SUSTAINABLE AGRICULTURE

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Allelopathic cover crops are the most promising application of allelopathic chemicals to weed control in agriculture. To access the allelopathic activity, new bioassay procedures named " plant box method", and "sandwitch method" were developed, and ca. 400 plant species and 1500 cultivars have been evaluated. There are many factors involved in weed control by cover crops, among which are competition for light and nutrients, in addition to allelopathy. A "weed suppression equation" including these factors was proposed, and leguminous cover crops such as hairy vetch, velvet bean, and gramineous cover crops, oats, rye and barley were found the most promising. Allelopathic chemicals from velvet bean was identified as L-3,4-dihydroxyphenylalanine, and from Crematis paniculata and Pulsatilla cernua, protoanemonin. Practical methods of using allelopathic cover crops to control weeds in abandoned fields were developed by field experiments, and found hairy vetch the most promising.

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AUXIN-BINDING PROTEIN 1 - A HISTORICAL PERSPECTIVE

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Rainer Hertel and his group discovered auxin-binding in microsomes of corn coleoptiles during 1970s and described later three binding sites where Site 1 was localized within the ER Several groups solubilized the auxin-binding protein from these microsomes and characterized it as related to Site 1. In 1980s we got data about its physiological functions, its molecular structure, and its distribution in corn. In more recent years we learned about gene structures of the auxin-binding proteins - a small gene family versus a single copy gene in corn - „ auxin-binding protein1 homologes in other plants and much more about the location of the functional ABP 1. What we do not know is the function of these proteins within the ER which may not be related to auxin and auxin action at all. We just get some new insights into the essential signal transduction from the auxin recognized by ABP 1 at the outer surface of the plasma membrane to the cytoplasm for the different cell responses.

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AUXIN INDUCED-CLUSTERING OF THE AUXIN-BINDING PROTEIN AT THE SURFACE OF MAIZE COLEOPTILE PROTOPLASTS: PRELUDE TO ENDOCYTOSIS?

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The predominant localization of the major auxin-binding protein (ABP1) of maize is within the lumen of the endoplasmic reticulum. Nevertheless, all the electrophysiological evidence supporting a receptor role for the ABP1 implies that a functionally important fraction of the protein must reside at the outer face of the plasma membrane. Using methods of protoplast preparation designed to minimize proteolysis, we report the detection of ABP at the surface of maize protoplasts by the technique of silver enhanced immunogold-epipolarization microscopy. We also show that ABP clusters following auxin treatment and that this response is temperature-dependent and auxin-specific.

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SYNTHETIC PEPTIDES AS PROBES OF AUXIN BINDING PROTEIN FUNCTION.

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The major auxin binding protein of maize (*Zm*ABP1) is a 22 kDa soluble protein with no hydrophobic domain long enough to span the membrane. Accordingly, it has been proposed that *Zm*ABP1 must interact with a surface localised docking protein, presumably via one or more solvent exposed domains. In order to test this idea, synthetic peptides were constructed which correspond to predicted surface regions of *Zm*ABP1 and their activity assessed in a electrophysiological, ion imaging and biochemical measurements. In intact *Vicia* guard cells, a peptide ($Pz_{152-163}$) based on the C-terminus of *Zm*ABP1 was able to cause a potent (I_{50}< 10 μM) and reversible inhibition of the inwardly directed K^+ channel when applied externally, whilst peptides corresponding to other domains were without effect. In parallel studies using BCECF and FLUO-3 as reporters, peptide $Pz_{152-163}$, but not other peptides, was found to elevate internal pH and Ca^{2+} with the same concentration dependency and reversibility. In studies with *Zea* microsomal membranes, only $Pz_{152-163}$ and peptides based around the C-terminus of *Zm*ABP1 were able to stimulate binding of the guanine nucleotide GTPγS. Interestingly, peptide $Pz_{148-159}$ which lacks the C-terminal -KDEL motif stimulated GTPγS binding whilst peptide A6.1n , of equivalent composition to $Pz_{152-163}$ but with residues 152 to 159 reversed, was inactive. In other studies with *Zea* membranes, the peptide stimulated GTPγS binding could be restored to NaCl-treated membranes by readdition of fractions enriched in immunologically detectable Gα subunits. In conclusion, the data support the idea that at least one signalling pathway mediated by *Zm*ABP1 proceeds via a G-protein linked receptor and subsequent regulation of cellular pH and Ca^{2+}.

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ENT-KAURENE SYNTHASE ACTIVITY IS LOCALIZED IN PROPLASTIDS AND LEUCOPLASTS OF THREE SPECIES OF HIGHER PLANTS

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The cyclization of geranylgeranyl diphosphate to *ent*-kaurene in two steps is an important event in gibberellin biosynthesis. The two reactions are catalyzed by two closely related enzymes, *ent*-kaurene synthase A and B, respectively. Although these enzymes have been studied in the fungus *Gibberella fujikuroi* and in immature seeds of higher plants, it has been difficult to demonstrate and study them in vegetative tissues from higher plants. The same has been true for the subcellular localization of *ent*-kaurene synthesis. Chloroplasts of vegetative tissues have yielded no A activity and, at the most, very low B activity. We obtained a very active cell-free system capable of converting [^{14}C]geranylgeranyl diphosphate to *ent*-[^{14}C]kaurene from the basal portions of five-days old shoots of wheat seedlings (*Triticum aestivum* L.). Since this tissue contains mainly proplastids, we proceeded to isolate plastids by Percoll gradient centrifugation using conditions suited to preserve proplastids, which are lighter and osmotically more sensitive than mature chloroplasts. Indeed, by means of marker enzyme activities, the synthesis of *ent*-kaurene from geranylgeranyl diphosphate could now be quantitatively assigned to the plastid fraction. The enzyme activity was located within the plastids, probably in the stroma. Plastid membranes had no activity. Plastid stroma prepared by the same methods from pea (*Pisum sativum* L.) shoot tips and pumpkin (*Cucurbita maxima* L.) endosperm, which latter tissue contains leucoplasts only, also yielded *ent*-kaurene synthase activity, whereas mature chloroplasts from wheat leaves and pea shoot tips did not contain *ent*-kaurene synthase activity. We therefore believe that *ent*-kaurene synthesis may be limited to proplastids of growing tissues and leucoplasts of seed tissues, which was confirmed by density gradient centrifugation and microscopical studies. Our results explain why previous attempts to locate *ent*-kaurene synthesis in chloroplasts have been unsuccessful.

094 KAURENE SYNTHASE A AND SEED DEVELOPMENT

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Gibberellins (GAs) are known to be involved in several aspects of plant development, including shoot elongation and seed germination. Recently, GA deficient mutants have been used to suggest that GAs are also required for seed development in pea (*Pisum sativum* L.) in the first few days after fertilization. For one of these mutants, *ls-1*, biochemical analysis suggests that GA biosynthesis is reduced in a developmental- and organ-specific manner by reduced conversion of geranylgeranyl pyrophosphate to copalyl pyrophosphate. This step is catalysed by the GA biosynthetic enzyme kaurene synthase A and this enzyme has been cloned from Arabidopsis. A putative kaurene synthase A has also been cloned from maize. To understand the regulation of kaurene synthase A expression in wildtype and *ls-1* plants, we have used RT-PCR on mRNA from developing embryos to clone fragments of putative kaurene synthase genes from pea and pumpkin. Preliminary results suggest that at least one (and possibly more) kaurene synthase A gene is expressed in embryos of both species. Fragments from pea and pumpkin have been isolated and have high amino acid similarity (75% and 63%, respectively) with the corresponding Arabidopsis kaurene synthase A fragments.

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BIOSYNTHESIS OF GIBBERELLIN AND INDUCTION OF α-AMYLASE ACTIVITY IN GERMINATING RICE SEEDS

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The biosynthesis of endogenous GA_1 and its role on the induction of α -amylase activity was investigated during germination of rice (Oryza sativa L.). The levels of 13-hydroxy GAs in the germinating rice seeds began to increase 3 days after the imbibition of seeds with water, at the time shoot started elongation. The level of GA_1 and α-amylase activity in the seeds increased simultaneously. The α-amylase activities in the dx and dy rice (dwarf) were less than that in the tall cultivar. The induction of α-amylase activity was inhibited with a treatment of seeds with uniconazol. The α -amylase activity in the seeds treated with uniconazol was recovered by additional application of GA_1. These results confirm that the endogenous GA_1 biosynthesized de novo regulates both shoot elongation and αamylase induction in germinating rice seeds. The biosynthetic site of GA_1 was suggested to be the shoots.

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MAIZE GA-MUTANTS: ISOLATION, CHARACTERIZATION, AND GENE CLONING AND EXPRESSION

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As an approach to studying the roles gibberellic acid (GA) plays in the growth and development of maize, we have isolated and characterized a number of GA mutants from maize. Our collection includes a new class of GA mutants. This new class of andromonecious dwarfs is recessive and non-responsive to GA3 treatment. Such a mutant, designated d*19, has all the characteristics common to maize andromonecious dwarfs. It also has additional floral phenotypes which will be discussed. A Mu2 tagged allele of *an1* was used, in conjunction with a reverse genetics screen of the "mutation machine", to clone the *An1* gene (The Plant Cell, 7:75-84). Expression studies of *An1* have been performed using quantitative RT-PCR. The results of these studies will be presented. A 485 bp product was generated by RT-PCR using template from an *an1* deletion mutant (*an1-bz2-6923*) and a select *An1* primer pair. Since *an1-bz2-6923* lacks *An1* transcript, this 485 bp RT-PCR product has been designated *An2*. The 485 bp product is 81% identical to *An1* at the nucleotide level, and 81% identical, 91% similar, at the amino acid level. Putative full length cDNAs for *An2* have been generated by a PCR screen of an "anchored" maize cDNA library. The characterization of *An2* will be presented.

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AFFINITY LABELLING OF GIBBERELLIN HYDROXYLASES

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The soluble 2-oxoglutarate-dependent dioxygenases of the later stages of gibberellin biosynthesis are inhibited by the acylcyclohexanedione class of plant growth retardants, such as prohexadione. These compounds have been shown to compete with 2-oxoglutarate for binding to the enzyme. A program of structure/activity studies with analogues of prohexadione led to the synthesis of a photoaffinity probe and an alkylating probe. These compounds retained the strong inhibitory properties of the parent compound in assays with several gibberellin hydroxylases. After radiolabelling with tritium, both compounds were shown by SDS-PAGE/fluorography to label partially purified *Cucurbita maxima* 3ß-hydroxylase and also a recombinant *Arabidopsis thaliana* 20-oxidase. The labelling was competed out by prohexadione and 2-oxoglutarate. The affinity reagents will prove useful for identifying 2-oxoglutarate dependent dioxygenases and also for examining the structure of the active site of these enzymes.

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IN SITU ANALYSIS OF *An1* TRANSCIRPT ACCUMULATION

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The anther ear 1 (*An1*) gene encodes a subunit of kaurene synthase, which cyclizes GGPP (geranylgeranylpyrophosphate) to form *ent*-kaurene in the first committed step of gibberellin (GA) biosynthesis (Bensen et al., Plant Cell 7:75-84). Maize mutants with defective GA biosynthetic activity are characterized by, among other things, a dwarf growth habit and short, broad leaves because of reduced cell elongation, and anthers on their ears because of failure of male organ abortion during diffferentiation of the female inflorescence. We will present in situ hybridization data which reveals *An1* transcript accumulation in tissues undergoing cell elongation. In roots, *An1* transcript is abundant in the cortical cells of the elongation zone, ca. 1 cm proximal to the root tip, as well as in the region of lateral root formation. The *An1* transcript also is abundant in the cells at the base of young, green leaves still within the sheath. These observations allow coordinatation of the mutant phenotype with the molecular histology of wild type plants.

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MOLECULAR CLONING OF GIBBERELLIN 20-OXIDASES FROM LEGUMES

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Gibberellin A_1 (GA_1) levels are high in developing seeds of *Pisum sativum* L. and *Phaseolus vulgaris* L. at six days and 15 days, respectively, after anthesis. Seeds at these developmental stages were used as a source of poly(A)$^+$ RNA for the synthesis of cDNA. The cDNA was used as a substrate for PCR with degenerate oligonucleotide primers designed on the basis of conserved amino acid sequences in 2-oxoglutarate-dependent dioxygenases. PCR clones encoding amino acid sequences similar to those in GA 20-oxidase from pumpkin (Lange et al., *Proc. Natl. Acad. Sci.* 91:8552, 1994) were used to screen appropriate cDNA libraries in λZAP. One full-length cDNA clone from *P. sativum* (G27-12, about 1400 bp) and two from *P. vulgaris* (J15-11 and J73-1, 1500-1600 bp), were isolated, each containing open reading frames showing considerable amino acid similarity to GA 20-oxidases from pumpkin and *Arabidopsis thaliana*. The clones were introduced in frame into pTrcHis vectors for expression as fusion proteins in *E. coli* and the products were tested for 20-oxidase activity using [^{14}C]GA_{12} and [^{14}C]GA_{53} as substrates. It was shown by GC-MS that the three recombinant proteins were capable of converting [^{14}C]GA_{12} to [^{14}C]GA_9, and [^{14}C]GA_{53} to [^{14}C]GA_{20} with equal efficiencies. This is in contrast to other GA 20-oxidases cloned previously from pumpkin, *Arabidopsis* and *Marah macrocarpus*, for which GA_{12} is the preferred substrate.

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GIBBERELLINS AND STEM ELONGATION OF PEA PLANTS GROWN UNDER LOW-IRRADIANCE STRESS

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Low-irradiance light enhances shoot elongation of many plants. We have investigated the changes in gibberellin (GA) levels (GA_{53}, GA_{44}, GA_{29}, GA_{20}, GA_{19}, GA_8 and GA_1) in shoots of pea cv. Alaska grown under different irradiances of white fluorescent light (100% [Control], 50% [R-1], 10% [R-2]) and darkness by GC/SIM using internal standards. The most evident growth response under reduced irradiance and in the darkness was an increase in stem elongation. The relative height for R-1, R-2, and dark-grown plants was 157%, 275% and 460% of the control plants. Compared with control plants, R-1 and R-2 plants contained slightly higher levels of GA_1, GA_8 and GA_{29}, while the GA_{20} level was markedly higher. In the dark, GA_1 and GA_8 levels also slightly increased, while GA_{20} and GA_{29} levels decreased compared with control plants, respectively. The GA_{53}, GA_{44} and GA_{19} levels were low and showed no significant changes. As a result of low irradiance, GA_1 accumulates slightly (2 to 3 fold increase) in shoots which results in enhanced stem elongation. In addition, low irradiance increases the responsiveness of the tissue to GA.

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ROLE OF GIBBERELLINS AND PHYTOCHROME IN SENESCING LEAVES OF *ALSTROEMERIA PELEGRINA* L.

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Sensescence in leaves of *Alstroemeria* cut flowers is retarded by both irradiation with red light and the application of gibberellins. Both far-red light and inhibitors of gibberellin biosynthesis abolished the effects of red light. Therefore, it is postulated that, with regard to leaf senescence in *Alstroemeria,* phytochrome action requires gibberellin metabolism. To investigate the effect of phytochrome on gibberellin content in *Alstroemeria*, we performed qualitative and quantitative analysis of endogenous gibberellins by means of GC-MS. We will present an inventory of free GAs in dark and red light treated leaves. The possible effect of phytochrome on the 3β-hydroxylation of GA9 to GA4 was tested by applying them to detached leaves, placed in the dark or under red light conditions, in combination with paclobutrazol and prohexadione (BX-112).

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ENDOGENOUS GIBBERELLINS IN THE IMMATURE FRUITS OF *Rhaphiolepsis umbellata* Makino var. *mertensii* Makino

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Endogenous gibberellins (GAs) of immature fruits of Japanese hawthorn (*Rhaphiolepsis umbellata* Makino var. *mertensii* Makino) were surveyed by combined gas-liquid chromatography / mass spectrometry, after purifying the acidic ethyl acetate-soluble fraction by several chromatographic procedures. Consequently, GA_{35}, GA_{50}, GA_{80} and GA_{84} were identified. Any GAs on early-non-hydroxylation pathway and on early-13-hydroxylation pathway except GA_{19} were not found. Based on the results, a possibility of the existence of early-11-hydroxylation pathway will be suggested in the fruits of Japanese hawthorn. Additionally, at least three GA-like compounds were detected after methyl ester / trimethylsilyl ether derivatization, whose characteristic fragment ions were M^+416, M^+-43, M^+-103, M^+-134; M^+506, M^+-15, M^+-103, M^+-134; and M^+592, M^+-15, M^+-90 and M^+-134, respectively. However, the mass spectra and Kovats Retention Indices of these compounds were not coincided with those of known GAs. The structures are now in elucidating.

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AN OPTIMIZED PROTOCOL FOR THE HETEROLOGOUS EXPRESSION OF GIBBERELLIN 20-OXIDASE ACTIVITY IN cDNA LIBRARIES

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Gibberellin biosynthesis occurs *via* a complex pathway, the later steps of which are catalysed by 2-oxoglutarate dependent dioxygenases. Recently, a cDNA encoding the gibberellin 20-oxidase was isolated from a λgt11-library derived from *Cucurbita maxima* cotyledons (Lange, Hedden and Graebe, PNAS 91, 8552-8556). 20-Oxidase activity was detectable even in single plaques. 20-Oxidase activity was also detectable in total lysates of *Escherichia coli*, strain Y1089, that were lysogenized with this λgt11-library. Conditions for the production, extraction and functional assay of the recombinant 20-oxidase were optimized for this simple expression system. Furthermore, we demonstrate that this protocol is also suitable for the heterologous expression of 20-oxidases from other sources and with different expression vectors.

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A NEW GIBBERELLIN, 1,2-DIDEHYDROGA$_{20}$ AS A PRECURSOR CANDIDATE FOR GA$_3$ FROM SOUR CHERRY SEED

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We identified a new GA, 1,2-didehydroGA20 and its isomeric lactone as well as ten known GAs, GA3, GA17, GA19, GA20, GA25, GA30, GA32, GA44, GA86 and GA87 in immature seed of sour cherry (*Prunus cerasus* L.) by GC-MS analysis. It was found that deuterium-labeled 1,2-didehydroGA20 was easily convertible to its isomeric lactone during purification procedures.

It has been known that GA3 is convertible from GA5 in some plants. However, co-occurrence of GA3 with GA5 has not always been demonstrated in these plants. Furthermore, to our knowledge, it seems that GA5 is not always convertible to GA3. This study using sour cherry seed demonstrated that GA3 co-occurs with 1,2-didehydroGA20, although GA5 could not be identified so far. These findings may allow us to speculate that 1,2-didehydroGA20 is a direct precursor of GA3 in the sour cherry seed. Biosynthetic pathways of highly-hydroxylated GAs in sour cherry are also discussed.

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CHARACTERISATION OF THREE CDNA CLONES ENCODING GIBBERELLIN 20-OXIDASES FROM *ARABIDOPSIS*.

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We recently isolated a cDNA clone encoding a gibberellin (GA) 20-oxidase from pumpkin cotyledons (Lange *et al.* (1994) Proc Natl Acad Sci USA, **91**: 8552-8556). The *E. coli* expression products catalysed the sequential oxidation of GA_{12} at C-20 to a carboxylic acid, yielding the inactive product GA_{25}. We have used a degenerate PCR approach to obtain cDNA clones that encode 20-oxidases on the pathway to bioactive GAs. Two related full-length clones (At2301 and At2353), with 50% amino acids conserved with the pumpkin 20-oxidase, were isolated from a cDNA library derived from shoots of the GA-deficient *gal* mutant of *Arabidopsis*. A third clone, YAP169, was identified by searching the EST database and was obtained from Dr. M. Delseny, Univ. Perpignan, France. Each cDNA clone was expressed as a fusion protein in E. coli: the products in each case catalysed the conversion of GA_{12} to GA_9, involving multiple oxidations at C-20 and leading to its loss as CO_2. GA_{53} was also metabolized, but at a lower rate. The three cDNA clones hybridise to mRNAs with tissue specific patterns of accumulation in wild-type plants: At2301 was expressed in stems and flowers, At2353 in flowers and developing siliques and YAP169 in siliques. At2301 and At2353 were very highly expressed in shoots of the GA-deficient mutant *gal*; YAP169 was expressed at a lower level. The abundance of all three mRNAs in this mutant was dramatically and rapidly reduced by the application of bioactive GA, suggesting that a feedback mechanism controls GA 20-oxidase gene expression. We have also produced transgenic *Arabidopsis* plants expressing sense and antisense copies of each GA 20-oxidase cDNA clone in order to study the effects of altered 20-oxidase gene expression on GA biosynthesis and plant development.

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COMPARABLE EFFECTS OF PROHEXADIONE-CA AND DAMINOZIDE ON GIBBERELLIN (GA) FORMATION IN PEANUT (*Arachis hypogaea*) SHOOTS

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Prohexadione-Ca (PRO), an acylcyclohexanedione, causes pronounced retardation of shoot growth in peanut and might be suited to fill the gap left in this crop after the withdrawal of daminozide (DAM). In order to know more about the mode of action of both compounds, we have analyzed the GAs from shoot increments of peanuts after treatment. GA_{19}, GA_{20}, GA_1 and GA_8 could be detected by full scan GC-MS spectra. In addition, GA_4, GA_9, GA_{44} and GA_{53} were tentatively identified. The quantitative immunoassay data so far available are given in the following table.

Treatment	Increase of Shoot Height [cm]	pMole Equiv./g FW GA_1	pMole Equiv./g FW GA_{20}
Control	18.0	1.00	2.00
PRO (500 g/ha)	3.4	0.30	11.30
DAM (1,500 g/ha)	3.6	0.25	13.30

These results suggest that DAM affects GA formation in a way similar to the one known for PRO: Primarily a dioxygenase is inhibited which catalyzes the conversion of GA_{20} (inactive) into GA_1 (active) and which needs 2-oxoglutarate as co-substrate. As with acylcyclohexanediones, this is most likely due to a structural similarity between DAM or its metabolite(s) and 2-oxoglutarate.

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GIBBERELLINS AND THE COLD REQUIREMENT OF TULIP

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The involvement of gibberellins (GAs) in the regulation of cold-induced floral stalk elongation and flower development was studied in tulip (*Tulipa gesneriana* L.). Using GC-MS, changes in GA levels were measured in sprouts during cooled (12 weeks 5°C) and noncooled (12 weeks 17°C) bulb storage, as well as after planting. GA_4 and GA_{24} were the major gibberellins, while GA_1, GA_9 and GA_{34} were present in lower amounts. During bulb storage, the level of GA_4 per sprout increased, especially in noncooled bulbs. At the time of planting, these sprouts contained more GA_4 and GA_1 than cooled sprouts. Since only cooled bulbs showed rapid sprout growth and developed a full-grown flower after planting, this growth cannot be related with high GA levels at planting. In the second week after planting, floral stalks of cooled bulbs contained more GA_4 than those of noncooled bulbs. The biological activity of GA_1, GA_4 and GA_9 was tested *in vitro* on sprouts of cooled and noncooled bulbs, in the presence or absence of the GA biosynthesis inhibitor paclobutrazol. The results demonstrated that sprouts from both cooled and noncooled bulbs can be responsive to exogenous GAs and may be a site of GA biosynthesis. The isolated sprouts did not develop a full-grown flower without the addition of GA. GA_4 was more effective than GA_9 in stimulating this flower development. Application of labelled GA_9 and HPLC- and GC-MS analyses of the metabolic products, indicated that sprouts are able to convert applied GA_9 to GA_4 and GA_{34} *in vitro*. No evidence was found for the production of labelled GA_1. The results support the idea that GA_4 is the major intrinsically active GA in the floral stalk elongation of tulip.

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EFFECT OF PRIMO GROWTH RETARDANT ON ENDOGENOUS CAMBIAL REGION GIBBERELLINS, INDOLE-3-ACETIC ACID, ABSCISIC ACID AND SECONDARY XYLEM FIBRE DEVELOPMENT IN *EUCALYPTUS GLOBULUS*

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Gibberellins GA_1, GA_4, GA_8, GA_9, GA_{19}, GA_{20}, GA_{29}, GA_{44}, GA_{81}, indole-3-acetic acid (IAA) and abscisic acid (ABA) were identified in cambial region extracts of *Eucalyptus globulus* Labill. by comparing mass spectra and Kovats retention indices with those from authentic standards. Using [17, 17- $^2H_2$] GAs as internal standards, GA_{19}, GA_{20} and GA_{44} were quantified at levels of 2-7 ng (g fresh weight)$^{-1}$. Other GAs were present at levels < 1 ng (g fresh weight)$^{-1}$. It seems likely that the early C-13 hydroxylation pathway predominates in *Eucalyptus* cambial region tissues. Localised stem injection of Primo (trinexapac-ethyl), a late stage acylcyclohexanedione type inhibitor of GA biosynthesis, was used to reduce endogenous cambial region GA levels. High doses of Primo also reduced the elongation of secondary xylem fibres and this was positively correlated with endogenous levels of both GA_1 (r_S=0.74, P<0.01) and GA_{20} (r_S=0.72, P<0.01). Correlations between endogenous IAA or ABA and fibre elongation were positive but weak and non significant. Gibberellins may thus be causal factors in secondary xylem fibre elongation in *Eucalyptus*.

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GIBBERELLIN DEACTIVATION AND THE *sln* MUTATION IN *PISUM*

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We have been studying gibberellin (GA) deactivation in the garden pea (*Pisum sativum* L.), using the mutation *sln*, coupled with feeds of labelled GA_{20}, GA_1 and GA_{29}, and analyses of endogenous GA levels. The *sln* mutation blocks the 2β-hydroxylation of GA_{20} to GA_{29}, and the further oxidation of GA_{29} to GA_{29}-catabolite, in maturing seeds, but only the first of these steps in the vegetative plant. Furthermore, in shoots *sln* does not reduce GA_{20} 3β-hydroxylation (to the bioactive GA_1), nor GA_{20} 2α-hydroxylation (to GA_{81}). Indeed, the endogenous level of GA_1 in *sln* shoots consistently exceeded that of wild-type shoots (even in the absence of the previously-reported influx of GA_{20} from *sln* seeds), although this increase was not sufficient to substantially affect internode length. In *sln* shoots the raised level of GA_1 is probably attributable to an increased availability of GA_{20} for 3β-hydroxylation, since competition from GA_{20} 2β-hydroxylation is reduced in the mutant. The raised GA_1 level does not appear to be due to a block in GA_1 2β-hydroxylation, since the endogenous level of GA_8 was not reduced in *sln* plants. In turn this indicates that the 2β-hydroxylation of GA_{20} and GA_1 may be catalysed by separate enzymes, supporting a previous report based on seeds (Smith and MacMillan 1986, Planta 167:9-18). There may also be separate members of a multi-gene family for the enzyme GA_{29}-oxidase, since *sln* affects the step GA_{29} to GA_{29}-catabolite in seeds, but not in the vegetative plant. Further, in maturing seeds the GA_{20} 2β-hydroxylase and GA_{29}-oxidase appear to be separate enzymes (even though both are affected by *sln*) , since GA_{20} and GA_{29} do not inhibit each other's metabolism, and GA_8 inhibits GA_{29} metabolism, but not that of GA_{20}. The significance of GA deactivation in regulating GA pool sizes, and the organ-specificity of GA-related genes, are discussed in light of the above results. It is suggested also that *Sln* may be a regulatory gene, and this possibility is being investigated currently in our laboratory, by firstly attempting to clone the GA_{20} 2β-hydroxylase.

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cDNA CLONING OF *ENT*-KAURENE SYNTHASE B FROM IMMATURE SEEDS OF PUMPKIN

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We purified *ent*-kaurene synthase B from the immature seeds of pumpkin (*Cucurbita maxima* L.). The enzyme was digested with protease and 5 different internal peptide sequences were determined. A 472bp cDNA fragment was specifically amplified by RT-PCR using degenerate oligonucleotide primers deduced from two of the internal peptide sequences. Using this cDNA fragment as a probe, we cloned a nearly full-length cDNA from a cDNA library constructed from mRNA of immature cotyledons. The cDNA showed high homology with other terpene cyclases, in particular with *ent*-kaurene synthase A from *Arabidopsis thaliana* (55% similarity, 30% identity). The putative divalent metal-pyrophosphate complex binding site (DDXXD) was also found.

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THE IDENTIFICATION, LEVELS AND METABOLISM OF GIBBERELLINS IN POTATO IN RELATION TO THE INDUCTION OF TUBERIZATION

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Endogenous gibberellins in potato (*Solanum tuberosum*) shoots were identified by GC-MS as GA_{53}-aldehyde, GA_{53}, GA_{44}, GA_{19}, GA_{20}, GA_{29}, GA_1, GA_8 and GA_{51}. Potato tuberization is promoted by short days and inhibited by applications of gibberellin (GA). Wild-type *ssp. andigena* require short days for tuberization, whereas their dwarf siblings tuberize under long days. The metabolism of [^{14}C]GA_{12} in shoots of wild-type and dwarf potato plants differed in that the dwarf plants had a reduced conversion of GA_{12} to GA_{53}, though no differences in [^{14}C]GA_{12} metabolism were detected between plants grown under short (10-h) versus long (16-h) photoperiods, in either wild-type or dwarf plants. We also analyzed the amounts of GA_1, GA_{19}, and GA_{20} in Norchip (NOR) and LT-1 cultivars grown under short (SD) or long (LD) days conditions. NOR tuberizes under both SD and LD conditions (better in SD), while LT-1 plants require SD conditions (tuberization is in the decreasing order $NOR_{SD} > NOR_{LD} \geq LT\text{-}1_{SD} > LT\text{-}1_{LD}$). The levels of GA_{20} were slightly higher, and those of GA_{19} lower, in LT1 than in NOR when the plants were grown either under long or short day-length. Plants of both cultivars grown in SD contained 2-3 times less GA_{19} and 1.5-2.5 times less GA_{20}, compared to plants in LD conditions. However there was no significant difference in amount of GA_1 between different day-length treatments, though LT-1 in LD had the most GA_1 (1.7 ng.g^{-1} FW). The higher GA_1 concentration in LT-1 plants, as well as more GA_{19} and GA_{20}, in LD grown plants is consistent with gibberellins having a negative role in potato tuber induction. However, the genotype effect is superior to any effect of GA_1 as determined by day-length as NOR plants grown under LD contain 3 times less GA_1 than LT-1 plants grown in SD conditions. That other factors must be involved is supported by the fact that we have mapped 10 genetic loci that promote tuberization.

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SELECTION OF GIBBERELLIN MUTANTS FROM A MUTAGENIZED POPULATION OF ABSCISIC ACID-INSENSITIVE *ARABIDOPSIS THALIANA*

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In an effort to isolate new stature mutants of *Arabidopsis thaliana* an EMS mutagenesis was conducted using the *abi3* genotype which is abscisic-acid insensitive (Finkelstein and Somerville, 1990, Plant Physiol. 94: 1172-1179). The selection of putative gibberellin (GA)-deficient and GA-insensitive mutants was facilitated because the *abi3* genotype does not display seed dormancy even in the absence of endogenous GA. Preliminary information on the biochemical and genetic characterisation of two GA-responding lines will be presented. Both are semi-dwarf. Response profiles to a range of *ent*-kaurenoids, C_{20}- and C_{19}-GAs suggest that they do not respond to *ent*-kaurenoids or GAs in the pathway up to and including GA_{12}. However both respond to GA_{15}, GA_9, GA_{36} and GA_4, all of which restore normal internode length. The identification and quantitation of GAs in these lines, and in two lines which are GA-insensitive will be reported.

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GIBBERELLIN METABOLISM IN MAIZE: THE *D1* GENE AND THE METABOLISM OF GA_{20} TO GA_5, GA_5 TO GA_3, AND OF GA_{20} TO GA_1

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In vegetative shoots of *Zea mays* L. (maize) gibberellin (GA) biosynthesis occurs mainly through the early 13-hydroxylation branch pathway which originates from GA_{12}. This presentation will focus on the later stages of this pathway and the control of specific steps in this pathway by the *d1* mutant of maize. Previous studies have shown that GA_{20}, GA_5, GA_3, and GA_1 are endogenous to maize vegetative shoots, that GA_{20} is metabolized to GA_1, and that the *d1* mutant controls the step GA_{20} to GA_1. Over the past two years we have studied the metabolism of [^{13}C,^{3}H]GA_{20} and [^{13}C,^{3}H]GA_5 in normal and *dwarf-1* seedlings. We report here that for normal seedlings GA_{20} was metabolised to GA_1 and GA_5, and GA_5 was metabolised to GA_3. Interestingly, no evidence was obtained for the metabolism of GA_{20} to GA_1 or GA_{20} to GA_5, or of GA_5 to GA_3 from *dwarf-1* seedlings. These results suggest that the steps, GA_{20} -> GA_5 -> GA_3, and GA_{20} -> GA_1, are controlled by a common enzyme, which is the product of the *D1* gene.

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CLONING A MAIZE DWARFING GENE BY TRANSPOSON TAGGING

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We have isolated a gibberellin-responding recessive dwarf mutant (*d-44*) from a Robertson's mutator line. The dwarf phenotype is expressed in the early seedling stage and disappears in later stages of growth. The height of the adult mutant approaches that of the normal; mutant plants are andromonoecious and have sparsely branched tassels. The dwarf seedling phenotype is reversed by treatment with the gibberellins, GA_1, GA_3, GA_{20}, and the GA precursors, *ent*-kaurenoic acid, *ent*-kaurenol and *ent*-kaurene. These growth responses suggest that the genetic block is early in the GA-biosynthetic pathway, the pathway that leads to bioactive GA_1. We identified by Southern analyses a 2.8 kb, *Mul* containing, BamHI restriction fragment which co-segregated with the dwarf phenotype. This 2.8 kb genomic fragment was cloned and used as probe to a maize seedling cDNA library. A 3.5 kb cDNA, which hybridizes to the genomic fragment, was isolated from the library. We have shown by classical genetic crosses that *d-44* is allelic to *an1*. We will present information on the sequence, and on the expression of this putative GA biosynthetic gene. Supported by grants from the Department of Energy, DE-FG03-90ER20016, and the National Science Foundation, MCB-9306597.

115 GIBBERELLINS AND PEA SEED DEVELOPMENT

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We are investigating the role of the gibberellins (GAs) in seed development of the garden pea *(Pisum sativum* L.). The GA biosynthesis mutations, *lh* and lh^i, reduce kaurene oxidase activity leading to reduced GA_1 levels in young shoots and a dwarf phenotype. The allele-specific interaction of lh^i with inhibitors of kaurene oxidation (e.g. paclobutrazol) suggests that the *Lh* locus may encode the major (or only) kaurene oxidase gene of pea and that this enzyme catalyses the three-step oxidation of kaurene to kaurenoic acid. In young seeds, endogenous GA_1 and GA_3 levels in the embryo/endosperm are reduced in the lh^i and *lh* mutants by ca. 90% and 60%, respectively. Seeds homozygous for lh^i grow more slowly than WT seeds and are more likely to abort during development, due to altered embryo and/or endosperm development. Fertilizing lh^i plants with WT pollen increases GA_1 and GA_3 levels more than ten-fold and restores normal seed development. The less severe *lh* allele does not affect seed abortion, but does reduce embryo growth. These results suggest that GAs play an important role early in pea seed development by regulating embryo growth, and that dramatically reduced embryo growth may result in increased seed abortion.

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SEED REGULATION OF GIBBERELLIN BIOSYNTHESIS IN PERICARP TISSUE.

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Pea pericarp (pod) has the capacity to metabolize ^{14}C-GA_{12} through the early 13-hydroxylation pathway. Removal of seeds from the pericarp inhibited the conversion of ^{14}C-GA_{19} to ^{14}C-GA_{20}, and 4-Cl-IAA (endogenous pea seed auxin) application substituted for the seeds in pericarp growth and conversion of GA_{19} to GA_{20} (Plant Physiol 99:S-2;100:88-94). To confirm these data, ^{14}C-GA_{19} metabolism was monitored in pea pericarp (2 DAA) with seeds (intact or split down the dorsal suture), split pericarp seeds removed (SPNS), and SPNS+4-Cl-IAA. Pericarp were harvested 2, 4, 8, and 24 h after ^{14}C-GA_{19} application. ^{3}H-GA_4 was added as an external standard for recovery determination. ^{14}C-GA_{19} metabolites were separated and monitored using a C_{18} HPLC-flow-through radiochemical detector. Further verification was carried out by rechromatographing the putative ^{14}C-GA's as methyl esters. Pericarp in all treatments metabolized ^{14}C-GA_{19} to ^{14}C-GA_{20} and ^{14}C-GA_{29} (biologically inactive GA); however, the pattern of metabolism over time differed between treatments. Pericarp with seeds, and SPNS+4-Cl-IAA continued to convert ^{14}C-GA_{19} to ^{14}C-GA_{20} throughout the incubation period (2-24 h). However, in the SPNS treatment, conversion of ^{14}C-GA_{19} to ^{14}C-GA_{20} was significantly lower than other treatments after 2-8 h of incubation, and no ^{14}C-GA_{20} was detected after 24 h. The GA_{29}/GA_{20} ratio was significantly higher in SPNS, and SPNS+4-Cl-IAA treatments than in pericarp with seeds at 2-8 h of incubation; after 24 h, only SPNS+4-Cl-IAA maintained a high GA_{29}/GA_{20} ratio. Therefore, conversion of ^{14}C-GA_{20} to ^{14}C-GA_{29} may also be seed regulated. These data support our previous findings that the conversion of GA_{19} to GA_{20} in pea pericarp is a seed regulated step and that the auxin, 4-Cl-IAA, can substitute for the seeds in the stimulation of the conversion of GA_{19} to GA_{20}. Supported by NSERC OGP0138166.

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THE SPECTRUM OF PHYSIOLOGICAL ACTIVITY OF SUBSTANCES SYNTHESIZED BY *SEPTORIA TRITICI ROB. ET DESM.*

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An ability of pure cultures of *Septoria tritici Rob. et Desm.* isolates to synthesize physiologically active substances was studied. Cultural filtrates and mycelium from 15 *Septoria tritici* isolates at various periods of fungus development were analised. Phytohormone content was measured in one assay test. The presence of basic biologically active substance groups in the mycelium and cultural liquid - abscicic acid (ABA), indole acetic acid (IIA), cytokinins and gibberllins was identified and confirmed by biological methods using HPLC techniques with pre-puritication on thin-layer chromatography. All isolates synthesize gibberellin with Rf 0.6 that corresponds with gibberellic acid. ABA is found in the mycelium and cultural liquid within the range of 3.4 to 111.6 ng/g of fresh mass; IIA - of 4.1 to 619 ng/g of fresh mass. Cytokinins (zeatin and zeatin riboside) are synthesized by fungi in small quantities: zeatin of 0.01 to 2.5 ng/g fresh mass, zeatin riboside of 0.03 to 3.4 ng/g freash mass. It was established that in their mycelium most of isolates accumulate IIA while in cultural liquid - gibberellins and ABA. The involvement of these biologically active substances in the penitration of fungi in tissues of a feeding plant is discussed.

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CLONING AND FUNCTIONAL EXPRESSION OF A GIBBERELLIN 20-OXIDASE cDNA FROM THE LONG DAY-PLANT SPINACH

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Spinach is a long-day (LD) rosette plant in which stem growth under LD is mediated by gibberellins (GAs). It has been suggested that major control points in GA biosynthesis in spinach are the later steps of sequential oxidation at C-20, ultimately leading to its loss as CO_2. Previously, cDNA clones encoding GA 20-oxidase from pumpkin and *Arabidopsis* have been isolated in our laboratory. Using degenerate primers derived from the conserved regions of pumpkin and *Arabidopsis* GA 20-oxidase, a PCR product was obtained from the spinach genomic DNA that has a high homology when compared with pumpkin and *Arabidopsis* GA 20-oxidase gene sequences. Using this product as a probe to screen a spinach cDNA library, a full-length cDNA with high homology to the GA 20-oxidase from pumpkin and *Arabidopsis* has been isolated. When this cDNA was expressed in *E. coli*, the fusion protein catalyzed the biosynthetic sequence $GA_{53} \rightarrow GA_{44} \rightarrow GA_{19} \rightarrow GA_{20}$. This establishes that in spinach, as in pumpkin and *Arabidopsis*, a single protein catalyzes the oxidation and elimination of C-20. With the cloning of a GA 20-oxidase from spinach, it is now possible to investigate at the molecular level how light controls the conversion of C_{20}- to C_{19}-GAs in this plant. (Supported by U.S. Department of Energy Grant DE-FG02-90ER20021 and by U.S. Department of Agriculture Grants 90-37304-6469 and 94-37304-0956).

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MOLECULAR CLONING AND FUNCTIONAL EXPRESSION OF THE *GA5* LOCUS OF *ARABIDOPSIS THALIANA*

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On the basis of our previous characterization of the *ga5* mutant of *Arabidopsis*, we predicted that the *GA5* locus encodes a GA 20-oxidase, a pivotal enzyme responsible for the formation of C_{19}-GAs in the latter stage of the GA biosynthetic pathway. Using a pumpkin GA 20-oxidase cDNA clone as a probe, a putative GA 20-oxidase clone was obtained from a genomic library of *Arabidopsis thaliana*. RFLP and YAC positional mapping results indicated that the putative GA 20-oxidase clone is tightly linked with the *GA5* locus. Furthermore, a point mutation, from G to A, which results in a premature stop codon, was found in the *ga5* locus. A cDNA clone corresponding to the putative GA 20-oxidase genomic clone was constructed with a RT-PCR method and the fusion protein encoded by the cDNA clone was able to convert GA_{53} to GA_{44} and GA_{19}, and GA_{19} to GA_{20}. These results indicate that the *GA5* locus encodes a multifunctional GA 20-oxidase. Expression of the *GA5* gene was enhanced by long days, and was reduced by GA_4 treatment, suggesting end-product repression in the regulation of GA biosynthesis. Evidence will also be presented that the *GAI* locus may play a role in the regulation of the expression of the *GA5*. (Supported by U.S. Department of Energy Grant DE-FG02-90ER20021 and by U.S. Department of Agriculture Grants 90-37304-6469 and 94-37304-0956).

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CLONING OF *ENT* -KAURENE SYNTHASE A FROM RICE

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The first important step in the gibberellin (GA) biosynthetic pathway is the conversion of geranylgeranyl pyrophosphate (GGPP) to copalyl pyrophosphate (CPP) by *ent*-kaurene synthase A. CPP is then converted to *ent*-kaurene by *ent*-kaurene synthetase B. To study the regulation of GA biosynthesis in rice (*Oryza sativa* L.), we started cDNA cloning of *ent*-kaurene synthase A using pGA1-29 (which encodes *ent*-kaurene synthetase A from *Arabidopsis thaliana*, Sun and Kamiya, *Plant Cell*, 1994) as a hybridization probe. A band was detected by genomic Southern analysis at low stringency using a 0.5-kb fragment of pGA1-29 that includes the conserved region of terpene cyclases as a probe. A cDNA library constructed from rice anthers (where GA biosynthesis is relatively high) was screened under the same conditions to isolate a 2.1-kb cDNA clone, pRKA1. Partial sequencing of pRKA1 revealed high homology with pGA1-29. A 2.8-kb mRNA was detected by Northern analysis, suggesting that pRKA1 is not a full-length clone. Screening to isolate a nearly full-length clone is now in progress. The enzyme activity in *E.coli* of the over-expressed protein encoded by the isolated cDNA will also be analysed.

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BIOSYNTHESIS OF GA_{73} METHYL ESTER IN THE SCHIZAEACEOUS FERN *LYGODIUM CIRCINNATUM*

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Biosynthesis of GA_{73} methyl ester (GA_{73}-Me), a principal antheridiogen in *Lygodium* ferns, was investigated. From the methanol extract of prothallia of *L. circinnatum*, GA_{73}, GA_{25}, GA_{73}-Me and GA_{88}-Me were identified by GC-MS after derivatization. While, from the culture medium of the prothallia, GA_{73}-Me, GA_9-Me, GA_{20}-Me, GA_{70}-Me, GA_{88}-Me, 3-*epi*-GA_{88}-Me and GA_{95}-Me were similarly identified. In the feeding experiment using *L. circinnatum* prothallia, [^{2}H]GA_{24} was converted into [^{2}H]GA_{73} and [^{2}H]GA_9, and [^{2}H]GA_9 into [^{2}H]GA_9-Me and [^{2}H]monohydroxy-GA_9 methyl esters such as [^{2}H]GA_{20}-Me, [^{2}H]GA_{70}-Me and [^{2}H]GA_{84}-Me. These results indicate that GA_{73}-Me is biosynthesized from GA_{24} via GA_{73}, and that neither GA_9 nor GA_9-Me is a precursor of GA_{73}-Me.

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EFFECT OF PACLOBUTRAZOL ON ENDOGENOUS LEVELS OF GAs, IAA, ABA AND CTKs IN WHEAT SEEDLINGS

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It has been shown that dwarfing and delaying senescence of wheat seedlings can be elicited by immersing the seeds in a solution containing paclobutrazol (PP_{333}) at a concentration of 5 mg / L for 16h (20℃). The dynamic contents of $GA_{(1+3+4+7)}$, IAA, ABA and CTKs(iPAs, ZRs and DHZRs) of leaves(1 / 0, 2 / 0, 3 / 0 and 4 / 0) were obtained by using six kinds of ELISAs (detection range:0.31–75pmol / L for GAs, 0.78–800pmol / L for IAA, 0.048–1.52pmol / L for ABA and 0.064–200pmol / L for CTKs) . These data are summarized as follows: (1) PP_{333} elicited a higher levels of CTKs and a lower levels of IAA and GAs continously. The free ABA level in each leaf was increased by PP_{333} significantly only during elongation stage, and then decreased gradually and obviously. (2) PP_{333} caused a lower ratio of GA / ABA or (GA+IAA) / ABA for each elongating leaf. As a result, the length of each leaf was shortened.On the other hand, due to PP_{333} treatment, CTKs / ABA ratio in each developed leaf was raised and leaf senescence retarded obviously.

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EFFECTS OF GIBBERELLINS ON SEED GERMINATION OF PHYTOCHROME DEFICIENT MUTANTS OF *Arabidopsis thaliana*

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Experiments were carried out to explore the role played by gibberellins (GAs) during the light-induction of *Arabidopsis thaliana* (L.) Heynh seed germination, using wild type (WT) and phytochrome-deficient mutants (*phyA*, *phyB* and *phyAphyB* mutants deficient in phytochrome A, B and A plus B, respectively). Seed germination of WT and phytochrome-deficient mutants was inhibited by uniconazole and prohexadione. This inhibition was overcome by simultaneous application of 10^{-5} M GA_4. The relative activities of GAs in the promotion of germination of uniconazole-treated seeds were $GA_4 > GA_1 = GA_9 > GA_{20}$. Wild type and the *phyA* and *phyB* mutants showed an increased response to a red light pulse in the presence of GA_1, GA_4, GA_9, GA_{20} and GA_{24} and there was no significant difference in activity of each GA between the mutants. Therefore neither phytochrome A nor phytochrome B appears to regulate GA biosynthesis from GA_{12} to GA_4 during seed germination. However, GA responsiveness appears to be regulated by phytochromes other than phytochromes A and B, since the *phyAphyB* double mutant retains the increased response to GAs.

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ABSCISIC ACID BIOSYNTHESIS IN A CELL-FREE SYSTEM PREPARED FROM *CITRUS SINENSIS* EXOCARP.

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The evidence for a carotenoid origin of abscisic acid (ABA) in plants is now almost conclusive. Current thinking is that ABA biosynthesis involves cleavage of either 9-*cis*-violaxanthin or 9-*cis*-neoxanthin, derived from all-*trans*-violaxanthin, to yield xanthoxin which is then converted to ABA via ABA-aldehyde. We recently identified 1′,4′-*trans*-ABAdiol and ABA by GC-MS as the major products of mevalonic acid and all-*trans*-β-carotene metabolism in cell-free extracts of chromoplast-rich exocarp of *Citrus*. The present paper describes the reaction sequence and identity of intermediates en route between all-*trans*-β-carotene and ABA in this cell-free system. HPLC-PDA analysis of the oxy-carotenoid fraction revealed accumulation of 9-*cis*-violaxanthin. Labelled and non-labelled β-carotene was converted into the neutral compounds xanthoxin alcohol and xanthoxin. The acid fraction contained xanthoxin acid, 1′,4′-*trans*-ABAdiol, ABA and trace amounts of PA. Identification of these metabolites was achieved by either UV spectrophotometry and combined capillary GC-MS or microchemical analysis and co-chromatography. In the presence of ancymidol, an inhibitor of cytochrome P450-type mixed function oxidase activity, accumulation of xanthoxin alcohol and xanthoxin occurred at the expense of xanthoxin acid, 1′,4′-*trans*-ABAdiol and ABA. Together these findings suggest that the synthesis of ABA involves mixed funtion oxidase enzymes and occurs along the pathway, all-*trans*-β-carotene → zeaxanthin → antheraxanthin → violaxanthin → 9-*cis*-violaxanthin → xanthoxin alcohol → xanthoxin → xanthoxin acid → 1′,4′-ABAdiol → ABA.

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FORMATION OF [^2H_n] ABSCISIC ACID FROM [G-^2H_n] CAROTENOIDS BY A CELL-FREE PREPARATION FROM AVOCADO

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A cell-free preparation from avocado mesocarp, which comprised cytosol, plastids and membrane fractions, routinely incorporated [^{14}C]mevalonate into ABA in darkness. Similar proportional effects on the amont of [^{14}C]ABA made by the cell-free preparation and by fruit slices were caused by AMO 1618 (increased incorporation by 50%) and (reduced to 20 to 40% of controls) by tungstate, sulphite, piperonyl butoxide, nicotine, naproxen and arachidonate. Addition of "cold traps" of β-carotene zea-, viola-, neo and 9-cis-neoxanthin and 2-cis-xanthoxin decrased the incorporation of [^{14}C]mevalonate into ABA.

Mustard (*Sinapis alba*) seedlings germinated in ^2H_2O (55 atom %) were extracted and the carotenoids isolated by HPLC. These deuteriated carotenoids were supplied to the cell-free system and [^2H_n] ABA was detected by GLC Negative Ion Chemical Ionisation Mass Spectrometry of the pentafluoro benzyl ester.

While it is possible that lipoxygenase activity could have cleaved all the carotenoids to produce artifactual C_{15} moieties which could then have been elaborated into ABA. This is considered unlikely as naproxen (a potent inhibitor of lipoxygenase) decreased the amount of ^{14}C in ABA by 60% but only neoxanthin contained a large amount of ^{14}C when reisolated. This suggests that carotenoids are cleaved by a lipoxygenase at this point.

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THE IDENTIFICATION OF NEW ABA BIOSYNTHESIS AND SEED DORMANCY SPECIFIC MUTANTS IN *ARABIDOPSIS THALIANA*

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In Arabidopsis only one locus *(aba1)* controlling abscisic acid (ABA) biosynthesis has been identified and characterized thus far. These *aba1* mutants were selected on the basis of their reduced gibberellin (GA) requirement for seed germination. Mutants deficient in ABA at two additional loci, were identified among mutants germinating in concentrations of the GA biosynthesis inhibitors tetcyclacis and paclobutrazol that inhibit seed germination of the wild type. The physiological characterization indicated the lack of seed dormancy and enhanced water loss of excised leaves, which is characteristic for ABA biosynthesis mutants in all species investigated. Biochemical analysis showed that these new loci *ABA2* and *ABA3* affect steps in ABA biosynthesis after the epoxidation of zeaxanthin, which is controlled by the *ABA1* locus. The selection of mutants with reduced dormancy led to the isolation of a mutant with an enhanced resistance to GA biosynthesis inhibitors but with wild type ABA levels and wild type sensitivity to ABA. The physiological characteristics of this *dor* mutant and its double mutants with various ABA biosynthesis and ABA insensitive (*abi*) mutants are described and the role of ABA in seed dormancy is discussed.

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CLONING OF AN ABA BIOSYNTHESIS GENE OF NICOTIANA PLUMBAGINIFOLIA BY TRANSPOSON TAGGING

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A new ABA deficient mutant has been identified, on the basis of his early germination phenotype, in an *Ac* mutagenized population of *N. plumbaginifolia*. The mutant is extremely sensitive to hydric stress and recovers the wild type phenotype when sprayed daily with an ABA solution. This mutant, *aba2,* is not allelic to the previously described *aba1* mutant of *N. plumbaginifolia* (1). It is impaired in ABA biosynthesis as a result of a deficiency in the epoxidation of zeaxanthin, the apo-carotenoid precursor of ABA. The same metabolic block has been described for *Arabidopsis thaliana aba* mutants (2, 3). The *aba2* mutation is closely linked to a transposed copy of *Ac.* This *trAc* allowed the cloning of an IPCR fragment adjacent to the *Ac* insertion, which was then used to screen a cDNA library of wild type *N. plumbaginifolia* plantlets. A positive recombinant phage was isolated and its insert was sequenced. Obtention of revertants in the progeny of *aba2* mutant and mutant complementation by the cloned cDNA confirmed the tagging. The ABA2 protein is imported into pea chloroplasts *in vitro,* as could be expected for zeaxanthin epoxidase. To our knowledge, this is the first report of cloning of an ABA biosynthesis gene.

1 Rousselin *et al.* (1992) Theor Appl Genet 85, 213-221
2 Duckham et al. (1991) Plant Cell Env 14, 601-606
3 Rock and Zeevart (1991) Proc Natl Acad Sci USA 88, 7496-7499

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BIOSYNTHETIC PRECURSORS OF ABSCISIC ACID

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We reported a new compound, named "adduct", that released MeABA[1] and have continued to examine its properties. Following extraction of avocado mesocarp in hot water and centrifugation the concentrate was chromatographed on preparative columns. Results from G-15 Sephadex indicated that adduct contained two components[1], < 1.1Kd. The larger was retained on carboxymethyl cellulose and on zinc iminodiacetate indicating that it was positively charged and probably contained a lone pair of electrons as in histidine. The smaller had a net charge of zero to one but was also retained by zinc iminodiacetate. Both compounds appear to comprise a small peptide, which may contain histidine, bound through what will become C-1 of the ABA moiety. The larger component, "enolate", appears to be bound to another molecule as an enolate ester[1]. The smaller component, "ketone", does not contain this linkage. The ketone releases a mixture of ABA and MeABA when exposed to H_2SO_4 (pH ~2) while the enolate releases a similar mixture when exposed to H_2SO_4 followed by base (pH>9). To obtain evidence for the biosynthetic origins of these components tomato seedlings were stressed in the presence of ^2H_2O. ABA from the adduct fraction had a higher specific enrichment of ^2H than the free ABA from the same plant indicating that adduct components were precursors. Data on the incorporation of ^{18}O from $^{18}O_2$ into adduct components and into free ABA will also be presented. Avocado mesocarp fed [2-^{14}C]-mevalonic acid contained ^{14}C labelled adduct components that released ABA, *t*-ABA, their 1',2'-epoxides and their methyl esters on hydrolysis.

1. A.G. Netting, R.D. Willows and B.V. Milborrow (1992), *Plant Growth Reguln.* **11**, 327-34.

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MOLECULAR ANALYSIS OF A NEW *VIVIPAROUS* MUTANT IN MAIZE

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The viviparous of mutants of maize typically affect synthesis or perception of abscisic acid in the developing seed. A new viviparous (vp) mutant that is viable and has no discernible plant phenotype has been recovered. Embryo culture studies indicate that the mutant is rescued by exogenous ABA and similar to wildtype with respect to sensitivity to the hormone. These results are consistent with a role in a seed ABA synthesis pathway. Complementation tests revealed three independent alleles, designated *vp*-2274, vp*-3250,* and *vp*-3286*. By using an internal fragment of *mu1* as a probe, a 2.5 kb *XhoI* fragment was identified co-segregating with the mutant in a *vp*-2274*. Sequencing confirmed the presence of a *mu1* insertion in the cloned fragment. A second *mu1* insertion was detected in the independent *vp*-3250* allele located 1.1 kb upstream of the *mu1* insertion in *vp*-2274* confirming the identity of the clone. Southern blots indicate that this gene belongs to a multigene family. Northern blots revealed a 2.7 kb transcript in wildtype embryos that is missing or altered in *vp*-2274* and *vp*-3250* embryos. Related transcripts are found in WT shoots, leaves and roots. Full length cDNA's have been cloned from embryos and roots. A BLAST search indicates that the gene encodes a protein related to a novel class of bacterial dioxygenase enzymes. The significance and potential function of this enzyme in relation to xanthoxin and ABA synthesis will be discussed.

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NITRILASE EXPRESSION DURING THE DEVELOPMENT OF THE CLUBROOT DISEASE IN CHINESE CABBAGE

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The infection of Chinese cabbage (*Brassica rapa ssp. pekinensis* cv. Granaat) with the obligate biotroph *Plasmodiophora brassicae* Wor. leads to tumorous swellings of the roots (=clubroot disease) as a result of increased cell division and hypertrophy. An increased auxin formation in infected tissue by enzymatic hydrolysis of the precursor indole-3-acetonitrile has previously been postulated (Rausch et al., 1981, Physiol. Plant. 52: 467-470). Based on a recently published nitrilase cDNA sequence of *Arabidopsis thaliana* (Bartling et al., 1992, Eur. J. Biochem. 205: 417-424), we have amplified a partial nitrilase cDNA fragment from Chinese cabbage by polymerase chain reaction. We also amplified genomic nitrilase fragments from Chinese cabbage and *Brassica oleracea*, another host for *Plasmodiophora brassicae*, with the same set of primers. Sequence comparisons indicate high homology in the exons which extended into the two included introns. The sequenced isoform of Chinese cabbage is most closely related to the NIT isoforms 1 and 2 of *Arabidopsis* (Bartel and Fink, 1994, Proc. Natl. Acad. Sci. USA 91: 6649-6653). Southern blot analysis with a Chinese cabbage cDNA probe revealed evidence for a nitrilase gene family in Chinese cabbage and *B. oleracea*. Using the homologous nitrilase cDNA fragment as a probe we have determined nitrilase mRNA in total RNA from *Plasmodiophora*-infected and healthy root and leaf tissue of Chinese cabbage. In both tissues an mRNA of approx. 1.3 kB was detected with similar amounts in infected and healthy tissues. The role of nitrilase during the development of the clubroot disease of the Brassicaceae is discussed.

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INDOLEACETONITRILE IN *ARABIDOPSIS*

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Recently, indoleacetonitrile (IAN) has been postulated as a posible precursor of the auxin indole-3-acetic acid (IAA) based on studies of stable isotope labeling patterns (1). The genes from *Arabidopsis* that encode the enzyme nitrilase have been cloned and the expressed enzyme was shown to convert IAN to IAA *in vitro* (2). In order to determine the significance of these studies, however, it was important to unabiguously identify IAN in *Arabidopsis* and to develop proper methods to quantify the levels of IAN in such plant materials. To address these needs, we synthesized ^{13}C-labeled IAN for use in GC-MS quantification of endogenous IAN following isolation under mild conditions. We developed isolation and derivatization methods for determination of IAN from *Arabidopsis* and showed the presence of IAN in *Arabidopsis* by copurification of the plant IAN with added ^{13}C-IAN with final conformation using full scan GC-MS analysis. Isotopic labeling methods for routine GC-SIM-MS IAN quantification were also developed. Using these techniques we also quantified the non enzymatic conversion of IAN to IAA under conditions used to hydrolyze IAA conjugates.

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1. Normanly *et al.* (1993) PNAS 90:10355
2. Bartel and Fink (1994) PNAS 91:6649; Bartling *et al.* (1994) PNAS 91:6021

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NITRILASE ACTIVITY IN CORN ENDOSPERM SUSPENSION CULTURES

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Recently there have been several reports on the involvement of nitrilases in the production of indole-3-acetic acid (IAA) in *Arabidopsis thaliana* as well as in plant-associated bacteria. These enzymes convert indole-3-acetonitrile (IAN) to IAA. Very little is known about how widespread this pathway is in the plant kingdom. We have been working with corn endosperm cells in suspension culture as a model system for studying IAA biosynthesis. These cultures do not require exogenous auxins and produce IAA at a very high rate (52 pmol/hr/g fwt). Cytoplasmic and membrane bound proteins were examined for nitrilase activity using three substrates: 3-cyanopyridine, 2-thiophene acetonitrile, and IAN. There was no observable activity in the membrane fraction with any of the substrates. Two different cytoplasmic protein fractions were prepared through ammonium sulfate precipitation. Only one of the fractions contained nitrilase activity. Both 3-cyanopyridine and 2-thiophene acetonitrile were converted to the corresponding acids. No activity was observed with IAN. Based on the *in vivo* biosynthetic rate of IAA, conversion of IAN to IAA would have been within the assay detection limits. Earlier experiments suggest that tryptophan is not a direct precursor of IAA in these cultures. The pathway by which this tissue biosynthesizes IAA remains unknown, but appears not to involve IAN.

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REGULATION OF INDOLE-3-BUTYRIC ACID BIOSYNTHESIS IN MAIZE

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Auxins are a class of phytohormones which play a central role in the growth and development of plants. Indole-3-butyric acid (IBA) is used extensively as a synthetic auxin for the induction of root formation, and only in the last few years was it identified as a natural constituent of plants. We have found that indole-3-acetic acid (IAA) is the precursor for IBA in maize (*Zea mays* L.). We have identified an *in vitro* system, in which microsomal membranes from maize are able to convert IAA to IBA. ATP and acetyl-CoA are necessary for the formation of IBA. The reaction product was identified after HPLC purification by GC-MS. The *in vitro* biosynthesis of IBA from IAA seems to involve an as yet unknown reaction product, which only accumulated when the membranes were isolated from dark-grown seedlings. Microsomal membranes from light-grown seedlings directly synthesized IBA without the release of an intermediate. We have observed that IBA synthetase activity and endogenous IBA content was increased, when the plants were cultivated under drought stress, whereas temperature stress did not enhance IBA formation. This was not found for IAA. The endogenous content of abscisic acid (ABA) was also increased under these culture conditions. Application of exogenous ABA also increased the activity of IBA synthetase at concentrations between 5×10^{-7} to 5×10^{-4}. Salicylic acid and jasmonic acid also increased IBA synthesis, but only at higher concentrations ($>10^{-4}$). Similar effects were observed after submitting the plants to osmotic stress. Sorbitol (12.5%) and NaCl (0.17 M) resulted also in an increase of IBA synthesis. This effect was not only observed in maize, but also in wheat (*Triticum aestivum*), pea (*Pisum sativum*) and amaranth (*Amaranthus paniculatus*). The possibility of a regulation of IBA biosynthesis by different culture conditions (light/dark) and by drought stress is discussed.

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THE EFFECT OF ENVIRONMENTAL FACTORS ON IAA TURNOVER IN NORMAL AND TRYPTOPHAN ANALOG RESISTANT LINES OF *LEMNA GIBBA*

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The change in indole-3-acetic acid (IAA) homeostasis in plants may be one of the key factors determining plant growth and development following altered environmental conditions. Turnover rate is a critical parameter of homeostatic control and it can be readly calculated based on isotope dilution where it obeys predicted first order kinetics. An inbred line (3F7-11) and an α-methyltryptophan resistant line (MTR1) of *Lemna gibba* were used to study the effects of temperature and light on IAA turnover. Six temperature treatments (5-35°C) were used. At low temperatures, IAA turnover was increased in 3F7-11, but no difference was seen in the analog resistant line. At 25°C, MTR1 had an approximately 10 fold higher rate of IAA turnover than the inbred line. IAA turnover time was similar under light and dark conditions in MTR1, however 3F7-11 turnover time in the dark was around 10 times faster than that in the light. A comparison of the changes in turnover in the normal and resistant lines suggests that biosynthetic processes are the key points of regulation of IAA turnover in relation to the environmental changes studies. *This work was supported by grant DE-AI02-94ER20153 from the U.S. Dept. of Energy.*

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AUXIN-REGULATED ACC SYNTHASE GENE EXPRESSION IN POLLINATED ORCHID FLOWERS.

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Ethylene regulates a range of postpollination responses in orchid flowers including perianth senescence, ovary growth, and ovule differentiation and development. To understand the initial events that occur following pollination and the perception of the pollination signal in the stigma, we previously isolated ACC synthase cDNAs (OAS1/2) from the gynoecium of pollinated orchid flowers. RNA blot hybridization analyses indicate that pollination induced the accumulation of OAS1/2 mRNAs in the stigma and labellum, but not in the ovary and perianth of pollinated flowers. Furthermore, these ACC synthase mRNAs accumulated in response to ethylene, suggesting a role in autocatalytic amplification of the primary pollination signal. Recently, we have isolated an additional distinct ACC synthase cDNA (Stigma AS) using reverse transcription-polymerase chain reaction amplification. The Stigma AS mRNA accumulated in the stigma in response to auxin in the presence or absence of the ethylene inhibitors AVG and NBD. Moreover, exogenous ACC did not induce the accumulation of the Stigma AS mRNA in the stigma. Thus, it is likely that the Stigma AS mRNA represents an auxin-regulated ACC synthase gene product responsible for transducing the primary pollination signal(s) and as such may be the key ACC synthase gene involved in the pollination response. Data supporting the role of the Stigma AS mRNA and pollen-borne auxin in the primary pollination event will be presented. This research was supported by grants from the USDA/NRICGP and BARD to S. O'N.

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ETHYLENE BIOSYNTHESIS IN *REGNELLIDIUM DIPHYLLUM*

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Ethylene biosynthesis in higher plants proceeds via the 1-aminocyclopropane-1-carboxylate (ACC)-dependent route. Prior to the elucidation of the higher plant pathway, reports in the literature suggested that an alternative pathway may exist in ferns. *Regnellidium diphyllum* is an aquatic fern which exhibits an elongation response when submerged. This response has been shown to be mediated, at least in part, by ethylene. Because of this response and because of the current state of knowledge of the higher plant ethylene biosynthetic pathway, we are re-investigating the pathway in ferns. Inhibitors of both ACC synthase and ACC oxidase are ineffective at decreasing ethylene production in *Regnellidium*. Addition of exogenous ACC does not increase ethylene production. However, ACC is present as revealed by thin-layer chromatography. Neglible conversion of [^{14}C]-ACC into [^{14}C]-ethylene occurs. *In vitro* ACC synthase activity is detectable, and this activity is inhibited by aminoethoxyvinylglycine (AVG), a competitive inhibitor of ACC synthase. We are investigating both the ACC synthase gene that is expressed in *Regnellidium* and the pathway that leads to ethylene production. (This research was supported by the U.S. Department of Energy through grant No. DE-FG02-91ER20021.)

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ETHYLENE AND ITS BIOSYNTHESIS IN SEED AND FRUIT OF *SECHIUM EDULE* SWARTZ.

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The intact fruit and isolated tissues of *Sechium edule* Swartz were analysed for ethylene production and for the content of 1-aminocyclopropane-1-carboxylic acid (ACC), the hormone precursor. Ethylene release follows patterns specific for each fruit and seed tissue. Seed teguments show the highest ACC content while fruit pericarp shows a very low ACC content. Moreover only seed teguments show ACC-synthase activity. The above results taken together indicate that the seed teguments are the only seed and fruit district capable of ACC synthesis. Considering the well known interaction between auxin and ethylene, the endogenous level of free and bound auxin were also measured in single seed tissues. Tissue competence for ACC synthesis was further investigated by determining the expression of the messenger for ACC-synthase. The ACC-synthase gene appears to be transcribed in all seed tissues, also in those that did not show ACC-synthase activity. However the ubiquitous transcription of the ACC-synthase gene in all seed tissues and the contrasting absence of the enzymatic activity in endosperm, cotyledons and pericarp, indicate that ethylene biosynthetic capacity, in *Sechium edule*, during the seed development, is regulated mainly at a post transcriptional level. We further observed that seed ethylene biosynthesis changes during development, reaching the highest values when the embryo starts its growing phase, and seed fresh weight rapidly increases. The comparison between the ethylene production and the endogenous levels of free and bound auxins during seed development, evidences that the burst in ethylene production and auxin content, in the developing seed, takes place when its fresh weight begins to increase, suggesting the involvement of ethylene during the seed development.

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ETHYLENE IN POST-POLLINATION RESPONSES IN A NON-CLIMACTERIC FLOWER

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Pollination of geranium (*Pelargonium Xhortorum*) flowers induces ethylene production in the pistils followed by petal abscission within 3-4 hours. Treatment of flowers with propylene, an ethylene analog, induced petal abscission, but not ethylene production, indicating that ethylene production in these flowers is not autocatalytic. The rise in ethylene production induced by pollination is accompanied by an increase in mRNA levels hybridizing to cDNAs coding for ACC synthase (ACS2) and two ACC oxidases (GEFE2 and GEFE4). Ethylene has no effect on levels of these mRNAs, again indicating lack of autocatalysis. The stigma, style and ovary contribute approximately equally to the total pistil complement of ACS2 mRNA. However, mRNAs hybridizing with GEFE2 and GEFE4 are differentially distributed, GEFE2 predominantly in the stigma and style, and GEFE4 in the style and ovary. Although ACC applied to the stigma is easily converted to ethylene, ACC is unlikely to be the signal from pollen that induces accumulation of ethylene-biosynthesis related mRNAs and ethylene production, since (1) no ACC is detectable in pollen; (2) ethylene biosynthesis is not autocatalytic, so a small amount of ACC would be insufficient to induce substantial ethylene production; (3) pollination with heat-killed pollen, which would still be expected to release heat-stable metabolites, induces only a small increase in ethylene production – 27% as much ethylene as live self pollen. It is possible, however, that ACC contributes to interorgan signalling. ACC accumulates in the stigma (but not the style and ovary) after pollination, providing a potential source of mobile ACC. All three organs express genes of the ethylene biosynthetic pathway.

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EXPRESSION OF ANTISENSE AND SENSE 1-AMINOCYCLOPROPANE-1-CARBOXYLATE OXIDASE RNA IN PETUNIA

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Ethylene production increases during pistil development and corolla senescence in *Petunia hybrida* flowers. Recently we have cloned and characterized a family of genes encoding 1-aminocyclopropane-1-carboxylate (ACC) oxidase from petunia. ACC oxidase catalyzes the conversion of ACC to ethylene. The petunia ACC oxidase genes are upregulated during corolla senescence, development of the pistil, and in response to wounding. The three transcriptionally active ACC oxidase genes share extensive homology within the coding region. In order to investigate the function of ethylene in pistil development and corolla senescence of petunia flowers we have sought to generate transgenic plants altered in the expression and activity of ACC oxidase. The ACO1 cDNA was cloned in the antisense and sense orientations behind the CaMV 35S promoter and constructs used to transform petunia via *Agrobacterium tumefaciens*. The cDNA was also expressed in *E. coli* and resulting recombinant protein used to raise antibodies against ACC oxidase. Transgenic plants were selfed and the progeny are currently being analyzed for ethylene production, ACC oxidase protein and mRNA levels and their effect on flower development.

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INCREASED ETHYLENE PRODUCTION IN RICE EXPOSED TO LONG TERM ELEVATED CO_2 - IS IT RELATED TO DEVELOPMENTAL CHANGES?

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An enigmatic effect of elevated CO_2 concentrations on plants is the repeated observation of hormone-like responses: changes in time to flowering, increased branching of shoots and roots and loss of apical dominance. Rice (*Oryza sativa* L.) provides a good example of this hormone-like response to high CO_2. Shoot elongation rates are faster at 700 than 350 $\mu L\ CO_2 L^{-1}$ until 21 DAP when the high CO_2 effect on elongation is reversed. This coincides with rapid rates of tiller production at elevated CO_2. Flowering is also accelerated, occurring seven days earlier at high CO_2. We explored the possibility that ethylene may have been involved in these developmental changes by measuring rates of ethylene release from blades of rice grown at either 350 or 700 $\mu L\ CO_2\ L^{-1}$ at different stages during antogeny. Blades were incubated in either water or ACC (1mM) prior to measurement of ethylene. Blades from plants grown at elevated CO_2 released more ethylene when incubated in either water or ACC. Maximum rates of release occurred in blades from high CO_2-grown plants between 14 and 21 DAP when shoot elongation rates were fastest. There was little ethylene released from blades of plants grown at ambient CO_2 over the same period. Additional studies with $CoCl_2$ led us to conclude that high CO_2 enhanced the capacity of blades to convert ACC to ethylene. The possibility that high CO_2 increases transcription of ACC oxidase is currently being explored.

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RHYTHMIC TEMPORAL REGULATION OF ACC OXIDASE IN *STELLARIA LONGIPES*.

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When genotypes of *Stellaria longipes* were grown under short day and cold (simulated-winter) conditions and then transferred to long day and warm conditions, we observed a daily rhythm in ethylene. To investigate the cause for such a rhythm in ethylene evolution, we examined the role of ACC oxidase. When ACC oxidase activity was assayed *in vitro*, it showed a circadian rhythm. The cDNA library made from *S. longipes* was screened and a clone encoding ACC oxidase was obtained. We then analysed the transcript levels of ACC oxidase transcripts. Northern blots clearly show oscillating levels of ACC oxidase mRNA transcripts. We conclude that expression of ACC oxidase is temporally regulated and may possibly be under the control of biological clock.

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THE FUNCTION OF E8 - AN ETHYLENE-INDUCED GENE FROM RIPENING TOMATO FRUIT.

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The E8 gene is expressed at high levels during tomato fruit ripening, and is transcriptionally regulated both by ethylene and by an ethylene-independent fruit ripening developmental program. The deduced E8 protein is a member of a family of dioxygenases and shares homology with ACC oxidase, the enzyme that carries out the last step in ethylene biosynthesis. Results from antisense experiments suggested that E8 may act to negatively regulate ethylene biosynthesis during tomato fruit ripening (Penarrubia et al., *Plant Cell* 4: 681-687, 1992). To learn more about the function of E8, we have over-expressed the E8 gene in transgenic tomato plants using the CaMV promoter. We have studied in detail 6 independently transformed lines that each carry a single copy of the transgene. We found that 4 of these lines have an abnormally low rate of transmission of the transgene to progeny. That is, among these lines, only 34% to 58% of the progeny of self-pollinated hemizygotes inherited the chimeric E8 gene, rather than the expected 75%. We examined expression of E8 in flowers, and found that high levels of E8 mRNA are present in anthers of wild type plants. Analysis of transgenic plants bearing an E8 driven reporter, β-glucuronidase, revealed that E8 is expressed in mature pollen grains. We are currently examining the effect of E8 overexpression on pollen viability, germination, and ACC biosynthesis.

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THE CLONING AND DEVELOPMENTAL EXPRESSION OF *S*-ADENOSYLMETHIONINE DECARBOXYLASE GENE IN CARNATION FLOWERS

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The development of carnation flower is associated with polyamine biosynthesis. Carnation flower senescence also is associated with a dramatic increase in ethylene biosynthesis. Both of polyamine and ethylene biosynthesize from same precursor, *S*-adenosylmethionine (SAM), while those are different in functions on development and senescence. We have got the 460bp-partial clone of *S*-adenosylmethionine decarboxylase, which converts SAM to decarboxylated SAM for polyamine biosynthesis, by PCR amplification. We used the degenerate PCR primers which were based on the conserved sequences of SAMDC gene from potato, human, and rat. A PCR SAMDC clone was used to screen a cDNA library prepared from mRNA isolated from the petals of carnation flowers in anthesis state. We have cloned cDNA of SAMDC. RNA gel blot analysis with PCR SAMDC clone indicated that transcripts accumulated in mature petals concomitant with increased SAMDC activity and polyamine accumulation. As compared with ethylene biosynthesis, the developmental expression of SAMDC gene in carnation flower will be presented.

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REGULATION AND FUNCTION OF A POLLEN-SPECIFIC ACC SYNTHASE GENE FROM PETUNIA

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At anthesis, petunia pollen contains large amounts of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC). This ACC is thought to contribute to the rapid burst of ethylene produced by the pistil following pollination. An analysis of ACC content in developing anthers revealed that ACC began to accumulate the day before anthesis, indicating its synthesis was a late event in pollen development. We employed degenerate DNA primers to conserved amino acid sequences of ACC synthase to amplify a cDNA from anther mRNA by RT-PCR. The resulting cDNA (pACS2) was sequenced and found to represent ACC synthase. Use of pACS2 as a hybridization probe revealed an increase in ACC synthase mRNA concomitant with the increase in ACC content. Further analysis indicated the ACC synthase mRNA was localized specifically to the haploid pollen grain. In an attempt to determine the function of ACC in pollen maturation or pollen-pistil interactions we have generated a series of transgenic petunias designed to inhibit the accumulation of ACC in pollen. For these experiments we have employed a pollen-specific promoter (LAT52) from tomato to drive the expression of antisense pACS2 mRNA or the coding region of ACC deaminase. The results of these experiments will be discussed.

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DIFFERENTIAL EXPRESSION OF TWO AUXIN-INDUCED ACC SYNTHASE GENES IN ETIOLATED PEA STEMS

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Auxin promotes ethylene biosynthesis in the stems of etiolated pea seedlngs by rapidly inducing the accumulation of 1-aminocyclopropane-1-carboxylate (ACC) synthase transcript and activity levels. We have cloned two genes for auxin-inducible ACC synthase, PS-ACS1 and PS-ACS2, that do not cross-hybridize. Transcripts of both genes accumulate rapidly, although with different patterns, after auxin treatment and are repressed by cotreatment with ethylene. We also find that in seedlings sprayed with auxin immediately after they are removed from a 4-h pretreatment with 20 μL/L ethylene, the transcript and activity levels of ACC synthase are increased 5- to 10-fold as compared to auxin treatment alone. This difference may be a result of the shift from a high ethylene environment to a low ethylene environment and may suggest that ethylene perception is an adaptive response. PS-ACS2 (2.1 kb) is present in all untreated tissues and increases in all portions of the stem after treatment with IAA. PS-ACS1, however, is not detectable in untreated tissues and only accumulates after auxin treatment in the upper portion of the stem. Moreover, a probe consisting of the 3'-untranslated region of PS-ACS1 hybrizes with two distinct bands (1.9 kb and 1.6 kb) on RNA blot analysis. These bands accumulate with different rates after auxin treatment. We are investigating whether the two bands may be a result of different transcriptional start sites. (This research was supported by the U.S. Department of Energy through grant No. DE-FG02-91ER20021.)

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A COMMERCIAL FORMULATION OF AMINOETHOXYVINYLGLYCINE (AVG) FOR USE ON APPLES

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(S)-trans-2-Amino-4-(2-aminoethoxy)-3-butenoic acid, or aminoethoxyvinylglycine (AVG), is being commercially developed by Abbott Laboratories for use on apples. AVG, a naturally-occurring plant growth regulator, inhibits ACC (1-aminocyclopropane-1-carboxylic acid) Synthase, the enzyme responsible for the conversion of ACC to ethylene. Our candidate AVG formulation is an organic soluble powder which has been shown to be safe, stable, non-phytotoxic, and highly efficacious in reducing preharvest apple drop. In over 40 field trials conducted with the formulation, AVG routinely out-performed the current industry standard, NAA (naphthaleneacetic acid), in controlling drop. For example, in one trial with cv. McIntosh, 50 grams AVG/acre applied four weeks before harvest resulted in 1.7% cumulative fruit drop two weeks after normal harvest while NAA applied at 10 ppm one week before harvest resulted in 20.4% fruit drop for the same period. AVG also helps maintain fruit quality at harvest, particularly when compared to NAA. Product chemistry characteristics, as well as field efficacy data, will be presented.

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POTAMOGETON PECTINATUS - A PLANT WITHOUT ETHYLENE

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It is generally accepted that growing vegetative organs of higher plants synthesise ethylene and that the gas promotes fast shoot elongation in aquatic and amphibious species when submerged. However, neither generalization applied to *Potamogeton pectinatus*, an aquatic species of cool climates that produces strongly elongating shoots from submerged tubers. Although stem elongation was strongly promoted by submergence, it did **not** respond positively to applied ethylene. Furthermore, no evidence of ethylene production was found using flame ionization gas chromatography (working sensitivity 0.01 ppm, v/v), nor laser-driven photoacoustic detection (working sensitivity 50 ppt, v/v). Submergence, physical wounding, auxin treatment, exposure to carbon dioxide (10 %, v/v) or to light failed to elicit ethylene biosynthesis. *P. pectinatus* was rich in the ethylene precursor 1-aminocyclopropane-1-carboxylic acid but lacked the ability to oxidise this ACC to ethylene.

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ETHYLENE BIOSYNTHETIC GENES IN *ACTINIDIA CHINENSIS*

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Actinidia chinensis Planch is a species containing diploid and tetraploid taxa that are closely related to the hexaploid kiwifruit (*Actinidia deliciosa* var *deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson). Both *A. chinensis* and kiwifruit are climacteric, and fruit ripening can be induced by exogenous ethylene. However endogenous ethylene cannot be detected until late in ripening, when the fruit are ready to eat. Ethylene is synthesised from its precursor L-methionine via *S*-adenosyl-L-methionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC). We have isolated three distinct SAM synthetase genes and two ACC oxidase genes from an *A. chinensis* ripe fruit cDNA library. A partial ACC synthase gene was isolated from cDNA by a PCR-based method. In order to investigate the role of ethylene in ripening, *A. chinensis* plants have been transformed with constructs comprising both ACC synthase and ACC oxidase genes, in sense and antisense orientations. We aim to reduce ethylene to a very low level, by antisense supression or co-supression by the sense transcript. To complement this work, we have undertaken a study of expression of the ethylene biosynthetic genes in a series of ripening *A. chinensis* fruit. Northern analyses showed expression of one or more SAM synthetase genes was induced immediately after application of exogenous ethylene. Transcripts of ACC oxidase were induced two days later, in the ripe fruit. ACC synthase transcripts were too rare to be detected by Northern analysis. We have begun a more detailed and quantitative study using RNase protection assays. We are using gene-specific probes for each of the three SAM synthetase genes, together with ACC synthase, ACC oxidase and a ubiquitin gene as an internal control.

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CYTOKININ SYNTHESIS IN CYANOBACTERIA

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It has recently been found in our laboratory that several cyanobacteria produce cytokinins which accumulate in the culture filtrate. The unicellular *Synechocystis* 7002 was found to produce both isopentenyladenosine and methylthioribosylzeatin. The filamentous, heterocyst-forming, nitrogen-fixing, *Mastigocladus laminosis* UTEX 1931 was found to produce both isopentenyladenin and isopentenyladenosine. These observations suggest a role for cytokinins in the growth and development of such evolutionarily ancient organisms as cyanobacteria and opens a new way to study the functions of cytokinins in general. Currently, we are trying to isolate the genes responsible for cytokinin synthesis in cyanobacteria. Based on the most conserved regions of 4 bacterial isopentenyltransferase and 6 transzeatine synthase genes, we have devised a set of primers for PCR reactions. The primer sets relate to the amino acid regions 32-37, 96-102, and 172-176 of the agrobacterial *tzs* gene and contain inosine at the most degenerate positions. We use both regular PCR, using a range of annealing temperatures, and Touchdown PCR. Isolation of these genes will make it possible to manipulate the synthesis of cytokinins in cyanobacteria. (Funded by the Feinstone Microbiology Research Fund at the U of M)

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INCORPORATION OF ^{14}C-ADENINE INTO CYTOKININS IN DEVELOPING MAIZE KERNELS

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Developing maize kernels exhibit peaks in cytokinin levels (Z and ZR) 8 to 12 days after pollination (DAP). However, the origin of these cytokinins is uncertain. Because in vitro-cultured kernels also exhibit these spikes, it is likely that most of these cytokinins are synthesized within the kernels. Yet incorporation of adenine into cytokinins in developing maize kernels has not been reported. Our objective was to determine whether whole kernels or kernel constituents (endosperm, embryo, pericarp) were capable of incorporating ^{14}C-adenine into cytokinins. Kernels were sampled from 8 to 17 DAP, partitioned into constitutive tissues, and placed for 6 hours in an aerated buffer containing ^{14}C-adenine. Radiolabelled-carbon was detected in relatively high amounts in Z, ZR, IPA and IPAR fractions from isolated endosperm. ^{14}C-adenine incorporation into ZR, IPA and IPAR was highest in 12-day-old endosperm (expressed per endosperm), and had declined by 14 DAP. Incorporation into Z was similar in 8,10,12 and 14 day-old endosperm. These results correspond well with spikes in cytokinin levels observed in intact maize kernels. Zeatin levels tend to peak at 8 to 10 DAP, while ZR levels peak later. These results suggest that peaks in maize kernel cytokinin levels are largely due to increased cytokinin synthesis in maize endosperm tissue.

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ACETOBACTER DIAZOTROPHICUS, A SUGARCANE ENDOPHYTE, PRODUCES CYTOKININ. Barbara J. Taller, Dawn H. Dawson and Chetan S. Patil. Molecular Sciences & Microbiology, University of Memphis, Memphis, TN 38152. E-mail: bjtaller@msuvx1.memphis.edu

Acetobacter diazotrophicus is a nitrogen-fixing bacterium which is an endophyte of sucrose-rich plants such as sugarcane and sweet potato. As many plant-associated microbes produce zeatin cytokinins, it was of interest to examine cytokinin synthesis by this endophyte. *A. diazotrophicus* PA1 5 (type strain) was grown to early stationary phase in LGI medium. Cytokinins were purified from the culture filtrate by XAD-2 chromatography, then fractionated by Sephadex LH-20 chromatography in 35% ethanol and in water. Pooled fractions from LH-20 columns were tested for cytokinin activity in the tobacco bioassay. Three cytokinin-active fractions were found, corresponding to zeatin riboside (ZR), zeatin and isopentenyladenine standards. When the putative ZR fraction was purified by phenylboronic acid-agarose chromatography and analyzed by reverse phase HPLC, the retention time was consistent with *trans*-ZR. *A. diazotrophicus* was previously found to produce IAA. Thus, in addition to nitrogen fixation, the growth promoting effect of the endophyte may be due to phytohormone production. (Funded by the Feinstone Microbiology Research Fund at The U of M.)

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BIOSYNTHESIS OF BRASSINOSTEROIDS IN CULTURED CELLS OF CATHARANTHUS ROSEUS

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Biosynthetic pathway of brassinolide was investigated using cultured cells of Catharanthus roseus. 22α-Hydroxy-6-oxocampestanol was found to be endogenous in cultured cells of C. roseus, and it was named cathasterone. Its endogenous levels were in the range of 2-4 ng/g fw, similar to those of brassinolide and castasterone. Feeding experiment with deuterium labeled substrate revealed that cathasterone was converted to teasterone, providing the evidence for cathasterone being the biosynthetic precursor of teasterone. Based on this study together with our previous studies, the most probable biosynthetic sequence of campesterol -> campestanol -> 6α-hydroxycampestanol -> 6-oxocampestanol-> cathasterone -> teasterone -> 3-dehydroteasterone -> typhaserol -> castasterone -> brassinolide in cultured cells of C. roseus was disclosed. Biological activity of brassinolide and its precursors was examined by rice-lamina inclination test. Activity increased according to the order of biosynthetic pathway. Introduction of a hydroxyl group in the side chain, the step of 6-oxocampestanol to cathasterone, exerted a substantial activity. Relative activity was as follows: 6-oxocampestanol (0.002), cathasterone (1), teasterone (20), and brassinolide (1200).

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DEVELOPMENTAL EXPRESSION OF ARGININE DECARBOXYLASE GENE IN CARNATION FLOWERS

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Polyamines have been implicated in differentiation processes and especially in flowering. Putrescine, the precursor of polyamine, is derived from ornithine by the enzyme ornithine decarboxylase in animals and microorganisms. However, plants and bacteria have an arginine decarboxylase (ADC), allowing the formation of putrescine from arginine via its decarboxylation product, agmatine. We cloned ADC partial clone, 627 bp, with PCR amplification using degenerate primers which were based on the conserved sequences of ADC gene from oat and tomato. In RNA gel blot analysis with this PCR clone, it was detected about 2 kb of transcripts for ADC gene. A PCR ADC clone also used to screen a cDNA library prepared from mRNA isolated from the petals of carnation flowers in anthesis state. RNA gel blot analysis with PCR ADC clone indicated that transcripts much accumulated in premature petals concomitant with increased ADC activity. We concluded the developmental expression of ADC gene was transcriptionally regulated. The developmental expression of ADC gene in carnation flower will be presented.

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INDOLE-3-ACETIC ACID DECONJUGATION IN *ARABIDOPSIS THALIANA*

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Indole-3-acetic acid (IAA), which promotes cell division and elongation, is found in plants conjugated to a variety of amino acids, peptides or carbohydrates. These conjugates represent ~95% of the auxin pool, and may allow rapid modulation of free IAA concentration, inactivate excess IAA, or function in IAA transport. *Arabidopsis thaliana* conjugates IAA primarily as amides, and exogenous application of several IAA-amino acid conjugates mimics free IAA, stimulating lateral root initiation while inhibiting root elongation. We isolated *A. thaliana* mutants insensitive to exogenous IAA-alanine (*iar1* and *iar2*) or IAA-leucine (*ilr1*). These recessive mutants respond normally to free IAA, and thus may be unable to cleave or transport auxin conjugates. We cloned the *ILR1* gene using a map-based positional strategy. *ILR1* encodes a 48 kDa protein that cleaves IAA-amino acid conjugates *in vitro* and is homologous to bacterial aminohydrolase enzymes. Our results confirm that IAA conjugates are inactive and must be cleaved to release active free IAA. *ILR1* is a member of a multigene family in *Arabidopsis* and DNA sequences similar to *ILR1* are found in other plant species, suggesting that IAA deconjugation plays a general role in control of plant growth.

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CHARACTERIZATION OF MUTANTS OF *ARABIDOPSIS* RESISTANT TO GROWTH INHIBITION BY IAA-AMINO ACID CONJUGATES.

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We are investigating the functions of IAA-amino acid conjugates in *Arabidopsis* by isolating mutants resistant to growth inhibition by IAA-phenylalanine. Two mutants were isolated; one from about 4,500 EMS-mutagenized seed and a second from the Feldmann collection of T-DNA treated seed. Genetic analysis indicates that the mutations are recessive. One mutation (*icr1*) is located at approx. 80cM on chromosome 3; mapping of the second (*icr2*) is in progress. Both mutants show cross-resistance to IAA-alanine but not to IAA-aspartate. Studies with radiolabeled IAA phenylalanine show that conjugate uptake is the same in seedlings of wild-type and both mutants. We have not yet been able to detect reproducible differences in conjugate hydrolysis between the mutant and wild-type strains. One of the mutants (*icr1*) also shows delayed germination and early flowering. Segregation analysis is in progress to determine whether these traits are associated with the conjugate-resistance phenotype. This research is supported by National Science Foundation Grant No. IBN93-20120.

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AN INDUCIBLE INDOLE-3-ACETYL-L-ASPARTIC ACID HYDROLASE FROM BACTERIAL STRAIN GK#12A: PARTIAL PURIFICATION AND CHARACTERIZATION

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IAA-amino acid conjugate hydrolases are presumed to have important roles in plant IAA regulation and have potential uses in alteration of IAA metabolism. A bacterial strain, coded GK#12A, was isolated by enrichment from a sewage sludge inoculation using IAA-aspartic acid as the carbon and nitrogen source. This crude enzyme hydrolyzed IAA-aspartate, but did not hydrolyze IAA-alanine. The hydrolase activity was inducible by IAA-aspartate or N-acetyl-L-aspartic acid, but not by IAA, $(NH_4)_2SO_4$, urea or indoleacetamide. The hydrolase was inhibited by 1 mM DTT or 1 mM Cu^{2+}, but unaffected by 1 mM Co^{2+}, 1 mM Mg^{2+}, 1 mM Mn^{2+} or 1 mM Zn^{2+}. Purification of the hydrolase by DEAE- and phenyl-preparative HPLC followed by gel filtration HPLC yielded a single 43 kD polypeptide by SDS-PAGE. *This work was supported, in part, by grant DE-AI02-94ER20153 from the U. S. Department of Energy.*

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THE NATURE OF TOBACCO ANIONIC PEROXIDASE INTERACTION WITH INDOLE-3-ACETIC ACID (IAA) , 2-METHYL-IAA AND IAA AMINO ACID CONJUGATES.

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IAA degradation by plant peroxidases has been studied since 1955, but the reaction mechanism still remains obscure. This study of a newly isolated tobacco anionic peroxidase overexpressed in transgenic plants gives new insight into the IAA oxidation mechanism. The oxidation of IAA, 2-methyl-IAA and IAA conjugates with D,L-aspartate, L-asparagine, D-phenylalanine and L-phenylalanine by the tobacco anionic peroxidase was studied by means of kinetic and spectral measurements in aerobic and anaerobic conditions. Anaerobic spectral studies at the fixed oxygen levels provided evidence of a direct interaction between the enzyme, oxygen and substrate. This results in the formation of IAA hydroperoxide inside the active center. 2-Methyl-IAA which produces radicals much easier than IAA itself was not a substrate for the oxidation reaction though it was readily oxidized via a peroxidative cycle according to the HPLC and oxygen uptake data. Thus, the 2nd position of the indole ring plays a key role in the oxidation mechanism indicating the real enzymatic character of the process is in contrast to peroxidation. Addition of hydrogen peroxide in the course of IAA oxidation led to the appearance of Compound II which was shown to accelerate the process of oxygen uptake eliminating a lag-period. At pH < 5, the tobacco peroxidase catalyzed the oxidation of IAA conjugates in the presence of hydrogen peroxide exhibiting the Compound II spectrum responsible for oxygen uptake. The oxidation was specific to the nature and stereo-configuration of the amino acid conjugated with IAA, also pointing out the existence of the enzyme-substrate complex. We conclude that the peroxidase active center is designed especially for the IAA oxidation reaction which is highly specific.

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rty-3, A T-DNA ALLELE: USE FOR THE CLONING OF A GENE INVOLVED IN AUXIN ACTION

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During the construction of transgenic *Arabidopsis thaliana*, an allele of *RTY* (*rty-3*) was generated. *rty-3* seedlings germinate normally but form large numbers of adventitious roots on the hypocotyl and petioles shortly after germination. Genetic analysis indicated that the phenotype of *rty-3* is due to a single recessive mutation and that it is allelic to *rty-1*. The phenotype of *rty-3* is more severe than that of other known alleles. *rty-1* contains elevated levels of IAA (King et al. Plant Physiol. Supplement Vol 102 Abstract 326) and it is hypothesized that the RTY product is involved in auxin metabolism. Analysis of the segregation of the *rty-3* mutation and the dominant kanamycin resistance marker contained on the T-DNA indicate that there is a single functional kanamycin resistance locus that is very tightly linked to the *RTY* locus, suggesting that *rty-3* is caused by the insertion of the T-DNA. Hybridization of Southern blots containing *rty-3* DNA with T-DNA probes indicates that *rty-3* plants contain a single T-DNA insert. We are taking advantage of the T-DNA insert in the *rty-3* allele to clone *RTY*.

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ROLES OF PEROXIDASE IN AUXIN METABOLISM

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The involvement of auxin catabolism by peroxidase has long been proposed but much of the evidence has come from in vitro studies. To better characterize on possible in planta functions of peroxidase in auxin metabolism, experiments associated with auxin-dependent physiology using peroxidase transgenic plants were performed. Differences between tobacco wild type and transgenic plants in auxin-associated root growth and development,and response of roots to auxin sensitivity and gravity were compared. Seedlings of wild type and anionic peroxidase over- and under-producing plants were grown from seeds on OMS medium. Shoot tips were transferred to GA7 jars containing OMS for adventitious root regeneration. Observations on the number of roots and the length of target root were made at various time after shoot tips were transferred. Preliminary results revealed peroxidase underproducer tend to produce more but shorter roots than others which may due to less endogenous auxin were decomposed by low level of peroxidases in planta. Moreover,growth chamber grown tobacco plants showed that phenotypically peroxidase overproducer suffered from severe wilting prior flowering and had much less root mass than wild type ones. To investigate their response to auxin and gravity, seedlings were grown vertically on OMS medium. Growth rate and degree of root curvature before and after gravistimulation, and prior and after IAA treatment were measured using the video digitizer system. Results suggested that peroxidase underproducers were more sensitivity to exogenous IAA than wild type.

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THE EFFECTS OF WOUNDING AND APPLIED 4-CL-IAA ON [^{14}C]GA_{19} METABOLISM IN PERICARPS OF *PISIUM SATIVUM*

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Gibberellin metabolism in garden pea *(Pisium sativum* L.) has been investigated by several groups focusing on different tissues. There are two parallel GA metabolic pathways in peas, with the early C-13 hydroxylation route the primary pathway to the formation of C_{19}-GAs, such as GA_{20}, GA_1, GA_{29} and GA_8. According to previous studies from this laboratory, the level of GA_{20} (and subsequently, GA_1) in the pericarp is probably regulated by remote signals from developing seeds. However, nothing was actually known about the effects of external factors on GA biosynthesis in the tissue. By feeding the pericarp (2 days after anthesis) with [^{14}C]GA_{19}, we observed a distinct wounding effect on the oxidation of GA skeleton at C-20, the key step to convert C_{20}-GAs to the C_{19} derivatives. In extracts from opened (cutting down and separating one suture line) or split (fully separating down both suture lines) pericarps, the level of [^{14}C]GA_{20} was reduced, relative to that from intact pods, 40 h after [^{14}C]GA_{19} application. The reduction (below detection, in certain circumstances) of [^{14}C]GA_{20} was always accompanied with an increased peak of un-metabolized [^{14}C]GA_{19}, based on radioassay results of C_{18} HPLC fractions. On the other hand, application of 4-Cl IAA (50 ng per pericarp side) promoted the conversion of [^{14}C]GA_{19} to [^{14}C]GA_{20} in the pericarp, converting the inhibitory effect of wounding on the oxidation reaction of the GA at C-20. Identification of [^{14}C]GA_{19} and [^{14}C]GA_{20} from related HPLC fractions was completed by GC-MS analysis with full mass spectra and KRIs.

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CONJUGATION OF ACC IN TOMATO

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The ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) has at least three fates in addition to oxidation to ethylene. Even in climacteric tomato fruit ACC can be sequestered as the free unmetabolized amino acid and can be conjugated. Extracts of both tomato fruit and seeds can conjugate ACC to 1-malonylaminocyclopropane-1-carboxylic acid (MACC) (Liu, et. al.,1985, Plant Physiol 77: 891) and to γ-glutamylACC (GACC), a new conjugate we have recently identified (Martin, et. al.,1994, Plant Physiol 105: 142). To elucidate the function of ACC conjugation in tomato plants, we have taken the approaches of: (1) measuring the two conjugating activities during tomato fruit development and ripening, seed germination and seedling development; (2) establishing the endogenous presence and the levels of ACC, MACC, and GACC during fruit and seedling development; and (3) establishing how the conjugating activities and the conjugates themselves are impacted in mutant and transgenic tomato plants altered in aspects of ethylene biosynthesis or perception. To measure the conjugating activities we developed facile assays for the ACC-N-malonyltransferase and the γ-glutamyltranspeptidase, which catalyze, respectively, MACC and GACC formation. To quantify the endogenous levels of ACC, GACC, and MACC, we: (a) developed a solid phase extraction method by which ACC, GACC, and MACC can be recovered from the same sample and resolved from each other in a single step; (b) employed a one-step derivatization procedure involving synthesis of the MACCdimethylester, the N-acetylGACCdimethylester, and the N-acetylACCmethylester to prepare samples for GC-MS analysis; and (c) developed isotopic methods for quantification of each by GC-MS. Supported, in part, by DOE DE-AI02-94ER 20153.

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INTRODUCTION OF THE ANTI-GIBBERELLIN ANTIBODY GENE INTO TOBACCO

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A single-chain Fv (scFv) gene encoding the variable heavy (VH) and light (VL) domains of an anti-gibberellin (GA) antibody was introduced into tobacco plant to examine a possibility to establish a new system for the study of gibberellin function. The VH and VL domains are derived from the mouse monoclonal anti-GA_4 antibody 8E(9), which specifically recognizes the structural feature essential for GA activity. The scFv gene was constructed by ligating genes of VH and VL to each end of the linker peptide (SG_4) gene. The scFv was first expressed in *E. coli* as a fusion protein with a histidine tag. The protein was recovered in an insoluble fraction, solubilized with guanidine-HCl, affinity-purified and dialyzed under a S-S forming condition. Although most of the protein was recovered as precipitates, the soluble fraction showed a slight binding activity to GA_4. Other conditions for protein refolding are being examined. The scFv gene was introduced into tobacco plant with the binary system using *Agrobacterium*, too. Northern hybridization analysis showed expression of the scFv mRNA (-800bp) in all kanamycine-resistant plants examined. No significant phenotypic alteration has been observed, so far. Endogenous GAs in the transgenic plants are being analyzed.

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DEVELOPMENTAL AND HORMONAL ASPECTS OF AN OAT MUTANT EXPRESSING GIANTISM

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As part of an investigation into the genetic/physiological basis for giantism found in a recently isolated oat (*Avena sativa* L.) mutant, a morphological and hormonal comparison was made between the mutant and its parents (two standard cultivars: Tibor and OT745) during growth and development. The mutant and its parents were grown in a controlled-environment chamber (21°C/17°C, day/night temperature; 16-h photoperiod; approximately 400 mol $m^{-2}s^{-1}$ photosynthetic photon flux at canopy height) Measurements of height, tillering and leaf stage were made and tissue samples were collected at weekly intervals until panicle emergence. The tissues in the mutant and the parents were analysed for endogenous gibberellins, ABA and IAA using HPLC, GC, bioassay and GC-MS. Panicle emergence occurred after 16 wk for the mutant, and 6 wk for the parents. At panicle emergence, the mutant averaged 17 leaves compared with 8 leaves for the parents; the height of the mutant was approximately 195 cm compared with 96 cm for the parents. The shift of the apex from vegetative to reproductive growth occurred at approximately 8 wk in the mutant and at 2 to 3 wk in the parents. The apex of the mutant showed a number of anatomical differences from those of the parents: it was longer and larger and produced more leaf primordia. Differences in both quantitaive and qualitative levels of gibbererllins were noted between the the mutant and the parents. In the mutant there were very elevated levels of less polar GAs at week 10 (after the shift to reproductive growth) relative to Tibor at any of the sampling periods on a per gram dw and on a per plant basis. The mutant would appear to have a faulty conversion mechanism from less polar GAs to GA_1/GA_3 based on the build-up of less-polar GAs and a very reduced concentration of GA_1/GA_3.

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GIBBERELLINS AND THE CONTROL OF SHOOT GROWTH IN *PINUS SYLVESTRIS*

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Previously, we found that applying $GA_{4/7}$ as a soil drench to seedlings increased longitudinal and cambial growth in the current-year terminal shoot and elevated the cambial region levels of GA_4, GA_7, GA_9 and IAA. In subsequent experiments, the application of $GA_{4/7}$ in ethanol around the circumference of shoots, i.e., ringing, was observed to stimulate cambial growth in debudded previous-year terminals treated apically with lanolin containing IAA, but not with plain lanolin, and this stimulation was not associated with an elevated IAA content in the cambial region. Ringing the previous-year terminal of seedlings with prohexadione (BX-112), which inhibited both current-year terminal elongation and cambial growth in the current- and previous-year terminals, also did not affect the cambial region IAA content; however it increased the level of GA_9 while decreasing that of GA_1, GA_3 and GA_4. In other work, endogenous GA_1, GA_3, GA_4, GA_9, GA_{12} and GA_{20} were identified by gas chromatography-mass spectrometry-mass spectrometry in elongating shoots. When applied in ethanol to the previous-year terminal, $[^2H_2]GA_9$ was converted to $[^2H_2]GA_{20}$, $[^2H_2]GA_4$ and $[^2H_2]GA_1$, while $[^2H_2]GA_4$ was metabolized to $[^2H_2]GA_1$ and $[^2H_2]GA_{20}$ was converted to $[^2H_2]GA_{29}$. The results indicate that, in Scots pine, 1) GAs play a role in the regulation of both longitudinal and cambial growth, 2) GA action requires IAA, but GAs do not act through increasing the IAA level, and 3) the late 13-hydroxylation pathway is the major route of GA biosynthesis.

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THE METABOLISM AND PHYSIOLOGY OF GIBBERELLINS IN *NEUROSPORA CRASSA*.

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Understanding the production and use of gibberellins in fungi will assist in expanding knowledge of the interaction between plant pathogens and their hosts. Cultures of *Neurospora crassa* were grown in Vogel's minimal medium containing various levels of nitrogen to enhance production of secondary metabolites. Both culture filtrates and mycelia were extracted for gibberellins (GAs). HPLC fractions were collected and tested in Tan-ginbozu rice bioassay. Large peaks of GA-like bioactivity were evident in the GA_1/GA_3 region. Small peaks were found in the GA_4 to GA_9 region. Nitrogen levels had no significant effect on GA-like bioactivity. When aliquots of HPLC fractions were subjucated to GC-MS, presence of GA_3 was confirmed and GA_1, GA_4, and GA_9 were tentatively identified. In order to characterize the GAs accurately, stringent methods of purification are currently being used. The effects of GA_3 and various inhibitors of GA biosynthesis such as CCC and AMO-1618 on mycelial growth and conidiation are also under investigation.

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INVESTIGATION ON GLYCOSYLATION OF GIBBERELLINS DURING SENESCENCE OF DETACHED LEAVES OF *RUMEX JAPONICUS*

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Exogenous 13-hydroxy gibberellins(GAs) played much more effective role in retarding the leaf senescence than exogenous 13-deoxy GAs or cytokinins in many species of *Rumex*, suggesting that the metabolism of 13-hydroxy GAs was closely connected with the senescence process in these plants. To determine rapidly and precisely GAs and their glucosides in the plants, monoclonal antibodies recognizing both non-derivatized 13-hydroxy GAs and their glucosides were prepared. Using these antibodies, enzyme-linked immunosorbent assay (ELISA) for direct determination of GA glucosides was developed as well as that for 13-hydroxy GAs. Based on these two ELISAs displaying linear detection ranges from 0.2 to 20pmol, the changes of the levels of endogenous GAs and their glucosides were investigated during the senescence of *Rumex japonicus* leaf discs (6mm) floating sterilized water in the dark at 30℃ . In the H_2O-treated leaves, rapid depletion of 13-hydroxy GAs and formation of their glucosides were observed, followed by rapid breakdown of chlorophylls measured as the index of leaf senescence. In the 6-benzyl amino purine (BA, 40 μmol / L) treated leaves, free GAs and the chlorophylls were maintainted at higher level, whereas their glucosides at lower level within first 3 days. This showed that the conversion of free GAs into their glucosides was weakened by 6-BA. Concerning the leaves treated by exogenous GA_3 (10 μmol / L) showing most effective retardation of senescence, uptake of exogenous GA_3 was followed by its glycosylation at very rapid rate. Free GAs and their glucosides showed great surplus relative to the contents of physiological levels. In spite of high level of GA glucosides, the senescence of GA_3- treated leaves was still significantly delayed. It is proposed that GA glucosides are inactive forms in senescing leaves. In conclusion, the glycosylation of free GAs is connected with the leaf senescence and the function of 6-BA with retarding the leaf senescence is probably related to delaying the process of glycosylation of free GAs.

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PREPARATION OF 8'-HYDROXYABSCISIC ACID, A POTENT BIOLOGICALLY ACTIVE METABOLITE OF ABA, FROM PHASEIC ACID. Garth D. Abrams, Suzanne R. Abrams, Dennis L. Barton, Lawrence R. Hogge and Douglas J. H. Olson. Plant Biotechnology Institute, National Research Council of Canada, 110 Gymnasium Place, Saskatoon, Saskatchewan, Canada S7N 0W9. E-mail: sabrams@pbi.nrc.ca

It is generally accepted that the oxidative metabolism of abscisic acid (ABA) to phaseic acid (PA) in plants proceeds *via* 8'-hydroxyabscisic acid (8'-HOABA), which readily cyclizes to PA. The instability of 8'-HOABA has heretofore prevented evaluation of its biological activity. We have found that in moderately acidic aqueous solutions at room temperature PA exists in equilibrium with *ca.* 7% 8'-HOABA, its metabolic precursor. Phaseic acid can be converted to 8'-HOABA by acid catalyzed ring opening: thus heating PA for 1h in glacial acetic acid at 100°C with 1 mole-equivalent of boric acid gives a mixture containing > 9:1 8'-HOABA:PA. Evaporation of the solvent traps the 8'-HOABA as a stable solid borate complex. Dissolution of the complex in water liberates the 8'-HOABA, which cyclizes to PA slowly under acidic conditions, but rapidly at neutral or basic pH. In the first tests of the biological activity of 8'-HOABA generated by this method, J. Zou *et al.* (Plant Physiol., in press) have demonstrated rapid induction of lipid and oleosin biosynthesis in microspore-derived embryos of *Brassica napus* L. cv Reston, comparable to that produced by ABA, but markedly greater than that caused by PA supplied under the same conditions.

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8'-MODIFIED ABSCISIC ACIDS AS HIGHLY POTENT, LONG-LASTING ANALOGUES OF ABSCISIC ACID

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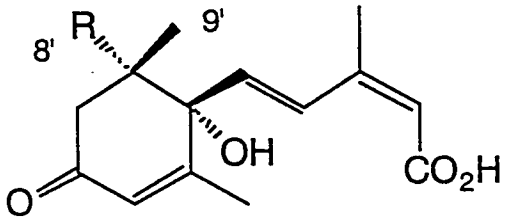
The majour pathway for abscisic acid (ABA) metabolism in plants involves enzymatic hydroxylation to unstable 8'-hydroxy-ABA, which is enzymatically or spontaneously isomerized to phaseic acid, which itself is biologically inactive. Blockage of this inactivation would result in high and long lasting biological activity. We synthesized 8',8',8'-trifluoro-ABA as an analogue resisting the hydroxylation, and 8'-methoxy-ABA as an anlogue resisting the isomerization. (+)-Trifluoro-ABA and (+)-methoxy-ABA inhibited the elongation of rice seedlings 30 and 4 times more strongly, respectively, than (+)-ABA. In causing stomatal closure, the former analogue was equally as effective as (+)-ABA, and the latter analogue was less effective than (+)-ABA. These analogues showed a good stability of the activity in the assay with rice seedlings, suggesting that these analogues resist the inactivation and keep the activity longer than (+)-ABA.



R=CH_3: (+)-ABA

R=CF_3: (+)-8',8',8'-Trifluoro-ABA

R=CH_2OCH_3: (+)-8'-Methoxy-ABA

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SYNTHESIS, BIOLOGICAL ACTIVITY AND METABOLISM OF 3'-FLUOROABSCISIC ACID

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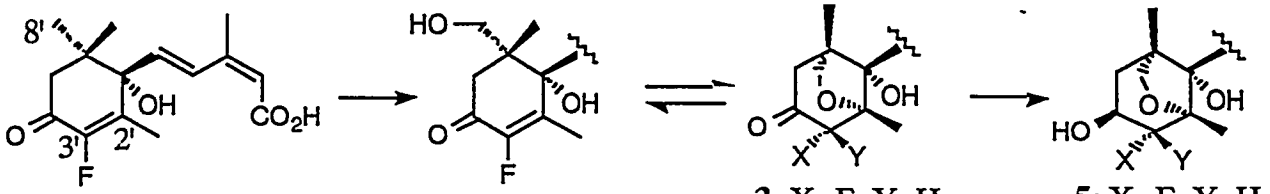
Abscisic acid (ABA) is metabolized to 8'-hydroxy-ABA which is so unstable as to be spontaneously cyclized to biologically inactive phaseic acid (PA) by the nucleophilic addition of the 8'-oxygen to the electron-deficient 2'-carbon. As an analogue that can resist the cyclization to PA, we synthesized (+)-3'-fluoro-ABA (**1**) which has higher electron density at the 2'-carbon than ABA. Inhibitory activity of **1** in lettuce seed germination and rice seedling elongation was almost equal to that of ABA. When **1** was applied to bean (*Phaseolus vurgaris*) shoots, 3'-fluoro-8'-hydroxy-ABA (**2**), 3'α- and 3'β-fluoro-PAs (**3** and **4**) and 3'α- and 3'β-fluoro-dihydro-PAs (**5** and **6**) were identified as the free metabolites. Metabolites **2**, **3** and **4** coexisted at equilibrium in the ratio of 7:6:1, while **5** and **6** were found in the ratio of 1:1. The (**2**, **3** and **4**)/(**5** and **6**) ratio was 1:1. This result indicates that **2** is more stable than 8'-hydroxy-ABA but not enough to have complete resistance to the cyclization, coinciding with the biological activity of **1**. The discrepancy between the **3**/**4** ratio (6/1) in equilibrium and the **5**/**6** ratio (1/1) suggests that the enzymatic conversion of **2** to **4** as well as the isomerization dependent on equilibrium is involved in the cyclization of **2**.



1 **2** **3**: X=F, Y=H; **4**: X=H, Y=F **5**: X=F, Y=H; **6**: X=H, Y=F

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LOW AND HIGH MOLECULAR WEIGHT CONJUGATES OF ABA IN THE CULTURE MEDIUM OF *AMARANTHUS* CELL.

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During the growth cycle of cell suspension of *Amaranthus tricolor*, ABA (detected by radio immunoassay), accumulated transiently inside the cells, reaching a maximum level after 4 days of culture. The decline might be explained by high intracellular ABA catabolism and also by an intense release into the culture medium ; in a 6 day-old cell culture, extracellular forms were quantitatively 1.5 fold more important than intracellular forms (Bianco-Trinchant *et al.*, 1994). Amongst these extracellular forms, besides free ABA and its glucose ester, conjugates non cleaved by alkaline hydrolysis could be partionned by alcoholic precipitation. ABA could be released upon enzymatic hydrolysis from high molecular weight conjugates, present in the pellet. Low molecular weight conjugates, present in the supernatant, were immunoreactive *per se*. Analysis revealed that this complex mixture was rich in xyloglucan oligosaccharides. These compounds were fractionated by ultrafiltration, with 10^4, 5.10^3,3 10^3, 10^3 molecular weight cut off membranes and separated by Bio-Gel P.2 chromatography and HPLC techniques.The only compounds which exhibited immunoreactivity were in the fraction containing xyloglucan oligosaccharides, polymerisation degree of which was from 12 to 18.

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FAST GERMINATION OF A BARLEY MUTANT IS CORRELATED WITH RAPID ABA TURNOVER

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To study the role of ABA in the germination of barley, a mutant with altered responsiveness to ABA was used. Gamma ray mutated barley (*Hordeum distichum* cv Kaya) grains were selected for an ABA insensitive mutant phenotype by testing germination in the presence of 100 μM ABA. Grains of M5 were analysed. Dissected embryos of the mutant germinated 20h faster than those of the wildtype (wt). The half maximal inhibitory concentration of ABA for germination was determined 10^{-4} M for the mutant and $5x10^{-6}$ M for the wt. To determine whether the observed difference was due to a difference in sensitivity to ABA, the ABA-induced Rab gene expression was studied in isolated embryos. The expression of these genes is specifically induced by ABA and can be used as marker for ABA response. The amount of ABA needed for the half maximal induction of Rab gene expression was $5x10^{-6}$ M in isolated embryos of both mutant and wt. This result suggested that ABA signal transduction pathways were not affected in the mutant. During imbibition of isolated embryos, the endogenous level of Rab mRNA slowly declined in wt whereas a rapid decline was observed for the mutant, since Rab expression follows the level of ABA, this indicates that the ABA levels in mutant and wt embryos were different. When isolated embryos were imbibed in water, in both mutant and wt a similar decline of ABA level in the embryo was observed, however the ABA level in the incubation medium (water) from the mutant was much lower and showed a more rapid decrease of detectable free ABA than that from the wt. Our results suggest that enhanced turnover of ABA outside the embryo is a determining factor in germination.

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CHANGES IN ENDOGENOUS CYTOKININS AND CYTOKININ OXIDASE ACTIVITY DURING PETUNIA SHOOT ORGANOGENESIS

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The biosynthesis of endogenous cytokinins and their degradation by cytokinin oxidase were measured in petunia leaves during the early developmental stages of *in vitro* shoot organogenesis. In both petunia lines (St40 and TLV1), the cytokinins iPAR, Z, ZR, and DHZ were detected at low levels before tissue culture and after 6 or 10 days in culture using HPLC and immunoassay quantification. In contrast, there were relatively high amounts of iP in uncultured petunia leaves which showed further increase during tissue culture; St40 showing a larger increase than TLV1. Cytokinin oxidase, a degradative enzyme which cleaves the N^6-saturated side chain from unsaturated isoprenoid cytokinins, was assayed in petunia leaf tissue. Cytokinin oxidase activity increased during shoot organogenesis in response to the presence of BA in tissue culture media. The preferred substrate, iP, greatly inhibited the degradation of [2,8-^{3}H]iP to adenine; t-Z was a weak inhibitor, while BA and DHZ did not inhibit degradation. The pH optimum was 8-9.5 in both petunia lines. In summary, this project has studied various aspects of cytokinin biosynthesis and metabolism during the developmental process of petunia shoot organogenesis. The most important conclusions are: 1) exogenous BA is taken up and rapidly metabolized, 2) BA and interconvertible metabolites peak and then decrease very early in shoot development, 3) endogenous cytokinin compounds can be found in leaf explants and some cytokinins increase during shoot development, 4) cytokinin oxidase activity can be found in petunia tissue, and 5) cytokinin oxidase activity increases in response to exogenous BA during organogenesis.

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IAA METABOLISM IN CYTOKININ OVERPRODUCING TRANSGENIC *N. TABACUM* PLANTS.

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Hormonal communications between shoots and roots, and various observations of the auxin and cytokinin physiology of calli, suggest that mutually regulatory interactions ocurr between these two sets of growth factors. To investigate some aspects of these interactions *in vivo*, we chose to study the influence of elevated cytokinin synthesis on IAA levels and IAA turnover in transgenic tobacco plants expressing the *ipt* gene of *Agrobacterium tumefaciens*. Endogenous IAA was quantified by LC-MS-SIM and rates of IAA synthesis, halflife and metabolic conversion were measured. In the *ipt* expressing plants we found lower IAA contents, correlating with reducded rates of IAA synthesis and IAA turnover. There was little or no difference in the profile of IAA catabolites. We also assayed activities of cytokinin oxidase, an enzyme possibly involved in cytokinin-auxin crosstalk and found altered patterns of activity. These data demonstrate interesting effects of cytokinin overproduction on both cytokinin and IAA metabolism.

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MAJOR CYTOKININS IN FRENCH BEAN: IDENTIFICATION, ORGAN DISTRIBUTION, AND REGULATION OF LEVELS BY LIGHT

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The composition of cytokinins in *Phaseolus vulgaris*, one of the best studied plants with respect to cytokinins, was re-investigated. The plant material was extracted by a procedure that ensures the inactivation of enzymes known to be able to degrade cytokinin nucleotides. A cytokinin fraction was obtained by immunoaffinity chromatography with antibodies that are able to bind isopentenyladenine, zeatin, cis-zeatin, dihydrozeatin and benzyladenine, as well as their ribosides, ribotides and 9-glucosides. The cytokinin nucleotides are present at levels much higher than nuclosides and free bases, a feature often reported for other plants. No cis-zeatin or dihydrozeatin compounds were detected, but high levels of isopentenyladenosine monophosphate, never before reported in this species, were found. Cytokinin levels were markedly higher in the stems and petioles than in the roots and leaves. Overall cytokinin levels increased with increasing light intensity, especially in the stems.

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CYTOKININ METABOLISM AND MORPHOGENESIS IN CUCUMBER SEEDLINGS

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Cytokinins are plant hormones that participate in the control of cell division and shoot organogenesis. Consequently, they have found widespread use in plant tissue culture. Cucumber hypocotyls, if cut within 5mm of the cotyledons, will generate basal adventitious shoots in response to exogenous cytokinin, whilst more basal regions produce roots and callus. The age at which the hypocotyls are cultured further influences ability to initiate shoots. There are therefore gradients of capacity to respond to cytokinin along the hypocotyl, and this can restrict efficiency of plant multiplication. The type of regeneration response may relate either to cytokinin concentration or metabolism in specific locations or to variations in sensitivity to cytokinins within the plant. We report here on changes in cytokinin concentrations in the various seedling parts during early development, and on the capacity of seedling tissues to metabolise exogenous cytokinins. Seedlings at 6 days old contain approximately 2.5 pmoles total cytokinin which is composed of zeatin, dihydrozeatin and isopentenyladenine compounds as determined by HPLC-radioimmunoassay. Feeds of [^{14}C]benzyladenine, [^{14}C]kinetin and [^{14}C]zeatin were made through roots of intact plants and the hypocotyl base of cut shoots. Analysis by HPLC-radiocounting indicated a substantial capacity to metabolise exogenous cytokinins. Principal products were ribosides, glucosides and polar compounds thought to include adenine and other products of side-chain cleavage. Up to 30 nmoles of cytokinin was metabolised in a 24 hour period, vastly in excess of the steady state endogenous concentration. Pulse-chase experiments suggest that cotyledons and the shoot apex are the principal sites of metabolism and roots are the sole site of synthesis of 7-glucosides. We conclude that these tissues have extensive capacity for metabolism and degradation of cytokinins. It remains to be seen whether the enzymes responsible are constitutive or induced by the cytokinin supply. Implications for optimisation of tissue culture systems are discussed.

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EFFECTS OF POLLINATION AND CPPU ON ENDOGENOUS CYTOKININS IN DEVELOPING FRUIT OF KIWIFRUIT (*ACTINIDIA DELICIOSA*)

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The endogenous cytokinin content of kiwifruit tissue was examined to determine the relationship between cytokinins and fruit growth. Cytokinins were extracted from fruit tissue and purified on a series of chromatography columns, resolved by HPLC, and quantified by RIA. The RIA results were validated, and the different cytokinins identified, by GC-MS. The levels of free bases, ribosides, nucleotides, O- and 9-glucosides were monitored throughout development of the control (fully pollinated) fruit. Fruit growth was manipulated by limiting pollination (to reduce seed number and therefore fruit size) and by the application of the synthetic phenylurea cytokinin N-(2-chloro-4-pyridyl)-N-phenylurea (CPPU) (to increase fruit size). Subsequent changes in endogenous cytokinins were monitored. Although unpollinated fruit (which abscised by 25 days post-anthesis) contained more cytokinin per g FW than control (pollinated) fruit when measured 10 and 20 days post-anthesis, changes in zeatin (which peaked in control fruit around 11 days post-anthesis) did not occur in unpollinated fruit. It is hypothesised than an increase in zeatin is the critical change in cytokinin metabolism required for the initiation of cell division and fruit growth. Contrary to expectation, CPPU neither increased endogenous cytokinin levels nor maintained them over time: zeatin was not detectable in the CPPU-treated fruit.

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METABOLISM OF ADENINE AND CYTOKININS IN AN APRT-DEFICIENT MUTANT OF *ARABIDOPSIS THALIANA* COMPLEMENTED BY PLANT AND BACTERIAL *APT* CODING SEQUENCES

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Formation of the riboside-5'-monophosphate is a general feature of the metabolism of cytokinins in plants. As part of a study of the biological significance of the nucleotide form of cytokinins, we have shown that the metabolism of benzyladenine is impaired in a mutant of *Arabidopsis thaliana* lacking adenine phosphoribosyltransferase (APRT) activity (Plant Physiol. 1991, 95:900-908). The mutants grow more slowly than the wild type, induce callus inefficiently and are male sterile due to aberrant pollen development, features which could result either from the absence of adenine salvage or from the reduction of the conversion of cytokinin bases to their corresponding nucleotides. Since the APRT activity produced by *E. coli* K12 does not act on cytokinin bases when assayed in vitro or in vivo, transformation experiments have been undertaken with both the *E. coli* and the plant *apt* coding sequences to investigate these two hypotheses. Heterozygous plants for APRT deficiency were transformed with the *A. thaliana apt* cDNA under control of the CAMV 35S promoter. Subsequent genetic analysis of several transformed lines indicates that the plant cDNA does complement the male sterility of the APRT-deficient mutant. The APRT-deficient mutant has also been transformed with the *E. coli apt* gene expressed from the CAMV 35S promoter. Physiological and biochemical analyses of these transformants will be presented (i.e. nondenaturing isoelectrofocusing of APRT activity, adenine and cytokinin metabolism studies).

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POTATO TUBER SHOOTS CONTAIN AN N-GLUCOSIDE OF *CIS*-ZEATIN

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The formation of N-glucosides are major metabolic fates of cytokinins like *trans*-zeatin, isopentenyladenine and dihydrozeatin in many plant materials, but have never been reported for *cis*-zeatin. This cytokinin with weak bioassay activity is found as a modified base in the tRNA of plants, but in some materials also as free *cis*-zeatin, its riboside and ribotide. Free *cis*-zeatins could conceivably arise from degradation of tRNA during extraction. We have now identified a 9-glucoside of *cis*-zeatin, which cannot have arisen this way. The compound was isolated from tuber shoots of *Solanum tuberosum* L., cv. Bintje, using immunoaffinity chromatography, with broad-affinity anti-cytokinin antibodies, and HPLC. GC-MS of the acetylated derivative showed fragments typical for zeatin and a hexose. Acid solvolysis released one moiety identical with *cis*-zeatin, and one identical with glucose. The UV spectrum was the same as the spectra for cytokinins modified at position 9 in the purine ring, and different from 2-, 3-, and 7-modified cytokinins. The presence of this N-glucoside shows that *cis*-zeatin has at least part of the pattern of metabolite formation as other cytokinins.

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CYTOKININ METABOLISM IN RELATION TO DEVELOPMENT OF PITH TISSUES IN TOBACCO STEM

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The presence of cytokinin or vascular tissue is essential for growth and differentiation of tobacco stem pith tissue in culture. Zeatin riboside (ZR) was identified as one of the principal cytokinins present in the xylem sap of tobacco (*Nicotiana rustica*). Therefore, [^{3}H]-ZR was fed to pith tissues excised from apical, middle and basal regions of tobacco stem in order to study its metabolism. The metabolites were characterized by thin layer chromatography and reverse phase-high performance liquid chromatography. After 24 hours, zeatin nucleotide (ZNT) was identified as the major metabolite of [^{3}H]-ZR in tobacco pith tissues with no clear concentration gradient along the stem. Pith tissues from middle stem had a higher proportion of ^{3}H associated with ZR (17.8%) than those from apical or basal stem. Adenine (Ade), adenosine (Ado) and adenosine 5'-monophosphate (AMP) were the minor metabolites in stem pith tissues. This is in contrast to a rapid metabolism of ZR to inactive compounds such as Ade, Ado and AMP in tobacco leaf tissue. These studies indicate that in stem pith tissue of tobacco, ZR is conserved as cytokinin nucleotides and ribosides.

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NITROGEN CONCENTRATION AND MAIZE KERNEL GROWTH AND DEVELOPMENT

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Nitrogen is essential for maximal grain yield in maize (*Zea mays* L.), but the exact physiological mechanisms involved are not well understood. Kernel size is largely determined by the number of endosperm cells and starch granules. Both parameters are determined early in grain development coinciding with high levels of zeatin (Z) and zeatin riboside (ZR). Cytokinin oxidase is responsible for kernel cytokinin metabolism. Therefore, our objective was to investigate the effect of nitrogen on cytokinin levels and metabolism and endosperm development. Maize kernels were grown *in vitro* at 25°C with 7.81 mM N (control) or with 0.00, 3.61, and 15.6 mM N. Nitrogen was supplied to media as amino acids. Kernel fresh weight analysis showed a 50% reduction for the 0.00 mM N treatment, and 20% reduction for 15.6 mM N treatment, compared to the control. No detectable differences were observed between the control and 3.61 mM N. Endosperm cell number counts revealed that at 9 DAP the control had much greater number of cells than either 0.0 mM N, 3.61, or 15.6 mM N treatments. However, by 15 DAP all three treatments had approximately the same number of cells. Cytokinin oxidase activity was determined between 7 DAP and physiological maturity. Enzyme activity in kernels from all treatments showed similar patterns of variation between 7 and 15 DAP, then decreased steadily for the control, 3.61, and 15.6 mM N treatments. In contrast, the cytokinin oxidase activity in the 0.0 mM N treatment was 30 to 45% higher than that of the control from 15 DAP to maturity. The effects of different nitrogen concentrations on cytokinin levels will also be presented. The present study suggests that absence of nitrogen in the *in vitro* medium significantly alters cytokinin metabolism in the kernel.

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ENDOGENOUS CYTOKININS IN DEVELOPING MAPLE SEEDS

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Cytokinins are known to be an important regulators of seed development. The aim of this investigation was to clarify the changes in cytokinin levels during maturation of two maple species seeds with different type of dormancy. *Acer saccharinum L.* seeds (absence of dormancy) and *Acer tataricum L.* seeds (deep dormancy) were studied. Cytokinin fractions were extracted by ethanol and butanol, purified by ion-exchange and thin-layer chromatography. Zeatin, zeatin riboside and dihydrozeatin were determined by means of high performance liquid chromatography. Zeatin riboside was dominant cytokinin in A.tataricum seeds at the beginning of development. During the maturation its level gradually decreased. Very low cytokinin levels were detected in mature A.tataricum seeds, but they were more than 3-fold higher after 3 months of stratification. To the end of stratification cytokinin content decreased again. Zeatin riboside and dihydrozeatin concentrations were found to be low at the beginning and at the end of A.saccharinum seed development. Cytokinin levels increased in the middle of maturation, when mitotic activity stopped. It was interesting that cytokinin amounts were low in both species seeds when they were ready to germinate.

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METABOLISM OF BRASSINOLIDE AND CASTASTERONE IN MUNG BEAN EXPLANT

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Metabolism of brassinolide and its biosynthetic precursor castasterone in explants of mung bean (*Vigna radiata*) was examined. Castasterone was not converted to brassinolide, but to unknown water-soluble metabolites in which major components seemed to be non-glucosidic and minor ones to be glucosidic. It is likely that castasterone is biologically active its own right without conversion to brassinolide. In constant, brassinolide was largely converted to 23-*O*-β-D-glucopyranoside. It seems that 23-*O*-glucosylation is important in deactivation of brassinosteroids in mung bean plants.

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BRASSINOSTEROID METABOLISM IN PLANT CELL SUSPENSION CULTURES

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Brassinosteroids are native plant growth regulators exhibiting a multitude of physiological activities and ubiquitous distribution in the plant kingdom. Therefore, they are considered as a new class of phytohormones. ^{3}H-labeled 24-epi-brassinolide (24-epi-BL) and 24-epi-castasterone (24-epi-CS) were used to establish the hitherto unknown pathways of brassinosteroid metabolism in cell suspension cultures of *Lycopersicon esculentum* and *Ornithopus sativus*. Altogether, 26 brassinosteroid metabolites were isolated from the cultured cells and the medium, and the structures unambigously were elucidated by NMR and MS analysis. In *L. esculentum*, both 24-epi-BL and 24-epi-CS were hydroxylated in the terminal part of the side chain at C-25 and C-26, respectively, followed by glucosidation of the newly formed hydroxyl groups, yielding novel pentahydroxylated brassinosteroid conjugates. 25-OH-24-epi-BL shows the 10fold bioactivity in the rice lamina inclination test as compared with 24-epi-BL, suggesting the 25-hydroxylation to be an activation step in the metabolism of brassinosteroids. Inversion at C-3, occurring in both cell cultures, follows a redox mechanism via 3-keto compounds, as was indicated by the presence of 3-dehydro-24-epi-CS as a metabolic intermediate in *L. esculentum*. This epimerization yielded 3,24-bisepi-CS and -BL, which are branching points in the metabolism of 24-epi-CS and 24-epi-BL in *O. sativus*. In part, these intermediates were hydroxylated at C-20, followed by side chain cleavage between C-20 and C-22, finally yielding 20-keto pregnanes. This completely elucidated metabolic sequence, resulting in the formation of pregnane type compounds from sterols, was established for the first time in a plant systems. Other portions of 3,24-bisepi-CS and -BL were conjugated with lauric acid, myristic acid, and palmitic acid, respectively, at 3β-OH in *O. sativus*. β-D-Glucosides at 2α-OH and 3β-OH of 3,24-bisepi-CS and -BL were formed in *L. esculentum*.

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GENETIC STUDIES OF AN *ARABIDOPSIS* MUTANT ALTERED IN ORGAN ELONGATION

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We are studying *gordo*, a seedling-lethal nuclear recessive mutant which may be allelic to *fass* (Torres-Ruiz and Jürgens, Development 120:2967-2978). If grown on enriched medium, all the multicellular organs of this mutant exhibit radial expansion instead of uniaxial elongation and its stunted inflorescence fails to set seed. Previous microsurgical and hormonal studies have implicated that endogenous auxin and ethylene may be the cause of *gordo*'s phenotype. *gordo* seedlings contain variable levels of free auxin, ranging from wild-type levels to six times the wild-type levels, and *gordo* seedlings evolve three times as much ethylene as wild-type seedlings on a per plant basis. *gordo*'s high levels of free auxin may be inducing its high levels of ethylene, which may, in turn, produce the *gordo* phenotype. We plan to further study the possible roles of auxin and ethylene in *gordo* by constructing double mutants with gordo and *axr1-12*, an auxin-insensitive mutant (Lincoln et al., Plant Cell 2:1071-1080), and *gordo* and *etr1-3*, an ethylene insensitive mutant (Chang et al.,Science 262:539-544). We will also examine the activity of hormone responsive promoters coupled with GUS reporter genes in *gordo* and wild-type *Arabidopsis* seedlings. We postulate that GORDO may be acting as a negative regulator to maintain wild-type auxin levels, and we hope to further describe how ethylene and auxin mantain the *gordo* phenotype by these genetic studies.

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MOLECULAR RECOGNITION OF AUXIN AND RELATED COMPOUNDS

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Molecular recognition approach in the auxin family (indol-3-ylacetic acid, IAA), the most intensively studied hormone, has been used. The final goal of the research is to offer a mechanism of hormone binding to the receptor(s). The study includes natural and synthetic analogues of alkylated and halogenated IAAs. Both, active and inactive anlogues have been included in the analysis. The problem has been tackled at three directions: to analyze the molecular geometry with the particular emphasis on the conformation, to study electrone redistribution by substitution on the phenyl moiety, and to correlate the physico-chemical parameters with bioassays. The analysis has been based on the results of X-ray structure analysis, computational chemistry, FT-IR spectroscopy and bioassays obtained on twenty compounds. In solid state folded conformation has been predominant in the most of the compounds studied. Molecular mechanics and molecular dynamics simulations recognized it as energetically favoured one. However, extended *ab initio* SCF (Ramek, Tomić & Kojić-Prodić) conformational analysis of IAA detected the planar molecule as the global energy minimum conformer. The charge distribution over indole NH has been mostly affected by the adjacent polar substituent. The results from *ab initio* calculations will be correlated with the N-H stretching frequency shift (FT-IR) of the selected derivatives in order to define the electronic properties of the pyrrole nitrogen.

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ISOLATION AND INITIAL CHARACTERIZATION OF CARROT CELL VARIANTS RESISTANT TO 2,4-D

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Eleven carrot cell lines resistant to high levels of 2,4-D were isolated. A physiological analysis showed that three of them present alteration in the efflux carrier, two in the influx carrier, two lines showed cross resistance to ABA and one showed cross resistance to both ABA and GA. Most of the lines revealed alterations in IAA conjugation mechanisms. Photoaffinity labeling with azidoIAA and azidoNPA revealed five bands in wild type cells (at 81, 58, 31, 24 and 23 KDa with azidoIAA and at 82, 49, 30, 29 and 15 KDa with azidoNPA). A similar analysis is being carried out with resistant lines.

Another approach involves the use of AFLP^{TM} (Amplified Fragment Length Polymorphism) to analyze possible differential expressions between wild-type and three resistant variants. The restriction fragments obtained by treatment with EcoRI and MseI were amplified using 16 different combinations of ^{33}P-labelled selective primers. Marked differences were found in 45 bands. The DNA eluted from those bands has been cloned and is being sequenced hoping to identify interesting genes.

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MOLECULAR CLONING AND CHARACTERIZATION OF AUXIN-BINDING PROTEIN IN *NICOTIANA TABACUM*

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Many studies have described auxin-binding sites in membrane preparations from various tissues and species. Membrane fractions from maize coleoptiles have been studied most extensively and several laboratories have characterized the major auxin-binding protein, Zm-ERabp1. Convincing evidences from electrophysiological studies have suggested that Zm-ERabp1 plays an important role in auxin perception, implying a critical function of this auxin binding protein at the external face of the plasma membrane and suggesting an interaction of this soluble protein with a docking transmembrane protein. Auxin induces early variations of the transmembrane electrical potential of tobacco protoplasts. It has been demonstrated that Zm-ERabp1 and the corresponding antibodies were able to modify the sensitivity of this response to auxin. To make further progress in our understanding of the auxin perception at the plasma membrane, we have tried to isolate auxin-binding proteins in tobacco. We have recently isolated two different tobacco cDNAs encoding proteins of 188 aminoacids showing 65% identity with Zm-ERabp1. Sequence analyses demonstrated that particular regions of Zm-ERabp1 are highly conserved in tobacco such as the A and B domains, the three cystein residues and the C-terminus KDEL sequence. The expression levels of Nt-ERabp1 and Nt-ERabp2 were determined in various tissues of tobacco plants. The specific mRNAs of these two genes exhibit very low accumulation levels, in accordance with the extremely low amount of the corresponding proteins in tobacco. The highest expression was found for Nt-ERabp1 in flowers. *E. coli* -produced and purified Nt-ERabp1 was used to prepare specific monoclonal antibodies. We already characterized several antibodies with auxin antagonist effect on the electrical response and one monoclonal antibody with agonist activity as it was described for the D16 antibodies. The production of the protein in eucaryotic expression system is in progress; the identification of the plasma membrane protein interacting with Nt-ERabp is under investigation.

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GENETIC STUDIES OF ETHYLENE SIGNAL-TRANSDUCTION IN *ARABIDOPSIS THALIANA*

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We are characterizing ethylene-insensitive mutants to study ethylene signal-transduction in *Arabidopsis thaliana*. A number of independently isolated mutant lines were classified into five allelic groups. The results of complementation tests on recessive mutants indicate that they are allelic either to the *EIN2* locus[(1)] or the *AIN1* locus[(2)]. Three dominant mutations were found to be not allelic to each other by allelism tests. One of them was allelic to *etr1-1*[(3,4)], and the other two, designated as *etr2-1* and *etr3-1* respectively, represent two new loci. The *etr2-1* mutant mapped to the same region on the top of chromosome 3 as the Q8 gene, a homologue of *ETR1*[(5)]. Sequence analysis of the Q8 gene from the *etr2-1* line revealed a single amino acid change in the hydrophobic domain of the gene[(6)]. Specific characteristics of individual mutant lines were studied by generating ethylene dose-response curves of seedling-growth. All alleles of *AIN1* showed a partial sensitivity to ethylene in the seedling-growth response. Gene dosage effects were characterized for the dominate mutations by constructing triploid lines (Wt/Wt/mt). Results indicate that increased dosage of wild-type *ETR1* can partially overcome the dominate effects of *etr1-1*. Double mutants of *etr2-1/ctr1-2*[(7)](constitutive triple response mutant) showed the constitutive ethylene response phenotype, while double mutants of *ctr1-2/ain1* showed a partial constitutive ethylene response phenotype. A model is presented for the possible functional relationships between these genes in the ethylene signal-transduction pathway.

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IDENTIFICATION OF NOVEL PROTEINS IN THE ETHYLENE SIGNAL TRANSDUCTION PATHWAY

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CTR1 is a gene encoding for a product involved in the ethylene signal transduction pathway in *Arabidopsis*. We are employing molecular approaches to identify novel elements using the *CTR1* gene as a starting point. Here we describe the use of the yeast two-hybrid interaction system to identify proteins that interact with CTR1. This system employs two plasmid constructs, the first being a "bait" plasmid containing a lexA DNA-binding domain fused to the gene of interest, and the second being a Gal4 activation domain fused to a library of cDNA clones. Positive interactions in this system are identified by transcription of two reporter genes. We have constructed bait plasmids containing different domains of *CTR1*. We have identified a number of cDNA clones that interact with these baits and they are being characterized.

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DIMERIZATION OF THE ETHYLENE-RESPONSE MEDIATOR ETR1 FROM *ARABIDOPSIS*

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Mutations in the *ETR1* gene of the higher plant *Arabidopsis* confer insensitivity to ethylene, indicating a role for the gene product in ethylene signal perception and transduction. The *ETR1* gene product has an aminoterminal hydrophobic domain and a carboxyterminal domain showing homology to the two-component signal-transduction proteins of bacteria. We report here that in both its native *Arabidopsis* and when transgenically expressed in yeast, the ETR1 protein is isolated from membranes as a dimer of 147 kD. Treatment with the reducing agent dithiothreitol converted the dimer to a monomer of 79 kD, indicative of a disulfide linkage between monomers. Expression of truncated versions of ETR1 in yeast confirmed that the high-molecular-mass form is a homodimer, and demonstrated that the aminoterminal region of ETR1 is necessary and sufficient for this dimerization. Site-directed mutagenesis of two cysteines near the aminoterminus of ETR1 prevented formation of the covalently linked dimer in yeast, consistent with a role in disulfide bond formation. These data indicate that ETR1 may use a dimeric mechanism of signal transduction in a manner similar to its bacterial counterparts, but with the addional feature of a disulfide bond between monomers.

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ETHYLENE SIGNAL TRANSDUCTION

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Ethylene signal transduction is likely to consist of constitutively expressed cellular factors which transduce the ethylene signal into a transcriptional response and components whose expression is hormonally regulated. Several constitutively present, early components in ethylene signal transduction have been identified by mutants isolated in *Arabidopsis thaliana* with the use of a simple screen, based on the classic ethylene response in seedlings known as triple response. In contrast, much less is known about the hormonally regulated components of this transduction pathway. This poster shows the differential display of eukaryotic mRNA to be a powerful method to investigate the hormonally regulated part of a signal transduction pathway. We demonstrated the reproducibility of the differential display and showed that low abundant mRNAs are detected as well. Since [^{35}S]dATP, commonly used to label the PCR products in the differential display, is volatilized at the high temperature used in PCR leading to a contamination of the thermal cycler and the environment, we demonstrated that different ways of labeling with ^{32}P instead of ^{35}S led to the same pattern of differentially expressed bands without radioactive contamination. We are using the differential display to isolate ethylene-regulated genes in *Arabidopsis thaliana* (Columbia). Several putative ethylene-regulated genes were identified. So far, four genes are shown to be differentially expressed on Northern blots. The cDNA of a gene whose transcription is repressed in the presence of ethylene contains a putative nuclear localization signal indicating that this gene might encode a nuclear protein involved in the downregulation of ethylene responses.

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REGULATION OF ETHYLENE BIOSYNTHESIS IN *ARABIDOPSIS* ETHYLENE OVERPRODUCING MUTANTS

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We used the seedling triple response to identify five mutants affected in ethylene production in *Arabidopsis*. These mutants, *eto* 1 through *eto* 5 (ethylene overproducing), define elements involved in the signal transduction pathway of ethylene biosynthesis. Eto mutant seedlings produce from 10 to 100-fold more ethylene than wild-type seedlings. We used gas chromatography to measure ethylene production by wild-type and Eto mutants at several developmental stages and to evaluate the spatial and temporal regulation of ethylene biosynthesis. By evaluating double Eto mutants and Eto/Ein (ethylene insensitive) double mutants we have begun to characterize the regulatory interactions between the mutations and the role of feedback in the regulation of ethylene production in *Arabidopsis* seedlings.

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PROTEIN KINASES EXPRESSED IN OAT ALEURONE CELLS.

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Aleurone cells of the cereal grain are induced by exogenously applied gibberellin (GA) to synthesize and secrete a range of hydrolytic enzymes, including α-amylase. It is our aim to understand the mechanisms by which GA controls both transcription and translation. Since protein phosphorylation is a mechanism often used to regulate such processes in eukaryotic cells, a study was initiated to investigate the protein kinases that are expressed in aleurone cells of germinating grain. PCR primers were designed to conserved regions of the protein kinase catalytic domain. Fragments from ten independent protein kinases were amplified from cDNA derived from GA_1-treated aleurone layers. Full length cDNA clones corresponding to two of these were isolated and sequenced fully. Clone Asmap1 encodes an kinase with a high degree of similarity to mitogen-activated protein (MAP) kinases. MAP kinases are central components of many signal transduction pathways in yeast and mammalian cells, linking extracellular signals to changes in gene expression. Clone Aspk11 showed homology to the mammalian ribosomal S6 protein kinase ($pp70^{S6K}$) and to a novel ribosomal protein kinase recently isolated from *A rabidopsis*. Transcripts hybridising to this kinase were markedly up-regulated by the treatment of aleurone cells with GA_1, while those hybridising to the MAP kinase increased in abundance in the untreated cells and were rapidly down-regulated by GA_1. Transcriptional regulation of Asmap1 and Aspk11 by GA may be indicative of these protein kinases having a role in GA signal transduction; this is currently under investigation.

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Gα AND β SUBUNIT GENES AND SIGNAL TRANSDUCTION IN *A. FATUA* ALEURONE

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Heterotrimeric GTP-binding proteins (G-proteins) are important components of signalling pathways in mammalian cells and there is now good evidence that this class of signal-transducing protein exists and functions in higher plants. This research project aims to identify and examine the role of G-proteins in aleurone cells. Gα subunit and Gβ subunit cDNAs were cloned by PCR and found to possess considerable nucleotide and deduced amino acid sequence similarity to known G protein subunits. Steady-state levels of poly $(A)^+$ RNA isolated from aleurone incubated for between 1 and 5 days with $1x10^{-7}M$ GA_4 were studied by northern analysis. A 700 bp Gα and a 430 bp Gβ PCR product were used as probes and revealed low-level expression of mRNAs which was unaffected by length of incubation. Mastoparan, pertussis toxin (PTX) and cholera toxin (CTX) are useful probes to study G-protein function. In this study, an analogue of mastoparan, Mas 7, stimulated α-amylase production in isolated aleurone protoplasts of *Avena fatua* in a dose-dependent manner similar to GA_4. Mas CP, a non-amphiphilic analogue differing from Mas 7 by a single amino acid, was ineffective in stimulating α-amylase. PTX and CTX at 1-2 μg/ml partially inhibited the response of aleurone protoplasts to Mas 7. However, they did not appear to have any effect on the response to GA_4. GA-induction of α-amylase is overcome by abscisic acid (ABA). This inhibition could be partly overcome by PTX and CTX. These data suggest the involvement of PTX- and CTX-sensitive G-protein(s) in both Mas 7 stimulation of α-amylase gene expression, and ABA inhibition of GA-induced α-amylase in aleurone and the existence of toxin-insensitive G-proteins or alternative signalling pathways initiated by GA.

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A RICE LEAF PROTEIN THAT CAN BIND TO GIBBERELLIN IS PHOSPHORYLATED

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A gibberellin A_1 (GA_1)-binding protein was identified from rice (*Oryza sativa* L. cv. Nipponbare) leaves by a ligand binding assay. The dissociation constant of GA-binding protein and GA complex was about 100 nM. This protein had a relative molecular mass of 47,000 and an isoelectric point of 5.1. The partial amino acid sequence of the protein was determined from both the N-terminal and internal regions. There were neither intra- or inter-disulfide bonds nor N-linked oligosaccharide chain in the molecule. The protein was localized in the cytosol fractions of leaves. The protein was immunologically detected as two polypeptides, which were relative molecular masses of 47,000 and 43,000, in the protein extracts from leaves. When protein extracts from the leaves of rice grown in the presence of exogenous GA were reacted with Ca^{2+}, Mg^{2+}, [γ-^{32}P]ATP and histone III-S, the protein kinase activity increased. In addition, the GA-binding protein identified was phosphorylated with Ca^{2+}, Mg^{2+} and [γ-^{32}P]ATP in the leaf protein extracts of rice grown in the presence of exogenous GA.

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GIBBERELLIN BINDING PROTEINS IN ALEURONE AND LEAF PLASMA MEMBRANE.

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Oat aleurone cells are a model system for studying the regulation of gene expression by the plant hormones gibberellin (GA) and abscisic acid (ABA). This poster will describe research that aims to characterise potential components of signal transduction pathways that link perception of GA with the transcription of α-amylase genes. The GA photoaffinity probe, [^{125}I]GA_4-17-SE-*p*-ASA, (Hooley *et al.*,1993 Aust.J.Bot. 20, 573) has been used to identify putative GA binding proteins in high purity plasma membrane isolated from aleurone cells and protoplasts of *Avena fatua* using the method devised by Walker *et al.*,1993 Physiol.Plant. 89, 388. *In vitro* photoaffinity labelling of aleurone plasma membrane gave high specific activity labelling of two polypeptides. Similar sized polypeptides were GA-photoaffinity labelled in plasma membrane isolated leaf tissue. Biologically active GA_4 and GA_1 competed for these labelling events. When plasma membrane was isolated from aleurone protoplasts following *in vivo* GA-photoaffinity labelling two different polypeptides were identified. Preliminary experiments have identified [^{3}H]GA_4-binding in oat aleurone plasma membrane.

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CHARACTERIZATION OF HIGH AFFINITY GIBBERELLIN-BINDING PROTEINS IN AZUKI HYPOCOTYLS

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Many interesting proteins which interact with plant hormones such as auxin and cytokinin have been isolated as membrane-bound proteins or buffer-soluble proteins. However, such binding proteins for gibberellins (GAs) have not been characterized in as much detail. We detected binding activity for tritiated GA_4 in a cytosolic crude fraction from *Azukia angularis* seedlings. An unequilibrated gel-filtration technique was used for the detection of activity that was abolished by a large excess of non-tritiated GA_4.

The GA_4-binding activity was retained on a hydrophobic interaction gel matrix under high salt conditions. On the other hand, it passed through an anion exchange gel matrix with the starting buffer containing 50mM NaCl. The dissociation constant value for GA_4 was measured by the above-mentioned techniques, and was approximately 10(-9)M. The isolation of the protein and inhibition tests with other GAs are now in progress.

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IDENTIFICATION OF GA-INDUCED GLYCOPROTEINS FROM *AVENA FATUA* ALEURONE

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Avena fatua aleurone is highly responsive to GA, setting into motion the production and secretion of a number of well characterised hydrolytic enzymes. This phenotypic response typifies the developmental change associated with exposure to this hormone. Our interest centres on the initial perception of GA and the early events which follow detection. A number of investigations suggest the existence of a plasma membrane receptor for GA which to date has not been identified. Our research aims to identify membrane glycoproteins that are induced by GA in advance of the production of the hydrolytic enzymes. We therefore labelled *de novo* synthesised proteins with ^{35}S-methionine in imbibed aleurone layers in the presence and absence of GA over a time period of 7 to 66 hours. The secreted proteins were not apparent until after 48 hours exposure to GA. Crude membrane preparations from 18 to 48 hour GA-treated aleurone layers were solubilised with detergent and subjected to lectin affinity chromatography. A number of GA-induced glycoproteins were identified. Concanavalin A bound a 70KDa polypeptide, wheat germ lectin a 58KDa polypeptide and peanut lectin a 58KDa and a 20KDa polypeptide. Scale-up preparations are underway in order to subject the proteins to microsequence analysis.

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REGULATION OF GA-BINDING PROTEINS ON DWARFISM OF RICE (*ORYZA SATIVA* L.)

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The levels of endogenous hormones (GA and ABA) in rice seedlings (7d) were measured by gas-liquid chromatography with flame ionization detector and the GA-binding proteins on membrane of young rice shoot were anylysed as described by Chiharu. The content of endogenous GA in semidwarf indica rice variety Guangchang' ai with sd-1 gene was about 80% of that in tall variety Baiyexian. The levels of endogenous GA in semidwarf varieties of Xinggui' ai having sd-g gene and Shuang' ai having both sd-1 and sd- g genes were respectively 2-fold and 1.8-fold as high as those in Baiyexian. The specific GA-binding proteins on membrane parts in tall variety Baiyexian was as high as 5754 dpm/100 mg proteins, accounting for 23.90% of the total binding proteins. The membrane-associated GA-binding proteins of Guangchang' ai, Xingui' ai and shuang' ai were much lwoer than those of Baiyexian. It was suggested that the dwarfism of rice may be determined not only by the content of endogenous GA, but also by the amount of GA-binding proteins.

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ANTI-IDIOTYPIC ANTIBODIES AS A TOOL FOR IMMUNODETECTION OF ABA RECEPTORS.

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According to Jerne's theory, anti-idiotypic antibodies are probes able to bind specifically to antigen receptors. We immunized Balb c mice with monoclonal anti-ABA antibodies conjugated with hemocyanin (KLH) or cross-linked via glutaraldehyde. Anti-idiotypic antibodies were detected by ELISA in the sera of 8 mice. In the serum of one of them, antibodies were also able to bind very efficiently onto rapeseed (*Brassica napus* L.) microsomal proteins adsorbed on microtitration plates. On western-blots from SDS-PAGE electrophoresis of these proteins, one band of about 20 Kd was found. Work is in progress in order to characterize and to purify the specifically-labelled rapeseed microsomal protein detected and to test its possible role as ABA-receptor.

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USE OF XENOPUS OOCYTE mRNA INJECTION SYSTEM FOR THE EXPRESSION OF PLANT HORMONE RECEPTORS

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In animal systems, several neurotransmitters interact with plasma membrane receptors to activate phospholipase C which results in the generation of IP_3 followed by release of Ca from internal stores. The *Xenopus* oocyte/mRNA injection system has and continues to be critical to the characterization and cloning of cDNAs for various animal neurotransmitter receptors. We believe that *Xenopus* oocyte/mRNA injection system can also facilitate the characterization and cloning of cDNAs for plant hormone receptors. The first plant hormone receptor chosen for study using this system is the ABA receptor. ABA has been shown to stimulate Ca release and there is evidence that its receptor is located in the plasma membrane. The methods used for this study include isolation of mRNA; injection of *Xenopus* oocytes and the two-electrode voltage clamp technique. When injected oocytes are monitored at a holding potential of -70 mV water injected oocytes gave no response to ABA [10^{-5} M] however, plant mRNA injected oocytes responded with inward currents similar to that observed from oocytes injected with cRNA for a neurotransmitter receptor. Other experiments are in progress to ensure that the response is due to the interaction of ABA with its receptor. This research is funded by NASA/NSF Joint Program in Plant Biology.

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IMMUNOLOGICAL DETECTION OF ABA-BINDING PROTEINS IN PLANTS

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ABA is found in all higher and lower plants, cyanobacteria, and fungi. The ubiquitous occurrence of ABA suggests the existence of an ABA receptor. We tested this notion with western blot analyses of plant material derived from dicots and monocots using anti-idiotypic antibodies which were amplified with alkaline phosphatase. Western blots showed the existence of ABA receptor-like proteins in barley aleurone cells and barley coleoptiles, corn roots and coleoptiles, sunflower roots, whole flax seedlings, watermelon hypocotyls, *Tradescantia* spec. leaves, inner epidermis of onion, whole *Arabidopsis* seedlings, and roots of *Phaseolus vulgaris*. Preincubation of the blots with ABA reduced binding of the anti-Idiotypic antibody in a concentration dependent manner but preincubation in IAA or GA_3 had no effect. The western blots also showed substantial variation in the concentration of the ABA-binding protein between different tissues and species. High concentration were generally found in leaf material, possibly due to the important role of ABA in the regulation of stomatal opening. Low concentrations of the ABA binding protein were detected in corn roots and watermelon hypocotyls. Our anti-idiotypic antibodies mimicked ABA by inhibiting the opening of stomata in excised epidermal peels of *Setcresea purpurea*. The data suggest that ABA binding proteins are located on the plasma membrane.

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ANTIBODIES AGAINST ABA—BINDING PROTEINS CAN INHIBIT EFFECT OF ABA ON ISOCITRIC ACID DEHYDROGENASE ACTIVITY IN MITOCHONDRIA OF MAIZE COLEOPTILES

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Isocitric acid dehydrogenase (ICDH), one of the important enzymes in Krebs Cycle, could be increased in activity by treating the isolated mitochondria from maize coleoptiles with (+)ABA. It implys that a certain binding site which can recognize ABA molecule may exist in mitochondria. For making sure of it, two kinds of polyclonal antibodies (PAb I and PAb II) against ABA—binding proteins fished out and purified with ABA—c_1 or ABA—c_4—BSA—Sepharose 4B columns have been respectively prepared (Zhou & Xia, et al, 1993) and then the PAb I was used in following experiment. After being isolated from maize coleoptiles grown in dark at 26℃ for 5 days. the mitochondria were treated with (+)ABA (range: 0—200 μmol/L), then a rise in ICDH activity could be obviously detected. When the mitochondria were incubated with PAb I (normal rabbit sera as control) for 15 minutes before treated with ABA, the increasing degrees of ICDH activity were decreased by 95%, 50% and 35% for 10, 100 and 200 mol/L ABA respectively. The results indicated that ABA effect on ICDH activity is mediated by ABA binding sites located in mitochondria.

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ZEATIN-BINDING PROTEIN INVOLVED IN TRANSCRIPTION REGULATION

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The aim of investigation was to isolate a receptor for *trans*-zeatin (tZ). 67±2 kDa protein was isolated from barley leaf cytosol by consequent purification including affinity chromatography on tZ-Sepharose. The protein was recognized by anti-idiotype antibodies from antiserum to tZ. It bound reversibly and specifically ^{3}H-tZ. In presence of tZ, tZ-binding protein (tZBP) activated transcription elongation in the system containing chromatin associated with RNA-polymerase I from barley leaves. Effect was strongly dependent on tZ concentration (maximum activation at 10^{-8} M). *Cis*-zeatin was not active. tZBP in concert with tZ activated RNA synthesis by RNA-polymerase I and RNA-polymerase II in nuclei isolated from barley leaves. This effect corresponded to cytokinin activation of RNA synthesis in intact leaves. 67±2 kDa tZBP fits to all requirements for cytokinin receptor in leaf cells. Our work was supported by RFFR, Grant 93-04-6881 and ISF, Grant MDJ000.

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SEPARATION OF A HIGH-AFFINITY CYTOKININ-SPECIFIC BINDING PROTEIN (CSBP) BY SPECIFIC AFFINITY CHROMATOGRAPHY

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We had confirmed the presence of a soluble high-affinity cytokinin-specific binding protein (CSBP), a plausible cytokinin receptor, in etiolated seedlings of mung bean (*Vigna radiata* Wilczek). Various cytokinins showed specific and mutually competitive binding activity to CSBP. The association constant (K_a) of CSBP with a very strong synthetic urea-type cytokinin, *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea (4PU30, forchlorfenuron), was 4×10^{10} M^{-1}, and the concentration of CSBP was equivalent to 3,000 molecules per cell (R. N. *et al.*, *B. B. R. C.*, 191, 543, 1993). We partially purified CSBP up to 100,000-fold, with 7 % of the yield, from the whole-cell extract. The purification procedure consisted of four chromatographic steps. At the first step, we utilized a Q Sepharose column. The fraction, which showed the specific binding activity to 4PU30, was applied to a *N*-phenyl-*N'*-(4-pyridyl)urea-linked Sepharose (4PU Sepharose) column, and the activity was eluted with 1 μM of zeatin. After zeatin was extensively removed from the active fraction with DCC and Sephadex G25, the fraction was applied again to a 4PU Sepharose column, and the activity was eluted with 1 μM of 4PU30. After 4PU30 was removed, the fraction was applied to an acetamide-linked Sepharose column, and the binding activity was eluted with the salt gradient. At this final purification step, the active fraction had specific and competitive binding activity to zeatin with the same affinity as 4PU30, and to benzyladenine with one-eighth of this affinity. The K_a of the activity in the fraction for 4PU30 was measured at 1×10^{11} M^{-1}. These properties of the binding activity was suggested to be identical with CSBP. The apparent molecular weight of CSBP in the fraction was 43 kDa on SDS-PAGE. Toward amino acid-sequencing, mass purification of CSBP is in progress.

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IDENTIFICATION OF A CYTOKININ-BINDING PROTEIN FROM PEA THYLAKOID MEMBRANES BY PHOTOAFFINITY LABELING WITH AN AZIDOCYTOKININ AGONIST

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Although cytokinins are involved in the regulation of a variety of physiological processes such as organogenesis, release of apical dominance, chloroplast differentiation, delay of senescence etc., little is known about their mode of action at the molecular level. Identification of cytokinin receptors is an essential step to understand the mechanism of cytokinin action in plants. So far, several cytokinin-binding proteins have been described, on the basis of specific and noncovalent binding of radioactive cytokinins. We have synthesized tritiated 1-(6-azido-2-chloropyrid-4-yl)-3-phenylurea (azidoCPPU) to search for such cytokinin-binding proteins by the technique of photoaffinity labeling. The use of this probe allowed us to specifically photolabel a 46 kDa peptide from pea chloroplasts, called Thy46. This peptide, localized in the thylakoid membrane of the chloroplasts, is also present in other plant species. A pharmacological study of the interaction of the peptide with different cytokinin agonist or antagonist molecules has been done. Molecules of the urea class are the most efficient competitors of the photolabelling and the efficiency follows the cytokinin activity of the molecules. The relative dissociation constant of CPPU towards the 46kDa peptide was estimated to be 0.5 μM. Purine derivatives are weaker competitors than urea derivatives, but the efficiency of the competition is also correlated with their cytokinin activity. Purification of Thy46 will be presented along with the partial amino acid sequences obtained.

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TOWARDS CLONING THE FUSICOCCIN-BINDING PROTEIN

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The fungal phytotoxin fusicoccin (FC) induces an immediate and strong increase of proton-motive force across the plasma membrane of all higher plants tested so far. The H^+-translocating ATPase providing the electrochemical gradient for secondary transport across the cell surface membrane is considered as ultimate target of FC-action. Physiological effects of the toxin appear to require the presence of a high affinity FC-binding protein (FCBP) which seems to be a common element in the plasma membrane of higher plants. The FCBP was purified from plasma membranes of the monocotyledonous plant *Commelina communis* as a complex with a radiolabeled FC-derivative. The preparation consisted of two polypeptides with apparent molecular masses of 30.5 and 31.5 kDa. Internal amino acid sequence information as well as immunological studies proved that both polypeptides are members of a eukaryotic family of conserved regulatory or signal transducing proteins known as 14-3-3 proteins. We therefore conclude that the FCBP is an oligomer composed of 14-3-3 homologs. In that it is a plasma membrane protein displaying characteristics of a receptor the FCBP is unique among known 14-3-3 proteins. Applying temperature-induced Triton X-114 phase separation 14-3-3 isoforms constituting the oligomeric FCBP are distinguishable from other cellular 14-3-3 homologs by their strong apparent hydrophobicity. The structural basis for this hydrophobical behaviour is under investigation. Results concerning the molecular cloning and functional analysis of the 14-3-3 isoforms comprising the FCBP will be presented.

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A BRASSINOSTEROID-INSENSITIVE MUTANT IN *ARABIDOPSIS THALIANA*

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Brassinosteroids are plant growth-promoting natural products with structural similarities to insect and mammalian steroid hormones. The wide distribution of brassinosteroids in the plant kingdom, their marked effect on cell proliferation and elongation at nanomolar levels, and their interactions with other plant hormones, suggest that these compounds play a role as endogenous plant-growth regulators. In order to investigate brassinosteroids as signals influencing plant growth and development, we have identified mutants in *Arabidopsis thaliana* that are insensitive to brassinosteroid action. Inhibition of root elongation by exogenous hormones is a common response in wild-type *Arabidopsis* seedlings, with auxin, ethylene, cytokinin and methyl jasmonate all showing this effect. We found that 10^{-7} M 24-epibrassinolide (a synthetic brassinosteroid) caused approximately 60% inhibition of primary root elongation in the Columbia ecotype of *Arabidopsis*. EMS-mutagenized seeds of *Arabidopsis* were screened for their ability to elongate roots in the presence of this concentration of brassinosteroid. One such mutant, BRI-1, has an extremely dwarf phenotype, with loss of apical dominance and almost complete male sterility. Based on the root elongation inhibition assay, BRI-1 is insensitive to brassinosteroids but retains sensitivity to auxins, cytokinins and gibberellins. Properties of BRI-1 and its use in dissecting the mechanism of brassinosteroid action will be discussed.

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CHARACTERIZATION OF FLORAL ORGAN ABSCISSION IN ARABIDOPSIS THALIANA

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A genetic approach is being developed to identify genes involved in processes controlling petal and sepal abscission in Arabidopsis. Abscission is the detachment of organs from the main body of the plant, and can be recognized as an important developmental program. Mutant plant populations generated by ems mutagenesis, T DNA insertion, and transposon tagging have been screened for delayed or accelerated abscission. Putative mutants are being characterized both anatomically and genetically. We propose four classes of mutants in respect to altered patterns of abscission. Class I mutants fail to develop an abscission zone; Class II mutants develop a normal abscission zone but display delays in development of abscission; Class III mutants initially develop a normal abscission zone but do not display further development, and Class IV mutants display ectopic cell separation. We will present preliminary characterization of mutants in these four classes, and a model for development and regulation of floral organ abscission.

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THE STUDY OF A NEW PLANT REGULATOR---OBRASSINOLIDE

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Brassinosterone, a precursor in the synthesis of brassinolide, exhibited more than 70% of the biological activity of brassinolide in rice test. Brassino-oxime, a product of condensation of brassinosterone with carboxymethoxylamine hydrochloride, showed 60% of that of brassinolide in the same test system at 10 umol/L concentration. In cucumbetr hypocotyl test. the brassino-oxime promoted elongation of cucumber hypocotyl by 20% as compared with control. The brassino-oxime was found to be competitive with brassinolide or brassinosterone. Combination of iodohistamine with brassino-oxime by Chloramine-T method formed the ^{125}I-labelled compound which was used for cucumber and rice tests. The results showed that the Rf 0.93 fraction of chromatography was effective in promoting both elongation and inclination significantly. The Rf 0.93 was an effective one. Its specific radioactivity was 73.8 GBq/mmol.Different fractions of mung bean epicotyl extract were tested for brassinosterone binding. The 10 000xg fraction showed stronger brassinosterone binding than other fractions. The brassinosterone binding to 10 000xg fractionm was saturated after 1 h incubation. The binding was temperature-dependent, showing a maximum at 4 °C. The amount of brassinosterone bound is dependent on pH, the optimal pH being 7.5. Trypsin treatment weakened the brassinlsterone binding. This result indicates that protein may be involved in the brassinosterone-binding site. Several analogues of brassinostertone could also bind to the brassinosterone-binding site to various extents.

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HORMONAL REGULATION OF CELL DIFFERENTIATION AND GROWTH IN ARABIDOPSIS THALIAN

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Arabidopsis Thaliana has been used to investigate the role of epibrassinolide in cell differentiation and regeneration. The results show that calli cultured on MS medium supplemented with 0.05 or 0.5 mg/L epibrassinolide and 0.1 mg/L KT formed green buds and shoots on the surface of all calli. The formation of green calli and the regeneration could be induce on medium supplemented with epiBR alone, but KT did not exhibit induction activity without epiBR. In addition ,we have found that calli induced by epiBR was different in protein SDS-PAGE patterns from those of control. Electron microscopic examination shows that relatively large amounts of starch grains with abnormal shape were contained in cell of calli cultured on medium with epiBR. However, the formation of green calli and the regeneration were inhibited when calli were transferred onto the medium supplemented with more than 5 mg/L epiBR. In order to elucidate the the mode of action of brassinolide as well as the relationship between BR and other plant hormone ,mutants resistant to the brassinolide have recently been isolated by soaking seeds in a solution of ethyl methane sulfonate. The results indicated that these mutants could germinate on medium with high concentration of BR. The plants of BR-insensitive mutants have abnormal root and leaf morphology as well as altered flower structure. None of these mutants have been characterized biochemically. Now further investigation is in progress to understanding the molecular basis of action of brassinolide.

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MONOCLONAL ANTIBODIES GENERATED AGAINST PEA GUARD CELLS THAT MODULATE ABA RESPONSES IN BARLEY ALEURONE PROTOPLASTS

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Both stomatal guard cells and those of the cereal aleurone layer represent target cells for ABA, in which ABA treatment results in an alteration of the cytosolic calcium content and the expression of ABA-regulated genes. We have generated a panel of monoclonal antibodies (MAbs) against guard cell protoplasts from the *argenteum* mutant of pea, *Pisum sativum* L., some of which have been characterised in terms of their cognate antigens (Knox *et al.* Planta, *in press*). JIM18 recognises a plasma membrane glycolipid antigen whose expression appears to be developmentally regulated. JIM19 and JIM20 both recognise glycoprotein epitopes which are detectable on the inner face of epidermal cells, but not those of the stomatal complexes, in epidermal peels of both pea and *Commelina communis* L. However, treatment of *Commelina* epidermis with cell wall-degrading enzymes exposes the JIM19 epitope. More than 50 of the MAbs have been assayed for their ability to modulate ABA-induced *RAB-16* gene expression in aleurone protoplasts of barley, *Hordeum vulgare* L. (Wang *et al.* Planta, *in press*). Co-incubation of protoplasts with ABA and five of the MAbs, JIM19,-91,-92,-93 and -94 inhibited ABA-induced *RAB-16* mRNA accumulation, whereas treatment with JIM90 enhanced it. The effects of JIM19 were specific, as there were no effects on the expression of a constitutive gene and only a slight effect on GA-induced amylase production. Both the *Commelina*-immunolabelling and the anti-ABA activity of JIM19 co-purified. The effects of JIM19 were concentration-dependent and could be counteracted by increased concentrations of ABA. JIM19 recognised a number of antigens on Western blots of aleurone protoplasts.

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SIMILAR EFFECTS OF CYTOKININS AND BLUE LIGHT ON GROWTH INHIBITION AND PROMOTION OF GTP BINDING IN *ARABIDOPSIS THALIAÑA*

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Cytokinins and continuous illumination with low fluence of blue light caused a similar significant inhibition of growth of *Arabidopsis* seedlings. The signaling mechanism that inhibits seedling growth is not clear. In animal cells, heterotrimeric G-proteins direct the flow of signals from the receptor to the rest of the cell. In order to assess whether GTP binding protein is involved in this process, we employed polyclonal antibodies that were raised against a decapeptide from the C-terminus of the α subunit of the G-protein transducin. Following the solubilization of the membrane fraction, we immunodetected a protein with a molecular mass of 36 kD (p36). Membranal proteins were adsorbed to DEAE-Sephacel anion exchange column and eluted by increasing concetnration of salt. Determination of GTP $\gamma^{35}S$ to the individual fractions revealed that a peak of high binding coeluted with the immunoreactive p36 fraction. The influence of several known hormonal and environmental effects have been studied. IAA, at physiological concentrations, had no effect of GTP binding. In contrast, similar promotive effects of GTP binding were found following treatment with either the plant hormone benzyl-adenine or blue light irradiation. Coincidentally with the promotion of GTP binding, cytokinins and blue light caused the mentioned significant inhibition of *Arabidopsis* growth. It is speculated that G-protein is involved and may initiate the signal transduction that leads to the physiological response of growth inhibition.

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PROBING CYTOKININ ACTION - I

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The moss *Physcomitrella patens* develops from spores as multicellular filaments. In response to cytokinin a percentage of side-branch initials (sbi) develop into multicellular leafy shoots (gametophores). It has been suggested that the calcium/InsP3 pathway is required for the transduction of the cytokinin signal (Saunders, M.J. In: Cytokinins: Chemistry, Activity, and Function, ed. by D.W.S. Mok and M.C. Mok. London: CRC Press, pp. 243-254, 1994). In order to test this hypothesis the wild type moss and a number of hormonal mutants have been (i) transformed with the cDNA for apoaequorin (ii) imaged using the calcium dyes Indo-1 and calcium-green (iii) analysed using ionophores. The transformed moss responds to cold-shock and any perturbation such as shaking or the addition of solutions by an increase in cytosolic calcium measured as aequorin luminescence. Calcium channel inhibitors have been used in order to ascertain the source of the calcium increases. Lanthanum chloride alters the response to cold-shock, causing a reduced but lengthened response in the wild type moss. In one mutant, lanthanum causes a dramatic increase in the cold shock response. No evidence has been obtained of a change in calcium in response to cytokinin. As cytokinin diffuses freely across moss membranes, this suggests that a rise in calcium is not associated with the entry of cytokinin into cells *per se*. The addition of ionophore to light-grown moss increases chloronemal side-branching. When added to moss filaments grown in the dark the ionophore mimics the effect of light in causing unbranched filaments to produce side-branch initials. This suggests that an increase in calcium is necessary for the production of side-branch initials but not for the formation of gametophores. Furthermore, time-lapse microscopy has been used to elucidate the first stages in cytokinin action and the calcium dyes indo-1 and calcium green have been microinjected into moss filament cells to obtain images of calcium distribution.

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CAGED PLANT HORMONES

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In recent years we have developed a number of plant hormone derivatives which were specifically designed to probe sites of hormone action at the cellular level. These include the membrane impermeant gibberellin analogues GA_4-Sepharose and GA_4-17-sulphonic acid, which show GA bioactivity for α-amylase induction in *Avena fatua* aleurone protoplasts, indicating that sites of GA-action in these cells are located at the plasma membrane [Hooley,R et al, Planta, 1991,183,274; Beale et al, Physiol Plant., 1992,85, A24]. We have now developed caged plant hormone as potential probes for intracellular sites of hormone action. Caged hormones are based on photocleavable *ortho*-nitrobenzyl esters. The preparation and properties of caged GA_4, ABA, NAA, JA and salicylic acid will be described. Analogous *para*-nitrobenzyl esters of the plant hormones are not photocleavable and thus are useful for control experiments. Photorelease of caged-ABA after microinjection into guard cells of *Commelina communis* caused the cells to close. In cells from plants grown at 25°C or above this closure was associated with an increase in cytosolic concentration of calcium ions as measured by Calcium Green-1 fluorescence [Allan et al, Plant Cell, 1994,6,1319]. These results suggest a possible intracellular site for ABA perception in this system.

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CALCIUM-BASED TRANSDUCTION PATHWAYS IN THE WHEAT ALEURONE: GIBBERELLIC ACID AND ANOXIA.

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Aleurone cells respond to both environmental and hormonal stimuli with characteristic and distinct changes in gene expression and cell fate. In order to understand the role of calcium in mediating these different transduction pathways, we have examined the response of wheat aleurone cells to gibberellic acid (GA) and anoxia. Gibberellic acid induces the production of α-amylase and other hydrolases and accelerates cell death, whereas anoxia induces the expression of alcohol dehydrogenase (ADH) and other proteins that promote the survival of cells under unfavorable environmental conditions. We have measured changes in intracellular Ca^{2+} using the fluorescent dye fluo-3 in wheat aleurone cells after stimulation by GA and anoxia. In separate experiments, we have measured message levels of α-amylase and of ADH. We have found that both GA and anoxia rapidly induce changes in cytosolic Ca^{2+}. Although the Ca^{2+} changes induced by GA require extracellular Ca^{2+}, those induced by anoxia do not. Although the timing and magnitude of the calcium change is similar for both stimuli the pathways can be distinguished by okadaic acid (OA), a protein phosphatase inhibitor. OA blocks GA-induced gene expression, Ca^{2+} changes and accelerated cell death. In contrast, OA has no effect on Ca^{2+} changes or gene expression induced by anoxia. These data indicate that there are multiple Ca^{2+}-based signal transduction pathways in aleurone cells and support the idea that multiple cytoplasmic mediators are necessary for GA signal transduction.

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INVOLVEMENT OF CALCIUM AND HETEROTRIMERIC G PROTEINS IN A NOVEL CLASS OF CYTOKININ RESPONSE MUTANTS, THE *ZEA* MUTANTS OF *NICOTIANA PLUMBAGINIFOLIA*

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Zea mutants were screened from EMS mutagenized lines on the basis of growth on high zeatin concentrations. On such concentrations wild type cotyledon development are inhibited, whereas *Zea* mutants show an important cotyledon and hypocotyl hypertrophy. We have shown that this cotyledon hypertrophy was highly specific to the presence of cytokinins. It could not be promoted by other hormones or inactive forms of cytokinins (Nogué et al. 1995 Plant Growth Reg. in press). This hypertrophy was linked to an accumulation of sugars, amino acids and organic acids as well as a deregulation of some light regulated genes from carbon and nitrogen metabolisms (Faure et al. Plant J 1994 5,4, 481-491).

In order to understand the nature of this cytokinin response, we have tried to identify the elements involved in cytokinin response pathway which could be affected in the *Zea* mutants. Preliminary evidence was obtained suggesting the involvement of calcium and calmoduline in the cytokinin response in the mutants either by their effects on seedlings development or by their effects on the expression of some cytokinin regulated genes. As animal calcium channels are mostly regulated by heterotrimeric G proteins, we also analyzed the pattern of different Gα isoforms in presence or absence of cytokinin. Two different isoforms were found to be regulated by cytokinins in the wild type and always absent in one mutant.

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THE RELATIONSHIP BETWEEN IAA, CALCIUM, AND PLASMA MEMBRANE H^+_ATPase ACTIVITY IN WHEAT COLEOPTILE

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Calcium (Ca^{2+}) is involved in numerous events during the life cycle of plants. The cytoplasmic free calcium may control regulatory systems for cellular responses to hormonal or environmental signals. In this study , wheat coleoptile segments were treated with 10 μM IAA and 1% sucrose, then the plasma membrane was prepared and the H^+_ATPase activity was examined with presence of different concentrations of $CaCl_2$ or EGTA or calcium channel blockers($LaCl_3$ or Verpamil). The results indicated that none of the tested componds had a significant effect on IAA activated plasma membrane H^+_ATPase activites in *vitro*. However, when the plasma membrane was prepared after the wheat coleoptile segments were treated with different concentrations of EGTA or calcium channel blockers, the results showed that both EDTA and calcium channel blockers inhibited IAA activated plasma membrane H+_ATPase activities in *vivo*. Further more, the inhibition by calcium channel blockers could be relieved by Ca^{2+} ionophore A23187. This suggested that calcium may involved in the auxin signal transduction pathway by changing the calcium level in cytosol; and calcium channel played a pivotal regulatory role in this process.

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CALCIUM-DEPENDENT PROTEIN KINASES FROM RICE LEAVES

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In plant cells, intracellular signal transduction may possibly regulate cell functions. Komatsu and Hirano (1993) reported protein kinase activity to increase during early seedling growth in rice (Oryza Sativa L.). Ca^{2+}-dependent protein kinases in rice leaves were partially purified by sequential chromatography and identified. These protein kinases phosphorylated the substrate, histone III-S, in the presence of Ca^{2+} with or without phosphatidylserine. The half-maximum concentration of Ca^{2+} for inducing protein kinase activity was 1 μM. The apparent molecular mass of Ca^{2+}-dependent protein kinase (CDPK) and Ca^{2+}- and phosphatidylserine-dependent protein kinase (Ca^{2+}/PS PK) were 45 kDa and 50 kDa, respectively, as determined by phosphorylation in SDS-polyacrylamide gel containing histone III-S. Ca^{2+}/PS PK differed from CDPK in rice leaves in that Ca^{2+}/PS PK had phospholipids dependency and the molecular mass of Ca^{2+}/PS PK exceeded that of CDPK. Examination was made of changes in CDPK and Ca^{2+}/PS PK activity in cytosol and membrane fractions during germination. The maximum activity of Ca^{2+}/PS PK in the cytosol fraction was at the time prior to immersion whereas the maximum activity of CDPK in the membrane fraction was noted at 6 days following immersion. The data suggest these protein kinases have some roles in the regulation of plant differentiation and growth through a signal transduction pathway.

Reference: S. Komatsu and H. Hirano, Plant Science, **94**, 127-137, 1993

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CALRETICULIN IN PLANTS: ADHESION AND SIGNAL TRANSDUCTION

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We are examining adhesion between the plasma membrane and cell wall in plants. Adhesion may play fundamental roles in gravitropism, pollination, response to stress, and development of polarity. An adhesive system would link the intracellular cytoskeleton to the extracellular wall, passing through plasma membrane receptors, providing not only structural links between the cells of a plant but also serving as a signal perception and transduction system. The specific aim is to examine one class of these *bona fide* receptors, integrins, and their putative second messenger molecule, calreticulin, which may transduce signals to nuclear transcription factors. Calreticulin is an intracellular Ca^{2+}-binding protein and contains C-terminal sequences (KDEL and HDEL) indicative of endoplasmic reticulum retention. However, by immunocytology, calreticulin is found in non-plant systems to localize to focal adhesion sites and also can be found in the nucleus. The species we study are the glycophyte *Arabidopsis thaliana* and the halophyte *Mesembryanthemum crystallinum*: the former due to its role as a model genetic system and the rapidly increasing availability of adhesion related genes and the latter due to the extremely strong adhesion which has been observed between the plasma membrane and cell wall of its NaCl-storing bladder cell. The root hairs of *Mesembryanthemum* also display strong adhesion in comparison to root hairs of glycophytes. We are utilizing cloned calreticulin cDNAs and anti-calreticulin antibodies to determine 1) the existence of integrin based adhesion and signal transduction in plants and 2) the physiological and developmental processes in plants in which integrins and attendant molecules would have a role. We are using immunocytology to answer structural/location questions concerning the pathway and anti-sense and constitutive expression constructs to answer functionality questions. However, interest is not only in the stress responses of these species: the analysis is utilizing transgenic *Arabidopsis* plants to examine the role of calreticulin in the putative integrin-based adhesion system involved in gravitropic response, pollination, and more fundamentally, plant development.

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CHIMERIC Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE IN PLANTS

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Calcium, a universal second messenger, regulates diverse cellular processes in eukaryotes. Changes in intracellular Ca^{2+} concentration can affect a number of physiological processes through the action of calmodulin (CaM), a ubiquitous Ca^{2+}-binding protein. Ca^{2+}/calmodulin-regulated protein phosphorylation is believed to play a pivotal role in amplifying and diversifying the action of Ca^{2+}-mediated signals. A chimeric Ca^{2+}/calmodulin-dependent protein kinase (CCaMK) gene with a novel visinin-like Ca^{2+}-binding domain was recently cloned and characterized (S. Patil, D. Takezawa, and B.W. Poovaiah, Proc. Natl. Acad. Sci., in press). CCaMK is a novel protein characterized by the presence of a catalytic domain, a CaM-binding domain and a visinin-like calcium-binding domain in a single polypeptide. CCaMK autophosphorylates in a Ca^{2+}-dependent manner and the Ca^{2+}-dependent autophosphorylation is decreased by the addition of calmodulin. In the presence of Ca^{2+}/calmodulin, CCaMK phosphorylates histones, syntide and myelin basic protein. In the absence of calmodulin, CCaMK can phosphorylate actin in a Ca^{2+}-dependent manner. To dissect the functions of each regulatory domain, deletion and point mutants of CCaMK are being used. The deletion of the visinin-like domain from CCaMK inhibits Ca^{2+}-dependent autophosphorylation. Site-directed mutants have been created to study the role of each of the three Ca^{2+}-binding sites of the visinin-like domain. The structural features and the biochemical properties suggest that CCaMK has dual regulatory controls and may play a unique role in Ca^{2+} signaling in plants.

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IDENTIFICATION AND PRELIMINARY CHARACTERISATION OF INOSITOL 1,4,5-TRISPHOSPHATE RECEPTORS FROM *CHENOPODIUM RUBRUM*

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Compelling evidence now exists which supports a role for the phophoinositide transduction pathway in signal-response coupling in higher plants. Inositol 1,4,5- trisphosphate ($InsP_3$) has been shown to increase in response to environmental stimuli such as light, abscisic acid and phytoalexins. $InsP_3$ elicits Ca^{2+} release from tonoplast vesicles and isolated vacuoles and electrophysiological measurements have confirmed the presence of $InsP_3$-gated Ca^{2+} channels in tonoplast from red beet storage root. These studies strongly suggest that $InsP_3$ receptors should exist in higher plants but to date, only a single report, of a high affinity binding site for $InsP_3$ in a detergent-solubilised membrane preparation from red beet, demonstrates that they do. Using a radioligand receptor assay, we have characterised two, distinct, high affinity $InsP_3$ binding sites in a membrane vesicle preparation from *Chenopodium rubrum*. These putative receptors both exhibit a Ca^{2+} dependence but differ in respect to pH optimum, response to other divalent cations and reside on membranes equilibrating at different densities in sucrose gradients. Displacement assays indicate similar Kd's, in the 200 - 500 nM range, and specificity for $InsP_3$ above other inositol phosphates. This work was partly supported by the grant No. 204/93/0633 from the Grant Agency of the Czech Republic.

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1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID STIMULATES POLLEN GERMINATION BY INOSITOL-1,4,5-TRISPHOSPHATE

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Pollen was collected from 5 species of plants -Cereis chinensis Bunge, Primus triloba Lindl, Sophora japonica L., Cucumis sativus L. and Amelianchier sinica (Schnied) Chun, and sowed in germination medium. The percentage of germinated ungerminated pollen grains was determined at 0.5,1,2 and 3 hours after sowing. In order to clarify whether a change in the free Ca^{2+} concentration and IP_3 signal messenger in the cytoplasm were involved in pollen germination, we monitored the steady-state Ca^{2+} levels with a new generation of Ca^{2+} indicator, fura-2-AM, loaded into pollen. The fluorescence of fura-2 was detected at 450 nm with alternative excitation at 340 nm and 370 nm using SPEX F212 Fluorolog. ACC, the immediate precursor of ethylene, stimulated pollen germination in vitro in all five species of plants tested. EGTA and phenothiazine inhibited the effects of increased germination rate by ACC. At the same time, it was observed that the Ca^{2+} levels in germinated and ungerminated pollen were 287 nM and 136 nM, respectively. When pollen was incubated in 2.5 mM ACC, however, the cytoplasmic Ca^{2+} levels were eleved to 450 nM. During incubation in germination medium containing 2.5 mM ACC and IP_3 3.6uM or LiCl 1 mM, large change in the $[Ca^{2+}]_c$ could be induced. With 3.6 uM IP_3, the Ca^{2+} level in the cytoplasm was 850 nM, and pollen germination was stimulated very rapidly. In the case of Li^+, an inhibitor of inositol monophosphatase, it was 155 nM, hence the ACC-stimulated pollen germination was inhibited. These results suggest an important function of ACC stimulating pollen germination in the control of flowering apart from its role in senescence of flower. The mechanism may be due to the rise in pollen $[Ca^{2+}]_c$ induced by IP_3. This also furnishes the first direct evidence for the involvement IP_3 as a second messenger in pollen germination.

This work was supported by the NSFC and the laboratory of Plant Physiology and Biochemistry, Beijing Agricultural University.

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THE EFFECTS OF 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID AND INOSITOL-1,4,5-TRISPHOSPHATE ON CYTOPLASMIC CALCIUM CONCENTRATION AND ROSE PETAL DEVELOPMENT.

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Protoplasts from rose (Rosa chinensis Jack cv zirong) petals at different developmental stages were loaded with fura-2-AM to measure the concentration of free $[Ca^{2+}]_c$ in the cytoplasm. The fluorescence of fura-2 was detected at 450nm using a Spex F212 fluorolog and with excitation at either 340nm or 370nm. The calcium concentration was calculated by means of the intensity ratio $R=I_{340}/I_{370}$. The results indicated that the concentration of cytosolic free Ca^{2+} at the bud, opening, withering and senescence stages were 8, 62, 12 and 15nM respectively. When cut flowers were treated with 1 mM ACC, $[Ca^{2+}]$ rapidly increased to 108 nM together with the ahead opening of flowers. Thus, the opening of the flowers appears correlated with a rise of $[Ca^{2+}]_c$. No change occurs in the low resting value of $[Ca^{2+}]$ during senescence, although the measured membrane fluidity is decreased at this period. The decline in membrane fluidity is a symptom of senescence. This suggests that unlike those rapid physiological responses which occurred over only a few minutes, the senescence is sustained for a long time. The rise in $[Ca^{2+}]_c$ may occur only at the onset of the senescence and quickly return to its resting value. When the protoplasts were treated with 3.6 uM IP_3 the $[Ca^{2+}]_c$ is decreased, especially at opening stage, but this kind of Ca^{2+} release can be inhibited by introduced La^{3+}, a potent blocker of plasma membrane calcium channels. Hence it seems that IP_3 can affect $[Ca^{2+} + Mg^{2+}]$-ATPase activity in the plasma membrane. Thus the turnover of phospholipid may play a central role in the controlling calcium homeostasis.

This work was supported by the NSFC and the laboratory of Plant Physiology and Biochemistry, Beijing Agricultural University.

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WOUNDING PROMOTION AND LANOLIN INHIBITION OF DIFFERENTIATION OF CAMBIAL CELLS INTO TRACHEARY ELEMENTS IN RED PINE (*Pinus resinosa* Ait.)

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Mature intact needles on branch cuttings of conifers promote cells of the vascular cambium to differentiate directly into tracheary elements. The differentiation response is localized to the junction of the needle trace with the cambium, and the tracheary elements typically appear opaque in unstained section and similar in appearance to primary-xylem elements; hence, they are readily distinguished from the neighbouring latewood tracheids of the secondary xylem. In pines, when the needles are removed by excision through the prophyll sheath, the tracheid-differentiation response does not occur; however, xylogenesis has been induced by applying inositol 1,4,5-trisphosphate (IP_3, bovine) to the needle-pair stump (Int. J. Plant Sci. 15:272-290, 1994). In the present work, it was observed that the tracheid-differentiation response at the trace-cambium junction occurred when the base of the needle-pair stump was punctured through the subtending bract into the short shoot with a 27G ½" needle (Becton Dickinson 305109). The response appeared to be qualitative in that none of the control (unwounded) stumps generated new tracheids whereas every wounded stump promoted a tracheid-differentiation response. The wound-induced tracheids were found both above and below the trace-cambium junction and were indistinguishable from those induced by an intact needle pair. Hence, it appears that a wounded short shoot, independently of its needles, generates a transmissible signal extending into and along the cambium and that this wound signal is sufficient for induction of cellular differentiation. No cell type other than tracheids has been observed to differentiate in response to the treatment. Plausibly, short-shoot wounding promotes or releases IP_3; this remains to be investigated. Lanolin (Fisher L7-500) applied to the apical ends of needle-pair stumps at the time of puncturing their short shoots completely eliminated the wound-induced tracheid-differentiation response. Lanolin has often been used as a carrier for topical application of phytohormones, but this research indicates that lanolin (mainly lanosterol and cholesterol) contains one or more components having a regulatory effect on plant cellular differentiation.

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AUXIN-INDUCED ACIDIFICATION OF THE CYTOPLASM IS A LIGHT-DEPENDENT PROCESS IN *AVENA* COLEOPTILE CELLS

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There have been a number of reports that auxins cause cytoplasmic acidification. We have examined this in *Avena* coleoptile cells by use of the pH-sensitive dye SNARF and either 2-wavelength photometry or confocal microscopy. By ratioing the fluorescence of SNARF an approximate pH can be obtained. In the dark, no auxin caused any change in cytoplasmic pH. In the light, there was a rapid and extensive acidification of the cytoplasm when the auxin was α-NAA, but not with 2,4-D and only slightly with IAA. The NAA-induced acidification was inhibited by the antiauxin PCIB and by TIBA. Neither FC nor ABA altered the cytoplasmic pH. Since growth is induced by auxin in the dark, we conclude that the cytoplasmic acidification is an auxin response unrelated to the induction of cell elongation.

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INDOLEACETIC ACID MODULATES INWARD K^+ CHANNELS AND THE PROTON PUMP IN ELONGATING *AVENA SATIVA* COLEOPTILES.

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IAA (10 μM) initiates a rapid biphasic change in the plasma membrane potential of elongating subepidermal cells of etiolated oat coleoptiles. The membrane begins to depolarize within 2 min, followed after 5-8 min by a gradual repolarization to a stable V_m 25-40 mV more negative than the original voltage. When $[K^+]_o$ was increased above 0.1 mM, the plasma membrane immediately depolarized by about 30 mV per decade; whereas when $[Ca^{++}]_o$ was raised, V_m became less responsive to changes in $[K^+]_o$ and slowly hyperpolarized. The auxin-induced changes in V_m were less at elevated $[K^+]_o$ but were restored by raising $[Ca^{++}]_o$. Our observations are consistent with the hypothesis that auxin affects both passive and active ion transporters — first, auxin increases the depolarizing influx of K^+ through inward K^+ channels, and later, auxin also stimulates active proton secretion. Elevated $[Ca^{++}]_i$ restores auxin-induced hyperpolarization in higher $[K^+]_o$ by reducing passive influx of K^+. The degree to which the plasma membrane hyperpolarizes in auxin depends on the extent to which passive currents returning into the cell balance active proton secretion.

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FLUXES OF PROTONS AND CALCIUM FROM OAT COLEOPTILES AND PROTOPLASTS INDUCED BY FUSICOCCIN AND AUXIN

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The mechanism of auxin mediated growth includes some effect of protons which are extruded through the plasma membrane. These protons acidify the cell wall, but the process by which the lowered wall pH causes the wall to become loosened is not yet clear. Application of the fungal toxin fusicoccin (FC) causes an immediate efflux of protons from the cell and an immediate growth stimulation. The application of IAA causes a proton efflux and growth stimulation both with a lag of about 10 minutes. For both FC and IAA we have observed exchanges of protons for Ca^{2+} in the wall (Arif & Newman, 1993 *Planta* **189**, 377-383). Current work is being undertaken, with greater sensitivity, to clarify the relationship between the proton and Ca^{2+} fluxes and their timing after the application of IAA. Fluxes from protoplasts are also being studied in order to distinguish between effects of IAA on the plasma membrane and effects on the cell wall. Results of this work will be presented.
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Characterization of the electrical response of *Avena sativa* L. coleoptile cortex cells to auxin treatment.

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Current goals of auxin physiology include identifying rapid electrical events in the plasma membrane and establishing their roles in the rapid enhancement of growth by auxin. The electrical response to auxin perfusion by cortex tissue of intact *Avena* coleoptile cortex tissue was examined using microelectrode measurements of the membrane potential. Applied at 10 μM, known auxins (IAA, αNAA, 2,4-D, and IBA), 2 anologues (βNAA and 2,3-D), and acetic acid transiently depolarized the potential (1-30 min) but only active auxins subsequently hyperpolarized the cells, possibly by increasing activity of the PM H^+ ATPase. The magnitude and duration of the depolarizations induced by the different compounds varied. Alpha NAA induced two depolarizations. The first reached a maximum within 2 to 3 minutes and the second between 6 and 10 minutes. The magnitude of the first αNAA depolarization varied with external concentration of Cl^- suggesting that this depolarization represents an efflux of Cl^-. Applied at 10 μM, the naturally occurring auxin, IAA, induced a single depolarization (maximal after 6-8 minutes) which was insensitive to external Cl^- but increased with lowered external pH. Applied at 100 μM, however, an early, Cl^- sensitive, depolarization was also evident. It is suggested that: (1) the putative Cl^- currents induced by αNAA and IAA are not essential for auxin induced growth and (2) all the compounds tested may act as weak acid proton ionophores.

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SPECIFICITY AND ACTION OF CYTOKININS IN *MEDICAGO SATIVA* ROOT HAIRS.

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Early events in the cytokinin signal transduction pathway involve membrane transport at the plasma membrane (PM). We have tested the importance of membrane transport in the root hairs of *Medicago sativa* L. Root hairs are ideally suited for the study of signaling, because they are both cytokinin-responsive and individually assayable cells within an intact tissue. Activity of cytokinins was confirmed by their ability to increase the growth rate of individual root hairs as measured by time-lapse video microscopy. Incubation with micromolar solutions of cytokinins caused a rapid hyperpolarization (<5 min) of the PM. The hyperpolarization of the PM was most reproducible with kinetin, which caused a -16 mV hyperpolarization of the PM at 3μM, and only -5.3 mV with 300 nM. *Medicago* seedlings grown in the presence of cytokinins for 24 h showed reduced growth of the primary root, increased root hair length, and initiation of the root hairs closer to the root tip. Primary root growth was reduced 9%, 30%, 39%, and 63% as compared to the control when grown in 30 μM of the cytokinins adenine, kinetin, isopentyladenine, and 6-benzyladenine, respectively. Our data indicate changes in Ψ_m of the PM correlate with cytokinin activity, and support the importance of membrane transport in cytokinin signaling.

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REGULATION OF CELL-FREE LIPID RELEASE FROM ENDOPLASMIC RETICULUM AND THE EFFECTS OF PLANT GROWTH SUBSTANCES.

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Membrane lipids are transported from sites of synthesis to target membranes to replace damaged lipids and to make membrane expansion possible during for example chloroplast development (lipid transport from the envelope to the thylakoid) and cell expansion (lipid transport from the endoplasmic reticulum to e.g. the plasma membrane and the tonoplast). Vesicular-mediated lipid transport from endoplasmic reticulum to other membranes of plant cells has been reconstituted in cell-free systems. The results showed that transport to the Golgi apparatus (Morré et al., 1991. Protoplasma 160:49-54) and the plasma membrane (Harryson et al., 1990. *In* Plant Lipid Biochemistry, Structure and Utilization [PC Quinn & JL Harwood, eds] pp 257-259, Portland Press Limited) were differently regulated. The first step of cell-free lipid transfer from the endoplasmic reticulum has been separately investigated. The release of [^{14}C]-labelled lipids, presumably as vesicles, was found to depend on soluble proteins (cytosol) and to be stimulated by acyl-CoAs (Sandelius & Räntfors, 1995. *In*: Plant Lipid Metabolism [J-C Kader & P Mazliak, eds] pp 210-212, Kluwer Academic Publ.). However, the release of [^{3}H]PI was unaffected by cytosol and inhibited by acyl-CoA indicating that lipids were sorted at the endoplasmic reticulum prior to release. Since several growth substances are involved in the regulation of cell, and consequently membrane, expansion, their effects were studied on cell-free lipid transfer. Preliminary data suggest that IAA, together with conditions which allow phospholipid synthesis, stimulates phospholipid release from isolated endoplasmic reticulum. Supported by the Swedish Natural Science Research Council.

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CALMODULIN LEVELS AND A PUTATIVE CAM- AND CYCLIC-NUCLEOTIDE-REGULATED ION CHANNEL IN BARLEY ALEURONE.

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Abscisic acid (ABA) and gibberellic acid (GA) regulate the synthesis and secretion of hydrolytic enzymes in the barley aleurone cell. An early event following exposure of aleurone cells to GA is a rise in cytoplasmic $[Ca^{2+}]_i$ to about 200-300 nM, a response that is dependent on an external supply of millimolar amounts of Ca^{2+}. ABA, which antagonizes the effects of GA on enzyme synthesis and secretion, resets cytoplasmic Ca^{2+} to resting levels of about 50-100 nM. Cytosolic Ca^{2+} interacts with Ca^{2+} binding proteins such as calmodulin (CaM) to regulate the activity of target proteins in the aleurone cell. Biochemical and biophysical approaches have identified a Ca^{2+}-ATPase on the endoplasmic reticulum and a K^+ channel in the protein body vacuole whose activities are regulated by Ca-CaM. We have determined the CaM steady state mRNA levels as well the CaM protein levels in barley aleurone cells after treatment with GA and ABA, and these data will be presented. We used a molecular approach to search for putative Ca-CaM regulated proteins. Expression cDNA libraries (made from barley aleurone mRNA) were screened with CaM conjugated to horse radish peroxidase. Seven positive clones were isolated that bound CaM in a Ca^{2+}-dependent manner. Clone CBP-13 (CaM binding protein C13) shows high homology at the amino acid level to olfactory channels and the *Arabidopsis* KAT1 potassium channel. The full length cDNA clone (2.7 kbp) has six characteristic transmembrane spanning domains. The carboxy terminus of CBP-13 has the conserved amino acids present in cyclic nucleotide binding domains, moreover, there is an amphiphilic helix downstream of the cNMP binding site, which has features of a CaM binding site. When the carboxy terminus of CBP-C13 is expressed in *E.coli*, the polypeptide binds CaM. Genomic Southern blots and screening of genomic libraries indicate that CBP-13 belongs to a multigene family. Nothern blots show that steady state mRNA levels of CBP-C13 increase in ABA treated aleurone layers. Experiments are underway to express CBP-C13 in *Xenopus* oocytes.

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GIBBERELLIC ACID AND ABSCISIC ACID REGULATE THE VACUOLAR pH OF BARLEY ALEURONE CELLS

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Aleurone tissue in the grain responds to gibberellic acid (GA) with the synthesis and secretion of hydrolases; abscisic acid (ABA) antagonizes this response. Barley aleurone cells contain prominent storage protein vacuoles (SPV) which provide the amino acids for the *de novo* synthesis of secreted hydrolases. Hydrolysis of storage proteins in the SPV is facilitated by proteases that have acidic pH optima; therefore changing the pH of the SPV would alter hydrolytic activity in this compartment. We have investigated vacuolar pH using BCECF, a pH-sensitive fluorescent dye, and fluorescence microscopy with ratio imaging analysis. BCECF can be loaded into the SPV of aleurone protoplasts non-invasively and does not alter the response of aleurone cells to GA, permitting the kinetics of pH change in response to hormone treatment to be measured in living cells. By comparing fluorescence signal from SPV in protoplasts with calibration curves generated both *in vitro* and *in vivo* using isolated SPV, accurate determinations of pH can be made. The pH of SPV in protoplasts not treated with GA is near 7, whereas vacuoles of cells pre-incubated in ABA have a higher pH. Treatment with GA causes a lowering of vacuolar pH to 6 or below. Data on the kinetics of this response and on the mechanism of acidification will be presented. This research supported by grants from the National Science Foundation and the National Research Initiative of the USDA.

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ANALYSIS OF A GUARD CELL cDNA LIBRARY.

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Guard cells are highly specialised sensory and effector cells whose function is to optimise gas exchange at the surface of the leaf. This involves their regulated swelling and shrinking in response to a variety of stimuli such as light, CO_2 and the hormone abscisic acid (ABA). The volume changes involved are brought about by the osmotic flux of water across the vacuolar and plasma membranes along osmotic gradients rapidly established by trans-membrane K^+ fluxes. Thus guard cells represent an excellent system for studying the processes of signal-response coupling, as it is likely they contain photoreceptors, ABA receptors, ion channel proteins and various components of signalling systems such as protein kinases and phosphatases, G-proteins and Ca^{2+}-binding proteins. We have designed a technique for isolating guard cells based on the arrested enzymatic digestion of epidermal strips of the pea mutant *Argenteum* and have constructed a guard cell library in λZAPII. This library is currently being screened and a number of strategies will be discussed. These include differential screening to isolate guard cell specific clones, reverse transcription-PCR using degenerate oligonucleotides and screening with a variety of heterologous probes representing components of the signalling pathway such as G-proteins, K^+ channels and the *ABI1* gene.

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THE EFFECT OF ABSCISIC ACID ON THE FLUIDITY AND SURFACE ELECTROSTATIC POTENTIAL OF MITOCHONDRIAL MEMBRANE

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So far, only few papers deal with the effect of ABA on biomembrane. Our data show that some biophysical parameters in plant mitochondrial membrane were significantly altered under the influence of ABA. The thermogram of mitochondria isolated from etiolated shoot of maize grown at 26℃ was determined with differential scanning calorimetor (DSC). It was found that an endothermal peak with range from 15℃ to 32℃ occured in the thermogram of untreated mitochondria. After ABA (38μmol/L) treatment during washing step, the peak was obviously changed into the range between 11—26℃. It indicated that the phase transition temperature of mitochondrial membrane could be reduced distinctly by ABA. The microvisicosity (η) of mitochondria was measured with the fluorescent polarization technique (FPT) and a fluorescent indicator, 1, 6—diphenyl — 1, 3, 5—hexatriene (DPH). As ABA concentration increased from 1μmol/L to 10μmol/L, the η (poise) decreased distinctly from 1.68 to 1.57. Using mitochondria from maize grown at 16℃, the η values measured at pH5.0 and pH7.2 were reduced for 7.16% and 14.46% respectively by 50μmol/L ABA. Both of results obtained with DSC and FPT indicated that the fluidity of mitochondria membrane could be obviously increased by ABA. The surface electrostatic potential (SEP) of mitochondria was measured with neutral red method. The SEP values of both soybean and maize were altered from —37.5mv to —22.5mv and from —60mv to —31.5mv respectively with 40μmol/L ABA. ABA effect on the activities of Na^+—K^+ATPase, succinate dehydrogenase and isocitrate dehydrogenase in mitochondria would also be reported.

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EFFECT OF BRASSINOSTEROIDS ON THE PLASMA MEMBRANE H^+-ATPase IN CORN SEEDLING ROOTS

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Brassinosteroids which are considered as a new class of phytohormones can influence the H^+-pump function in plasma membranes of shoot and root cells. It was demonstrated by an electrode technique that 24-epibrassinolide (EB) at growth-activating concentrations promoted H^+ efflux and K^+ influx in roots. We investigated EB effect on the H^+-pump mechanism which is representated with the plasma membrane H^+-ATPase. It was found that EB didn't influenced it in vivo but increased its activity and K^+-stimulation in vitro at seeds soaking, roots exposition, and shoot sprincling. EB effects on molecular characteristics of the enzyme including pH-optimum, kinetic parametres, and sensitivity to inhibitors and the activator have been studied. Transduction of EB signal on the H^+-ATPase protein is under discussion.

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THE EFFECT OF T-DNA GENES ON HORMONE BALANCE AND DIFFERENTIATION - STUDIES ON SILVER BIRCH

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The *Agrobacterium* T-DNA contains genes affecting both the auxin and cytokinin balance of the host plant cells either by creating new biosynthetic pathways or by changing the sensitivity to the endogenous phytohormones. When expressed in the host plant, these genes cause well known symptoms of the crown gall and hairy root diseases. The aim of the present study was to investigate the effect of T-DNA genes on the IAA and IPA levels, as well as on the differentiation ability of silver birch (*Betula pendula* Roth.) tissues. The ELISA based IAA and IPA measurements were performed from tissues transformed with *A. rhizogenes* strain R1600 or with *A. tumefaciens* strain A281 growing either on growth regulator free media or on media containing thidiazuron (TDZ). As controls, samples were measured also from untransformed silver birch calli on TDZ-media. When the IAA: IPA ratios were examined, no correlation could be found with shoot formation ability of the tissues. The IPA levels were, however, on an average higher in the differentiating tissues than in the callusing tissues. Exogenous cytokinin supply seems to decrease endogenous IPA biosynthesis, even if the tissue contains the IPA producing cytokinin gene (*ipt*) from *A. tumefaciens*. As a conclusion, the effect of phytohormones on the differentiation of the silver birch tissues is a complex phenomenon, and can not be explained barely by the IAA:IPA ratio. Corcerning the transferred T-DNA genes, the *ipt* gene seems to be functional in silver birch tissues based on the high IPA levels found. Further on, if exogenous cytokinin is provided, the transferred bacterial cytokinin gene may be either depressed or even deleted.

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ALTERED GROWTH AND WOOD CHARACTERISTICS IN TRANSGENIC HYBRID ASPEN (*POPULUS TREMULA* X *P. TREMULOIDES*)

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The effect of the plant hormone indole-3-acetic acid (IAA) on plant growth and development was studied in transgenic hybrid aspen (*Populus tremula* x *P. tremuloides*) trees expressing *Agrobacterium tumefaciens* T-DNA IAA-biosynthetic genes from a bidirectional mannopine synthase 1´2´ promoter. Several transgenic lines with an altered phenotype and wood composition were regenerated. All the lines were affected in their growth pattern, including alterations in stem elongation, leaf enlargement, stem diameter growth and resumption of axillary bud growth. Two lines showing the most distinct phenotype were characterized for wood formation and IAA distribution and content. Microscopic examination of stem sections from the basal part of these transgenic trees revealed reduction in xylem width, together with several qualitative changes in the xylem structure and composition. These traits were correlated with elevated levels of free and conjugated IAA in mature leaves and roots. IAA level was not elevated in bulk samples of the extraxylary mature stem, and the factors affecting IAA-concentration at the site of wood formation in the transgenic trees are discussed. Our results show the potential for manipulation of wood quality by altering the size and density of different xylem elements through controlled changes of IAA content, and emphasize the importance of the regulatory sequences directing the expression of introduced genes.

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INHIBITION OF THE AUXIN-REGULATED *MAS* PROMOTER IN TRANSGENIC TOBACCO PLANTS

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The activity of the mannopine synthase (*mas*) promoter was investigated in transgenic tobacco plants using luciferase *luxA&luxB* as reporter genes. The results suggest that an endogenous inhibitor, referred to as tobacco shoot inhibitor (TSI), can counteract the effect of auxin on *luxA&luxB* expression. Here we report on an attempt to isolate and characterize TSI. The active substance was separated from methanolic extracts of tobacco stems and immature inflorescence using a Sephadex LH-20 column. TSI activity was assayed by applying column fractions to stem sections of *luxA&luxB* plants, and measuring the decrease in luciferase activity. TSI was eluted from the column as a single peak. Fractions of this peak were further purified by HPLC. Results on the characterization of the substance will be presented.

240 INCREASED BOLL PRODUCTION IN COTTON PLANTS TRANSFORMED WITH AN ISOPENTENYL TRANSFERASE GENE DRIVEN BY A TOMATO THIONIN GENE PROMOTER

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Evidence suggests that sink strength of various reproductive and vegetative tissues may be influenced, directly or indirectly, by levels of the plant growth regulator, cytokinin. In an effort to increase the sink strength of cotton flowers and young bolls, and thereby reduce boll abortion, we have transformed cotton plants with a chimeric gene encoding isopentenyl transferase (*ipt*). Expression of the *ipt* gene was directed primarily to ovaries by utilizing the regulatory regions of a tomato thionin gene highly expressed in ovary tissues. The chimeric thionin:*ipt* gene had been previously used to increase soluble and total solids in processing tomatoes, presumably through a mechanism of increased sink strength (Martineau *et al.* 1995 Production of High Solids Tomatoes Through Molecular Modification of Levels of the Plant Growth Regulator Cytokinin, *Bio/Technology*, in the press), and expression of the tomato thionin promoter in transgenic cotton plants had been previously verified using *gusA* as a marker (Martineau *et al.*, unpublished). In a greenhouse experiment, the most dramatic of the seven original transgenic cotton plants examined set 96 flowers compared to a range of 30-53 set among five control plants. The final number of harvestable bolls was 58 on the most dramatic plant and ranged from 20-38 on the control plants. The increased boll load and other phenotypic characteristics of these plants (short stature, decreased internode length, increased production of vegetative branches) have been observed through three sexual generations. These results suggest that production of increased levels of cytokinin, as a result of the expression of the chimeric thionin:*ipt* gene, has not only increased flower sink strength but also decreased apical dominance in the transformed plants.

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INSECT RESISTANCE MEDIATED BY CYTOKININ PRODUCTION IN TRANSGENIC PLANTS EXPRESSING THE BACTERIAL CYTOKININ BIOSYNTHESIS GENE

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Cytokinins as well as other plant hormones have commercial applications as bioregulators and in combination with endogenous hormones have been shown to affect natural plant defense mechanisms. Since current interests are focusing on combined effects of natural defense mechanisms of plants and biotechnology for crop improvement, we evaluated cytokinin's role as a modulator of insect resistance in transgenic plants carrying a cytokinin biosynthesis gene that was engineered for leaf expression by mechanical wounding or insect feeding. We were able to demonstrate that the herbivorous *Manduca sexta* larvae consumed up to 70% less of the leaf material from transgenic plants expressing the cytokinin gene as compared to control plants. The effect on newly hatched *Myzus persicae* (green peach aphid) nymphs feeding on transgenic plant material was more dramatic in that only about 35% of the nymphs matured and of those only about 60% were able to reproduce. The green peach aphid is an efficient vector for transmission of plant viruses. Insecticidal activity was associated with the leaf surface extracts of transgenic plants and upon further fractionation yielded high molecular weight compounds that are in the process of purification. The activity in the extracts is stable at 4 C for more than 2 months in water but appears to be light sensitive. Coating of normal leaf disks with 0.1% solutions of the partially pure extracts completely retards insect feeding and, in most cases, kills the hornworm within two hours of exposure. The insecticidal activity may only be specific for certain orders of insects such as Lepidoptera and Homoptera but not Coleoptera since it appeared not to have an affect on the alfalfa weevil.

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CYTOKININS AND CYTOKININ-BINDING PROTEINS IN TRANSGENIC PLANTS.

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The responses of plant cells to cytokinins are thought to involve receptors that may be represented by cytokinin-binding poteins (CBPs). A good model system for studying the role of these proteins in mode of cytokinin action is transgenic plants expressing the bacterial gene *ipt* for cytokinin biosynthesis and having different hormonal status. We used five morphologically similar clones of transgenic potato plants with permanent expression of *ipt* gene. The cytokinin content in the leaves of these plants assayed by ELISA. The increase in the level of cytokinins (zeatin and zeatin ribiside) was revealed in all the clones studied. Using anti-idiotype antibodies (as antibodies to CBP) we have shown that the high amount of cytokinins in plants of clone 1339-3A correlated with high cytokinin-binding capacity of purified protein fraction and ribisomes isolated from these plants. Some data will be provided to compare the correlations obtained with the effects of a transient accumulation of cytokinins following thermal induction in transgenic tobacco plants where the *ipt* gene was placed under the control of a heat-inducible promoter from *Drosophila hsp* 70 gene.

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REGENERATION POTENTIAL AND HORMONAL STATUS OF TRANSGENIC TOBACCO PLANTS POSSESSING THE 6B GENE OF AGROBACTERIUM TUMEFACIENS STRAIN AKE10

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The auxin and cytokinin biosynthesis genes, located on the T-DNA of the A. tumefaciens Ti plasmid, are responsible for the formation of crown gall tumors on plants. Additional genes that modulate this phytohormone effect have also been identified. We have introduced one such gene, the 6b gene from A. tumefaciens AKE10, under the control of its own promoter, into tobacco leaf discs . The 6b gene was faithfully integrated into plant DNA and was expressed at the mRNA level. Leaf discs, grown on hormone-free MS medium, produced shoot-forming calli that spontaneously developed into mature plants, several leaves of which displayed morphological abnormalities. When leaf discs from normal leaves of transgenic plants were again placed onto hormone-free medium, numerous shoots developed, indicating that the transgenic plants possessed a high regenerative potential. The 6b gene, under the control of the strong CaMV-35S promoter, induced unorganized calli on hormone-free MS medium. The resultant calli contained high intracellular cytokinin levels that were comparable to that in shooty calli expressing the cytokinin gene. This result suggests that the 6b gene may act to modulate the hormonal balance in tobacco.

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DEVELOPMENT OF A HETEROLOGOUS TRANSPOSON MUTAGENESIS SYSTEM IN *NICOTIANA PLUMBAGINIFOLIA*.

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Transposable elements (TE'S) are mobile segments of DNA which can move from one position in the genome to another. The movement of TE's into genes can result in a recognisable mutant phenotype. If a clone for the TE is available this can be used as a probe to isolate the mutant allele which can then be used to isolate the wild type gene. The best characterised TE's are found in maize and *Antirrhinum*. To extend the technique to other species these elements have been transformed into and shown to be active in, for example, *Arabidopsis*, carrot and tobacco. The objectives of this work were to develop a heterologous transposon mutagenesis system in *Nicotiana plumbaginifolia* using the maize *Ac* element as a transposon tag. This is a diploid species and each plant produces a mass of tiny seeds allowing for large scale screening of mutants. Germination screens were developed to isolate progeny showing altered germination characteristics. Transgenic *N. plumbaginifolia* plants containing the *Ac* element were generated via the *Agrobacterium* vector system using the leaf disc transformation technique. The vector contained a kanamycin resistance gene for the selection of transformants and a streptomycin resistance gene only expressed in cells where excision had occurred. Using the phenotypic antibiotic assays and molecular techniques such as Southern blotting and a PCR-based excision assay the maize element was shown to be active in this species. Four mutants were isolated during analysis and screening of progeny from the transgenic plants: an albino mutant, a female sterile mutant, a male sterile mutant and a leaf morphology mutant. Initial characterisation of the mutants will be discussed.

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BROAD RESISTANCE TO PLANT VIRUSES IN TRANSGENIC PLANTS CONFERRED BY ANTISENSE INHIBITION OF A HOST GENE ESSENTIAL IN SAM-DEPENDENT TRANSMETHYLATION REACTIONS

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S-adenosylhomocysteine hydrolase (SAHH) is a key enzyme in transmethylation reactions that use S-adenosylmethionine (SAM) as a methyl donor. Based on the importance of SAHH in a number of SAM-dependent transmethylation reactions, particularly the 5'-capping of mRNA during viral replication, SAHH has been considered as a target of potential antiviral agents against animal viruses. In order to test the possibility of engineering a broad type of resistance to plant viruses, we expressed the antisense RNA for tobacco SAHH in transgenic tobacco plants. As expected, transgenic plants constitutively expressing an antisense SAHH gene showed resistance to infection by various plant viruses. Among those plants, about half exhibited some level of morphological change (typically stunting). Analysis of the phisiological changes in those plants showed that they contained excess levels of cytokinin, and that pathogenesis-related (PR) proteins were induced. Because cytokinin is found to induce aquired resistance, there is also a strong possibility that the observed resistance was induced by cytokinin.

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STRUCTURE-ACTIVITY CORRELATIONS FOR RING-FLUORINATED INDOLE-3-ACETIC ACIDS

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As a part of molecular recognition studies on auxins, a series of indole-3-acetic acids (IAAs) fluorinated at ring-positions 4, 5, 6 or 7 was examined. The substituent profoundly influences the electron distribution in the aromatic ring system, but does not require much more space than the hydrogen atom it replaces (van der Waals radii: H, 1.20 Å; F, 1.35 Å). Indeed, X-ray structure analysis and molecular mechanics calculations confirmed that the conformations of all the fluoro-IAAs studied are essentially the same as for the unsubstituted parent compound, with the side-chain CH_2—COOH bond perpendicular to the ring plane (folded conformation). According to the results of molecular dynamics simulations, this remains an energetically preferred, highly populated conformation in aqueous solution, although there is considerable rotation about all single bonds in the side chain. The dose response curves for 5-F-IAA, 6-F-IAA, and 7-F-IAA in the *Avena* coleoptile straight-growth test were essentially superimposable to that for unsubstituted IAA, with a flat maximum at 10 μmol/L (range of concentrations examined: 1 to 100 μmol/L). In contrast to these results, the optimal concentration for 4-F-IAA was 1 μmol/L, and the optimal growth response was about 40% larger than for the other compounds tested.

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ALTERATIONS OF AUXIN PERCEPTION IN TOBACCO PROTOPLASTS TRANSFORMED BY THE *ROLB* GENE FROM *AGROBACTERIUM RHIZOGENES*

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Expression and physiological effects of the *rolB* gene from *Agrobacterium rhizogenes* were simultaneously investigated in mesophyll protoplasts from tobacco plants. The kinetic study of the expression of *rolB* mRNA following exogenous auxin application showed that auxin transiently stimulated *rolB* expression, mRNA levels starting to accumulate 6 to 9 hours after auxin supply and increasing 300-fold after 12 to 18 hours. The parallel study of the auxin sensitivity of *rolB*-transformed protoplasts, as assayed by their electrical response to the hormone, showed that the auxin treatment generated within 6 hours an increase in sensitivity by a factor up to 100,000. In untransformed protoplasts, the same auxin treatment induced an increase in auxin sensitivity that never exceeded 30- to 50-fold. These results reflect a strong cooperative effect of auxin and *rolB* in transformed protoplasts. The possible origin of such alterations in auxin sensitivity were investigated. On one hand we compared accumulation and metabolism of exogenous auxins on wild-type and *rolB*-transformed tobacco protoplasts. Accumulation of free 1-[^{3}H]-naphthaleneacetic acid (NAA) was equivalent in both types of protoplasts. Naphtyl-ß-glucose ester, the major NAA metabolite in protoplasts, reached similar levels and was hydrolyzed at the same rate. Essentially similar results were obtained with exogenous indoleacetic acid. The *rolB*-dependent behaviour of transgenic tobacco protoplasts is thus not a consequence of modified intracellular auxin concentrations. On the other hand, we showed that protoplasts expressing the *rolB*-induced increased sensitivity differed quantitatively in their responsiveness to antibodies directed against ZmER-abp1, the major auxin-binding protein from maize coleoptile. This suggests that alterations of the auxin reception-transduction pathway at the plasma membrane of *rolB*-transformed protoplasts may account for their increased auxin sensitivity.

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LATERAL ROOT PRIMORDIA DIFFERENTIATION IN RESPONSE TO AUXIN IN TOBACCO SEEDLINGS TRANSGENIC FOR *ROL* GENES FROM *A. RHIZOGENES*

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We have undertaken a simple study involving primary roots of tobacco seedlings containing various *rol* genes to ascertain whether these genes altered the capacity of the plants to form lateral root primordia (LRP) in response to exogenous auxin. Exposure of 11 day old control seedlings to a range of auxins revealed a classic response with 20-25 LRP being formed/cm within 72 hr of exposure to 10^{-4} M IBA, IAA or NAA as compared to 2-4 LRP/cm when exposed to auxin concentrations of 10^{-7} M or less. Seedlings transgenic for *rol* A or *rol* B genes showed little, if any, difference in sensitivity to auxin compared to controls with respect to the time frame of LRP formation or the concentrations which induced LRP differentiation. However, seedlings transgenic for *rol* C did show a moderate, but statistically significant increase in sensitivity to auxin with 10^{-5} M IBA causing the differentiation of 20-25 LRP/cm in roots of *rol* C transgenics as opposed to 12-15 LRP/cm in non-transgenic controls. The latter results may be explained, at least in part, by the observations of Nilsson *et al.* (1993) that transgenic tobacco expressing the *rol* C gene had similar auxin levels but reduced cytokinin levels relative to wild-type controls. Thus, a lower level of exogenous auxin is needed to achieve the optimal auxin : cytokinin ratio for maximal LRP induction in seedlings transgenic for *rol* C than in non-transformed controls. A reduced cytokinin : auxin ratio *in vivo* may also help explain why the root systems of plants expressing the *rol* C gene are often excessively branched compared to those of wild-type controls.

Reference. Nilsson O *et al.* (1993) Plant Physiol. **102**, 363-371.

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THE TOMATO GENE *diageotropica* AFFECTS RESPONSES TO BOTH AUXIN AND CYTOKININ

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The *diageotropica* (*dgt*) mutant of tomato is a single-gene, recessive mutant with a complex pleiotropic phenotype. Hypocotyl segments of *dgt* are insensitive to exogenous auxin in elongation and ethylene-synthesis assays, which has led to the proposal that the primary effect of the *dgt* mutation is to reduce the sensitivity of the tissue to auxin via an altered perception mechanism (Kelly and Bradford, 1986, Plant Physiol. 82: 713-717). Cytokinins counteract auxin effects in many physiological systems. We have found that application of exogenous cytokinin to wild type tomato plants leads to the appearance of *dgt*-like morphological traits. High concentrations of exogenous cytokinin also reduce the responses of wild type hypocotyl segments to auxin in elongation and ethylene biosynthesis assays. We used a tissue-culture system to concomitantly compare the sensitivity of *dgt* and wild-type tissues to auxin and cytokinin. Initiation and growth of wild type calli showed clear optima for both auxin and cytokinin concentrations while growth of *dgt* calli was unaffected by a wide range of auxin and cytokinin concentrations. In addition, *dgt* calli contain numerous vascular elements which are absent from wild-type tissue. We conclude that the *dgt* gene affects the response to both auxin and cytokinin. In order to further compare the effects of cytokinin and the *dgt* mutation on the transduction of the auxin-signal, we are currently investigating the expression of auxin-induced genes in callus and hypocotyl tissues. Supported by NSF-Grant # BIR-9314619

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CELLULAR RESPONSES TO AUXIN IN WILD-TYPE AND TRANSGENIC TOBACCO ALTERED IN IAA CONTENT AND/OR AUXIN SENSITIVITY

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Both the hormone level and hormone sensitivity have been suggested as limiting factors in plant hormonal responses. The present study was undertaken to investigate the effects of an increased level of the plant hormone IAA, as well as the combined effect of an increased IAA level and increased IAA sensitivity, on auxin-dependent responses having different temporal characteristics. Three transformed genotypes were compared to the wild-type tobacco SR1: the IAA-overproducing lineC, expressing the *iaaM* and *iaaH* genes of *Agrobacterium tumefaciens*; the line BBG, expressing the *rolB* gene of *A. rhizogenes* and a chimeric prolB-Gus gene; and a line resulting from a cross between C and BBG plants. Results will be presented on the general appearance of these genotypes, as well as the IAA content in protoplasts and different tissues, the mRNA levels of four auxin-regulated genes (*parA*, *parB*, and two genes of the *Nt-aux* family), the auxin-dependent effects on the modification of protoplast ΔEm, the initiation of protoplast division in culture, and the initiation of adventitious roots on leaf discs. The possible influence of the endogenous IAA level on different dose-dependent auxin responses, as well as the possibility of an autoregulatory effect of IAA on its own reception or signal transduction, will be discussed.

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GENETIC DISSECTION OF THE RELATIVE ROLES OF AUXIN AND GIBBERELLIN IN THE REGULATION OF STEM ELONGATION IN INTACT LIGHT-GROWN PEA PLANTS

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To evaluate the roles of auxin and GA in the process of stem elongation in intact pea (*Pisum sativum* L.) plants, we have examined the stem elongation responses to exogenous IAA and GA in the light-grown, dwarf, GA-response *lkb* mutant in comparison with the GA-deficient dwarf *le* mutant and their near-isogenic wild-type tall plants. In *le* plants IAA elicited a fast initial response characterized by a sharp peak in growth rate after a lag of about 20 min, followed by a prolonged slow decline, whereas the GA response had a much longer lag (about 2-3 h), and was rather weak initially, but increased gradually with time. The growth responses induced by exogenous GA and IAA were largely additive. The growth effect of GA was preferentially in the nascent internodes, with that of IAA mainly occurring in the relatively more mature, but still elongating internodes. In contrast, dwarf *lkb* plants responded poorly to applied GA as compared with *le* mutants, but were highly responsive to exogenous IAA. Exogenous GA could produce a substantial promotion of elongation in *lkb* plants, but only conditional upon the addition of exogenous IAA. Exogenous IAA stimulated stem growth exclusively by cell extension whereas GA treatment of *le* plants produced an increase in both cell length and cell number. Although GA alone produced a relatively insignificant effect on the growth of *lkb* plants, with a negligible effect on cell length, it was markedly effective in increasing cell number in this system. Along with the previous report that *lkb* plants contain low levels of endogenous IAA (Mckay et al., 1994), our findings collectively suggest that the growth suppression in dwarf *lkb* mutants is mainly due to a deficiency in endogenous auxin, and that the GA response may be relatively normal in this mutant type. These results further imply that both auxin and gibberellin are needed for normal stem elongation in intact plants.

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THE BINDING SITE OF ABA-BP IS IN A SHALLOW CAVITY

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We have shown, that the enone function of immobilised C_1-conjugated ABA can be used as UV-inducible crosslinker. We also found, that ABA-binding proteins (ABA-BPs) in soluble protein extracts of *Arabidopsis thaliana* seeds were bound by this conjugate. [^{3}H]-ABA was of no use as UV-inducible affinity crosslinker. In order to design a biotin-labelled ABA-probe knowledge about the depth of the ABA-binding pocket was required. The ABA-N-hydroxysuccinimide ester was used to conjugate ABA to ω-amino acids, linked to NH_2-functions on either protein or activated microtiter plates. The effect of the spacer incorporation was analysed using a competitive immunoassay. It was found, that ABA-NHS was remarkably stable at physiological pH (half life approx. 10 h) and conjugated more efficiently to NH_2-functions on proteins than published methods. Competition between ABA-BPs and anti-ABA antibodies for immobilised ABA-C_1-ω-amino acid-NH_2-matrix conjugates was higher for spacers of five or seven chemical units than for zero, three or four units. ABA-C_1-4-amino butanoic acid methyl ester was tested in inhibiting germination of wild type *Arabidopsis thaliana* seeds. The activity was comparable to the activity of ABA methyl ester, but it remains unclear whether the activity could be attributed to the conjugate or to ABA hydrolysed from the conjugate.

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ABA-BP ACTIVITY COINSIDES WITH EMBRYONAL ABA PEAK IN DEVELOPING SEEDS

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ABA levels in developing seeds occur in two distinct peaks, the second of which has been shown to be of embryonic origin. This second rise in ABA indicates a switch from developmental to maturing genetic programming. A competitive immunoassay, using monoclonal anti-ABA antibodies as inhibitors of ABA-BP binding to immobilised ABA-C_1-conjugated proteins, has been used to trace the occurrence of ABA-binding proteins in developing seeds of ABA-insensitive and -deficient mutants *Arabidopsis thaliana* (ecotype Landsberg erecta) relative to wt. In seeds harvested in May-June a significant difference in ABA-BP activity was found between wt, *abi1* and *abi2* on one hand and *abi3-1*, *aba1* and *aba4* on the other. In 11 dap seeds the first three contained relatively more ABA-BPs than the second three, whereas the opposite was true for 14 dap seeds. Mature seeds contained about the same levels of ABA-BP activity in all mutants relative to wt. No significant differences were found in seeds harvested in November, but they did show the same trend. The effect is thought to be season-dependent. The results confirm the notion that, ABA-BP levels are correlated with the ABA signal. In *abi3-1*, *aba1* and *aba4* the deposition of reserves and embryo growth is lagging behind wt seed development. Again, our results are in agreement with this observation.

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THE IMPORTANCE OF THE C-1 CARBOXYLIC ACID GROUP OF ABSCISIC ACID FOR BIOLOGICAL ACTIVITY.

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Abstract:--The amide and N-methyl amide analogs of (+)-abscisic acid were prepared from (+)-ABA methyl ester by reaction with ammonia and methylamine respectively. The amides were tested against (+)-ABA and its methyl ester in growth inhibition, cress seed germination inhibition, transpiration, and pH bioassays where they were found to be inactive. Feeding the amides to a corn cell suspension culture which rapidly metabolizes ABA to phaseic acid led only to recovery of the unchanged amides, with no hydrolysis to ABA or metabolism to other products observed. Since the amide analog of ABA is very similar to ABA sterically and electronically, the major difference being the reduced acidity of the analog, it appears that the C-1 carboxylic acid functionality is very important for ABA activity and metabolism. Most previous studies of ABA analogs which have been modified at C-1, including the methyl ester, were derivatives which were convertible to ABA in vivo via hydrolysis and/or oxidation.

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VICILIN AND NAPIN CHIMERIC GENES, INITIALLY SENSITIVE TO ABA, LOSE SENSITIVITY FOLLOWING DESICCATION IN TRANSGENIC TOBACCO SEED

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Drying of seeds, when imposed prematurely, elicits a switch in metabolism; events unique to development, such as synthesis of storage protein, are terminated, while syntheses associated with germination and growth are initiated. Previously, we have shown that the 5' regulatory regions of genes encoding two storage proteins are down-regulated following desiccation in transgenic tobacco. In developing transgenic seed, the expression of chimeric GUS genes containing the vicilin or napin promoters is responsive to premature drying and declines upon subsequent rehydration, in contrast to chimeric genes containing a constitutive viral promoter. Following desiccation, this region may become insensitive to positive controlling factors. We are interested in testing the hypothesis that the maturation drying period, by effecting changes in the content of, or sensitivity to, ABA may partially, or wholly remove the effects of this 'positive modulator' of developmental gene expression. Toward this end, we studied the enhancement of GUS reporter gene activities in transgenic tobacco by exogenous ABA before and after drying. A racemic mixture of ABAs at 10 *u* M caused a 2-3 fold increase in GUS activity in developing transgenic seed expressing chimeric genes containing the vicilin or napin promoters. However, when these seeds were prematurely dried prior to their culture on ABA medium, enhancement of GUS activity was virtually abolished. Use of optically pure ABAs, revealed that the enhancement in GUS activity in developing fresh seed was due largely to the (+) form of ABA. ABA had a slightly inhibitory effect on GUS activities in transgenic seed expressing chimeric constructs containing the viral 35S promoter, whether or not premature drying was applied. Thus, vicilin and napin chimeric genes, initially sensitive to ABA, become *relatively* insensitive to the hormone after desiccation.

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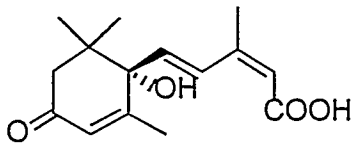
A METHYL ETHER DERIVATIVE OF ABA EXHIBITS SELECTIVE ACTIVITY IN VARIOUS BIOLOGICAL ASSAYS. PA Rose[1], AJ Cutler[1], B Lei[1], AC Shaw[1], DL Barton[1], MK Loewen[1], SR Abrams[1], and MK Walker-Simmons[2]. [1]Plant Biotechnology Institute, National Research Council of Canada, 110 Gymnasium Place, Saskatoon, SK, Canada, S7N 0W9. [2]Agriculture Research Service, United States Department of Agriculture, 209 Johnston Hall, Washington State University, Pullman, Washington, 99164-6420. E-mail: prose@pbi.nrc.ca

The plant hormone abscisic acid [S-(+)-ABA, **1**] regulates many aspects of plant growth including dormancy, transpiration and responses to stress. We are synthesizing optically pure analogs of ABA to obtain highly active and selectively active ABA agonists, and to help develop probes for ABA-binding proteins. We have recently developed a chiral synthesis of a new ABA analog that has a methyl ether replacing the hydroxyl group at the C-1' carbon, **2**. We have compared the biological activity of the methyl ether analog to that of natural ABA in a variety of biological assays. In some assays such as growth inhibition of maize suspension-cell cultures, the methyl ether exhibits strong activity, comparable to ABA itself. In others, such as reducing transpiration and inhibiting embryo gemination in wheat, the methyl ether is only weakly active. Since biological activity depends in part on the rate of conversion to inactive degradation products, the metabolic fate of the methyl ether in corn cells has been determined. The results of these studies will be described and the implications for designing future ABA agonists discussed.



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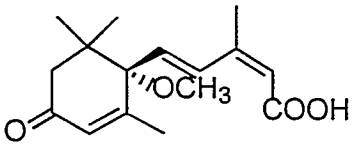

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EFFECTS OF CLINOROTATION AND MICROGRAVITY ON EXPRESSION OF SAUR AND GH3 GENES IN TRANSGENIC PLANTS

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Seedlings grown under altered gravity conditions exhibit increased lateral root formation and ethylene production. To gain some insights about the involvement of auxin in these observed phenomena, we decided to examine expression of two auxin-inducible genes, SAUR and GH3, under altered gravity conditions. Transgenic seedlings that express SAUR promoter-GUS or GH3 promoter-GUS genes were grown on clinostats or a spaceflight (Spaceflight STS-63, February, 1995) for 7 days, and expression of SAUR and GH3 genes were determined by histochemical staining and fluorometric assay for GUS enzyme activity. Preliminary results indicate that expression of SAUR gene was 2 to 3 times higher in seedlings grown on clinostats than the stationary controls. Expression of GH3 gene was enhanced in cotyledons and root elongation region of seedlings grown on clinostats and the spaceflight as compared to the stationary or ground controls. If clinostat-grown seedlings were treated with exogenous auxin, expression of GH3 gene was induced to a significantly higher level than the stationary controls. These results suggest that seedlings grown under altered gravity conditions have elevated auxin levels and/or an increased sensitivity to auxin in tissues where SAUR and GH3 genes are expressed.

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POLLINATION-INDUCED ETHYLENE PRODUCTION BY CARNATION FLOWERS

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In *Dianthus caryophyllus* flowers, cell to cell communication between the pollen and the pistil induces ovary development and corolla senescence. The production of elevated ethylene by the style is the first measurable postpollination response, and occurs with 1 h of pollination. After pollination, ethylene production by the other floral organs follows a sequential pattern beginning with styles, then the ovary and lastly petals. Ethylene production by pollinated styles can be defined temporally by 3 distinct peaks. Carnation flowers have multiple styles and unpollinated styles on a pollinated flower also produce increased ethylene corresponding to the first and third peaks exhibited by pollinated styles. Increased ethylene by the unpollinated style was detected within the first hour following pollination of another style on the same gynoecium. This response was pollen-specific and could not be elicited by wounding the style. This provides evidence for very rapid signalling between styles following pollination. Inhibition of ethylene action in the carnation style by treating individual styles with diazocyclopentadiene (DACP) prevents pollination-induced corolla senescence. When either pollinated or unpollinated styles from a pollinated gynoecium were treated with DACP, the early increase in ethylene was unaffected, but sustained ethylene production was inhibited. This result indicates that the final increase in ethylene production by both the styles and petals of pollinated flowers is an autocatalytic response to the rapid increase in ethylene following pollination.

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ETHYLENE AND INTERORGAN SIGNALING DURING FLOWER SENESCENCE

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The roles of ACC and ethylene in interorgan signaling during flower senescence in petunia and orchid (*Cymbidium*) flowers were investigated by studying the translocation and distribution of stigma-applied radiolabelled 1-aminocyclopropane-1-carboxylic acid (ACC) and α-amino*iso*butyric acid (AIB) and by ethylene diffusion measurements.
In pollinated or stigma-wounded *Petunia* flowers no significant translocation of ACC and AIB from the stigma to the other flower parts was observed. Also in isolated pistels no translocation of these chemicals was observed. Following stigma wounding, a burst of ethylene was produced by the pistil as well as by the other flower parts indicating that gaseous ethylene may be the translocated factor. Following pollination no ethylene was recovered from the corolla indicating that in pollinated petunia flowers other factor(s) play a role in interorgan communication.
In *Cymbidium* flowers emasculation and pollination induce a burst of ethylene from both the central column (stigmatic region) and the petals. ACC and AIB were found to be largely immobile in intact flowers as well as in isolated gynoecia indicating that ACC is not the signal responsible for interorgan communication during pollination- or emasculation-induced senescence. It was shown that gaseous ethylene rapidly diffuses from the stigmatic region to the petals. Creation of alternative outlets (incisions) for the produced ethylene in the labellum or the central column significantly delayed senescence symptoms supporting the view that, in orchid flowers, ethylene produced in the stigmatic region following emasculation or pollination diffuses to other flower parts where it induces the senescence process.

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IMPORTANCE OF THE CHIRAL CENTRES OF JASMONIC ACID IN BIOLOGICAL RESPONSES IN PLANTS

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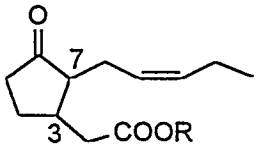
Jasmonic acid has two chiral centres, one at C-3 and one at C-7. In jasmonates obtained from plant sources, the stereochemistry at C-3 is R, and at C-7 is either R or S. Jasmonic acid is involved in numerous plant responses such as senescence, wounding, seed germination, seed storage product deposition and interaction with other hormones like ethylene. The specific chemical structures of jasmonates that trigger these responses is not known.



R=H, CH_3

To determine the importance of stereochemistry of jasmonates, we have separated the optical forms at C-3, and have made the C-7 centre non-epimerizable by introducing a methyl group or a fluorine atom. Optically pure jasmonates and methyl analogs which are either R or S at C-3 and C-7, with known absolute stereochemistry, have been tested for jasmonate agonism or antagonism in physiological and gene expression assays in canola, wheat and tobacco. The results of these structure/activity studies will be discussed in the context of the shape of the receptors involved in jasmonic acid responses in plants.

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ABSCISIC AND JASMONIC ACID EFFECTS ON THE PROTEIN SYNTHESIS IN SEEDS OF *ACER SACCHARINUM L.*

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Acer saccharinum L. seeds are very sensitive to dehydration and on the termination of the maturation period they germinate remaining still nondormant. We studied the protein synthesis in cotyledons and embryo axis at the final stage of maturation and under effects of abscisic (ABA) and jasmonic (JA) acid treatment. The protein syntnesis in vivo was measured by means of [^{35}S]-methyonine introduction in isolated protein. Radioactively labelled material was analysed using the two-dimentional gel electrophoresis method. The protein synthesis intensity increased along with seed maturation. JA and ABA treatment inhibited the protein-synthesing apparatus and JA effect was much more stronger than that of ABA. However, along with a general effect of inhibitor both hormones promoted the synthesis of similar proteins within the range of masses of 14-67kDa.

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JASMONIC ACID EFFECT ON PROTEIN SYNTHESIS IN MATURING *ACER TATRICUM L.* SEEDS

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Changes in protein synthesis in *Acer tatricum L.* embryos at different stages of seeds development were studied. Protein synthesis intensity increased until 90th day after anthesis and than decreased when seeds became dormant. Jasmonic acid treatment stimulated protein synthesis intensity at the final stages of seed maturation. Mechanism of this process will be discussed.

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EXPRESSION OF JASMONATE-RESPONSIVE GENES IN ARABIDOPSIS THALIANA WILDTYPE AND JASMONATE-INSENSITIVE MUTANTS

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Jasmonic acid and its methyl ester are plant signal molecules with a number of different effects on plant growth and development. Jasmonates act through regulation of transcription as well as translation initiation. Little is known, however, about the signal transduction pathway which links the jasmonates to the modification of transcription/translation. To address this question two approaches were taken: 1. we studied the regulation of the expression of a jasmonate responsive gene and 2. we investigated jasmonate-insensitive mutants of Arabidopsis thaliana. 1. Arabidopsis thaliana contains a gene (*Atvsp*) encoding a protein homologous to VSPa and VSPb, jasmonate-inducible proteins in soybean. *Atvsp* is expressed constitutively at high levels in flowers and buds and is inducible by methyljasmonate and wounding. Similar to the soybean *Vsp* genes, expression of *Atvsp* is regulated by sucrose, light and phosphate. 2. Mutants of Arabidopsis thaliana were screened for jasmonate-insensitivity on methyl jasmonate-containing medium for decreased inhibition of root growth. The effects of jasmonate treatment on the expression of jasmonate inducible genes in two mutants were characterized. Both mutants show altered inducibility of the *Atvsp* gene in leaves.

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JASMONIC ACID LEVELS AND WOUND GENE EXPRESSION IN LINOLENIC ACID DEFICIENT AND LOX COSUPPRESSED *ARABIDOPSIS*

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Jasmonic acid (JA) has been proposed as a key regulator of plant responses to pathogens and insects based on its ability to induce a variety of defense genes including proteinase inhibitors, flavonoid and sesquiterpenoid biosynthetic enzymes, thionin and osmotin. Recent models suggest that both JA-dependent and JA-independent pathways may exist for wound gene induction. In order to test this hypothesis, we synthesized ^{13}C-JA for use as an internal standard to quantitate endogenous JA levels and used a wound induced *Arabidopsis* gene, *AtVSP*, to determine if JA-independent wound gene expression could occur in wounded *Arabidopsis* plants deficient in linolenic acid or lacking detectable *AtLOX2* protein. No accumulation of JA was observed in wounded linolenic acid deficient plants (control JA levels were 10 to 40 fold lower than wt). In addition, no detectable *AtVSP* mRNA was observed in control or wounded linolenic acid deficient plants. In plants with no detectable *AtLOX2* protein, the wound induced accumulation of JA and *AtVSP* mRNA was substantially impaired (control JA levels were similar to wt). These results suggest that JA is essential for the expression of *AtVSP*. This work was supported by NSF-IBN-9407092 and WSU ARC to JAB and by TAES and NRICGP Grant 37100-9014 to JEM.

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JASMONATE-INDUCED LIPOXYGENASE FORMS ARE LOCALIZED IN CHLOROPLASTS OF BARLEY AND *ARABIDOPSIS* LEAVES

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Barley leaves respond to application of (-)-jasmonic acid (JA), or its methylester (JM) with the synthesis of abundant proteins, so-called jasmonate induced proteins (JIPs). Western blot analysis of crude leaf extracts revealed a remarkable increase upon JM treatment of a 100 kDa lipoxygenase (LOX), and appearance of two new LOX forms of 98 and 92 kDa. The temporal increase of LOX-100 upon JM treatment was clearly distinguishable from the other two detectable LOX forms. The JM induced LOX forms of barley leaves were compared with those of *Arabidopsis* and soybean leaves. Both dicot species showed a similar increase of one preexisting LOX upon JM induction. Additionally, leaves from soybean responded with synthesis of a newly formed LOX of higher molecular mass.

Immunofluorescence analysis and isolation of intact chloroplasts confirmed the localization of JM-induced LOX forms in barley leaves. Immunofluorescence experiments carried out with *Arabidopsis* and soybean revealed a similar plastidic location in *Arabidopsis* but a different situation for soybean. In untreated soybean leaves LOX was mainly restricted to vacuoles of paraveinal mesophyll cells. Additionally, LOX forms could be detected in cytoplasm and nuclei of bundle sheath cells. Upon JM treatment cytosolic LOX was detectable in spongy mesophyll cells, too.

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THE EFFECT OF JASMONIC ACID, SUCROSE, AND LIGHT ON THE PHOTOSYNTHETIC PIGMENTS OF POTATO PLANTS GROWN *IN VITRO*

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In our previous results we demonstrated the involvement of 1μM jasmonic acid (JA) in the metabolism of photosynthetic pigments of cv. Sante *in vitro* grown potato plants which showed hyperhydric symptoms induced by JA (Plant Science, 103, 11-17, 1994). In this study the research was extended to different concentrations of JA, sucrose, and light. The potato cv. Sante leaf node cultures were grown *in vitro* on Murashige and Skoog medium supplemented with 0.1, 1, and 10 μM JA, 1% or 3% sucrose, at Osram Cool White (55-70 $\mu mol/m^2/s$) or Osram Flora (80-100 $\mu mol/m^2/s$) light in different combinations. Pigments were analysed with high performance liquid chromatography. The plants grown on 1% sucrose at the lower light synthesise the highest amount of chlorophyll, while the lowest amount of carotenoids and chlorophyll was found in those grown on 3% sucrose at the higher light. Application of JA resulted in a decrease of total pigment content per dry weight in all studied conditions except in the plants grown on 1% sucrose at the lower light where only 1 and 10 μM JA were inhibitory. This indicates that the amount of sucrose in the medium and the light conditions contribute to the "source" regulation of photosynthesis. The involvement of JA might be mediated directly through pigment metabolism or through a more indirect way with increased sucrose transport due to the increased root system in JA treated plants. The presence of the xanthophyll cycle pigments violaxanthin, antheraxanthin and zeaxanthin, assumed to have a protective function in stressed photosynthetic tissue, was demonstrated in all samples. The extent of conversion of violaxanthin to zeaxanthin was highest in JA treated plants grown on 3% sucrose, indicating the stressed condition of the plants.

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EFFECT OF METHYLJASMONATE ON ETHYLENE PRODUCTION IN TOMATO HYPOCOTYL SEGMENTS AND FRUITS.

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Effect of methyljasmonate(MeJA) on ethylene production in tomato (Lycopersicon esculentum Mill) hypocotyl segments and fruit were studied. 450 μM of MeJA showed 50% of inhibitory effect after 3 hr of incubation period. Such inhibitory effect was due to the decrease in ACC synthase activity but not due to ACC oxidase activity. In contrast, MeJA stimulated ethylene production in tomato fruits. When 30 μl/ml MeJA was treated in a gaseous state, ethylene production increased double and continued until 4 days. To elucidate the mechanism of MeJA on ethylene production, ACC synthase and ACC oxidase activities were tested after MeJA treatment. MeJA increased the activities both of ACC synthase and ACC oxidase, and induced ACC oxidase mRNA accumulation in fruit tissue. These data suggest that MeJA plays distinct roles in the ethylene production in different tomato tissues. It may be possible that MeJA affects differently the mechnisms of signal transduction leading to the ethylene biosynthesis.

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SEQUENCING AND CHARACTERIZATION OF A cDNA AND GENOMIC DNA-FRAGMENTS CODING FOR THE JASMONATE-INDUCIBLE 37 kDa PROTEIN OF BARLEY

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Barley leaf segments respond upon treatment with jasmonic acid (JA) or its methyl ester (JM) with the synthesis of a specific set of proteins (JIPs: jasmonate-induced proteins). We isolated a novel cDNA-clone (pHvJS37) by differential screening of a lambda gt10 cDNA-library constructed from mRNA of JM-treated barley leaf segments. The ORF encodes a 39,9 kDa polypeptide. Antibodies raised against the *in vivo* JIP37 cross-react with this 39,9 kDa polypeptide; this suggests that both are identical.. No significant similarities to other protein or DNA sequences were found in DATA-bank searches. Northern blot analysis showed that the JIP37 transcripts accumulate not only upon JM-treatment but also upon ABA- or sorbitol-treatment or desiccation stress. Immunohistochemial methods localised this JIP37 protein to the nucleus, the cytoplasm and the vacuole. One of two genomic clones isolated from a lambda-ZAP genomic library contains the full coding sequence (4 exons, 3 introns) and 1250 bp of the 5'upstream region. Characterization of this clone has been done by transient expression studies after transformation of reporter gene fusion constructs (GUS) using barley aleurone protoplasts. Interactions of promoter fragments with nuclear proteins are being studied.

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THE EFFECT OF METHYL JASMONATE ON ETHYLENE PRODUCTION, CO_2 EVOLUTION AND PHENOL OXIDASE ACTIVITY IN JONAGOLD APPLES

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The role of jasmonates in apple ripening is still obscure. It was found previously that methyl jasmonate (JA-Me) stimulated ethylene production in preclimacteric apples and inhibited the process in postclimacteric apples of some cultivars. In this work some further details on the physiological action of JA-Me in apples cv. Jonagold are presented. Apples were harvested at the beginning of October and stored at 0°C until treatment between the end of December and the end of February. JA-Me at concentration of 1.0, 0.5, 0.1, 0.05, and 0.01% in lanolin paste were applied to the surface of intact apples cv. Jonagold. During five days from the treatment, samples of cortex with a skin (area about 2.0 cm^2) were cut off at a depth of about 2 mm and used for determination of ethylene production, ACC oxidase activity, CO_2 evolution, browning of tissue, and phenol oxidase activity. JA-Me initially (1-2 days after treatment) stimulated ethylene production and the ACC oxidase activity and then (3-5 days after treatment) these processes were inhibited; the lower concentration of JA-Me caused the higher production of ethylene. Simultaneously, within the period of 5 days, JA-Me stimulated CO_2 evolution, flesh browning and phenol oxidase activity. The metabolic significance of these findings will be discussed.

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WOUND-INDUCED FREE RADICAL FORMATION IN CORN SEEDLINGS

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Recent studies carried out at the University of Sheffield (Sheffield, England) have established a correlation between the onset of senescence and loss of viability in plants with a development of stable free-radicals which can be observed by Electron Paramagnetic Resonance (EPR) spectroscopy. We have explored a possibility of free-radical formation following root injury of corn seedlings (*Zea mays* L.). Free radical signals were detected by EPR spectroscopy at 9.5 GHz. In all cases, we have observed a single-line free radical signal (g-value around 2.004) super imposed with six-lines EPR spectrum from Mn^{2+} ions naturally present in the plant tissues. We have determined a consistent increase in the intensity of single-line EPR signal after inducing the root injury. This signal is presumably attributed to a quinone-derived radical and, as speculated, is associated with stressed respiratory transport chains. Based on results of our observation, we can suggest that free-radical reactions can be induced by the root injury. Further, an appearance of free-radical signal in the root may serves as an indication of a stress, both natural and induced. If the precursors of observed free-radical signal can be determined (this work is in progress), this will provide a better inside on free-radical processes in plants followed by an injury. This work used the resources of the Illinois EPR Research Center (NIH P41-RR01811).

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INVOLVEMENT OF JASMONIC ACID IN ELICITOR-INDUCED PHYTOALEXIN PRODUCTION IN SUSPENSION-CULTURED RICE CELLS

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Exogenously applied (±)-jasmonic acid (JA; 10^{-4} M) induced the production of, a major phytoalexin, in suspension-cultured rice cells (*Oryza sativa* L. cv. BL-1), although the level of momilactone A was lower than that in the rice cells treated with an elicitor (*N*-acetylchitoheptaose; 10 μg ml^{-1}). On the other hand, the endogenous levels of JA in the elicited rice cells increased rapidly and transiently prior to the accumulation of momilactone A. The treatment with JA biosynthetic inhibitors reduced the production of momilactone A in the elicited rice cells, and the addition of (±)-JA recovered and raised the productivity of momilactone A to higher levels than that in the rice cells treated with the elicitor alone. These results strongly suggest that JA functions as a signal transducer in the phytoalexin production of the elicited rice cells.

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AN ELICITOR FOR PATHOGENESIS-RELATED PROTEINS IN *SILENT LATIFOLIA* IN SUSPENSION CULTURE.

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Cells derived from hypocotyl callus of *Silene latifolia*, the host plant for the smut fungus *Microbotryum violaceum*, were grown in suspension culture in modified Murashige-Skoog medium. Cell wall preparations from *M. violaceum* and infected and non-infected *S. latifolia* buds were incorporated into the culture medium and, after 3-12 days of culture, the activity of chitinase, ß-1,3-glucanase and peroxidase was determined by gel electrophoresis. Cell wall preparations from infected *S. latifolia* buds but not from *M. violaceum* induced enzyme activity. We conclude that the fungal hyphae, which grow only intercellularly, as seen with electron microscopy, may partially hydrolyze the host cell wall and the resulting wall hydrolysates may serve as elicitors.

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SYSTEMIC ACTIVATION OF PHOSPHOLIPASES IN WOUNDED TOMATO AND BROAD BEAN PLANTS

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Plant défense systems are activated upon exposure to pathogens or physical wounding by herbivores. Lipids and lipid metabolites have previously been implicated in these defense responses. Activation of phospholipases, which generate lipid metabolites important in the defense response, was measured in response to physical wounding. The first leaves of tomato or broad bean seedlings at 2 leaf stages were wounded and production of lipid metabolites was measured in the wounded leaf and in a second non-wounded leaf. In the wounded leaf, phosphatidic acid increased approximately 4 fold within 5 min whereas the lysophospholipids, lysophosphatidylcholine and lysophosphatidylethanolamine, increased over 2 fold within 15 min of wounding. Similar changes in these lipids were observed in the second non-wounded leaf. Our results suggest that the wound signal is propagated to the outside of the wounded leaf within 5 min in these plants, and it activates phospholipases, which can produce some important precursors of defense molecules or second messengers in wound signal transduction in plants. (Supported by a grant from Korea Ministry of Education)

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STEM ELONGATION AND BUDDING ENHANCEMENT INDUCED BY SALICYLIC ACID.

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Recently some reports have shown that exogenous application of salicylic acid (SA) to plants induces a large variety of responses, among them flower and tuber induction. We tried to study these effects using two systems easy to work *in vitro*: leaf discs of *Streptocarpus nobilis* (a short-day plant) for flowering, and stem segments of *Ullucus tuberosus* for tuberization. Leaf discs of *Streptocarpus* were cultured in MS basal medium with IAA (0.1 $mg.L^{-1}$) and BAP (0.35 $mg.L^{-1}$) and 2% sucrose, under long or short days. Stem segments of *Ullucus*, with a node, were cultured in MS basal medium without growth regulators. In the case of *Streptocarpus*, SA did not replace short days to induce flowering, but the number of floral and vegetative buds was significantly increased with addition of SA (0.5-2.0 μM). The most remarkable result was that in the presence of SA, floral scapes were significantly elongated. When SA (0.5-1.0μM) was applied to *Ullucus* cultures, tuberization was not affected but the axillary buds developed shoots significantly higher than the controls. A complementary experiment showed that SA does not stimulate elongation in avena hypocotyl, a system highly sensitive to auxins. Attempts are in development to verify this effect in other systems and to found an eventual relationship between SA and the control of plant hormones activity.

275 ENDOGENOUS FUSICOCCIN AND FUSICOCCIN-LIKE SUBSTANCES

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Diterpenoid fusicoccin (FC) was discovered as a metabolite of a phytopathogenic fungus Fusicoccum amygdali Del. (Ballio et al., 1964). Exogenous fusicoccin is a hormone-like regulator for many plants (Marre, 1979). All higher plants studied possess plasmalemmal fusicoccin binding sites, or receptors. There are endogenous ligands for these binding sites (Aducci et al., 1980, Aducci et al., 1990; Babakov et al., 1994). We have proposed that FC may be an endogenous plant growth regulator. To prove this, we have used two approaches. The first, a conventional one, involves extraction of target substances from plant material, purification (HPLC), and identification (GC/SIM). We have found FC A in cabbage and maize (Muromtsev et al., 1986; 1989). Another approach consists in chemical identification of ligands revealed by radioreceptor and radioimmunoassays. For this purpose we used extracts from sterile cultured transformed horseradish roots, which ruled out the microbial origin of FC and FC-like substances (Muromtsev et al., 1994). Several HPLC fractions contained FC-like ligands, and in one fraction (with the FC A retention time) FC A was identified by means of mass spectrometry. These data give serious grounds for considering FC and FC-like substances as phytohormones.

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ADDITION OF CYTOKININ TO ALFALFA ROOTS CAUSES CORTICAL CELL EXPANSION AND TRIGGERS MsENOD40 GENE EXPRESSION IN BOTH THE WILD-TYPE AND MUTANT ALFALFA

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Plant phytohormones have been postulated to be involved with nodule development which occurs during the legume-*Rhizobium* nitrogen-fixing symbiosis. To study the relationship between phytohormones and nodulation, we examined early nodulin gene expression after alfalfa plants were treated with either Nod factor (the *Rhizobium*-produced signal molecule for nodulation) or phytohormones. *Msenod40*, but not *Msenod2*, expression was triggered by Nod factor treatment in uninoculated alfalfa roots. Among the different phytohormones, only cytokinin induced *enod* expression. In addition, cytokinin treatment or inoculation with a *R. meliloti* transconjugant carrying the *Agrobacterium tumefaciens trans*-zeatin secretion (*tzs*) gene resulted in MsENOD40 and MsENOD2 gene expression in the non-nodulating alfalfa mutant, MNN1008. Root outer cortical cells expanded in response to these treatments in both wild-type and mutant alfalfa and their cytoplasms became re-oriented to form "cytoplasmic bridges". NPA treatment of MNN1008 also resulted in the formation of nodule-like structures that contain MsENOD40 transcripts. These results indicate that a change in hormone levels induces early nodulin gene expression in legume roots and furthermore, this change in hormone levels is autonomous and downstream of *Rhizobium* and/or Nod factor perception.

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INTERCELLULAR COMMUNICATION BY NOVEL SEX PHEROMONES DURING SEXUAL REPRODUCTION OF THE HETEROTHALLIC *CLOSTERIUM PERACEROSUM-STRIGOSUM-LITTORALE* COMPLEX

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Closterium, a unicellular conjugatophycean alga, has thought to exploit various diffusible substances for the intercellular communication that is essential for its conjugation. A glycoproteinaceous sex pheromone, designated protoplast-release-inducing protein (PR-IP), that was released from mating-type plus (mt^+) cells during the sexual reproduction of the *C. peracerosum-strigosum-littorale* complex was isolated (Planta 1990) and the machanism whereby this protein induces the release of protoplasts from mating-type minus (mt^-) cells was characterized (J. Phycol. 1992, Planta 1993). In addition, cDNA clones for the 19-kDa and 42-kDa subunits of PR-IP were isolated (Plant Physiol. 1994). On the other hand, another sex pheromone, that was released from mt^- cells, was purified (Plant Cell Physiol. 1993, 1994). This pheromone, designated PR-IP Inducer, was a glycoprotein, constitutively released from mt^- cells in the light and responsible for the release of PR-IP from mt^+ cells. The dose-response curve showed that the amount of PR-IP released from mt^+ cells was in proportional to the concentration of PR-IP Inducer with an exponential relationship. The results of northern hybridization analysis revealed that the transcripts of cDNAs encoding either subunit of PR-IP appeared transiently only when mt^+ cells were treated with PR-IP Inducer in the light and that their patterns of accumulation were identical (Planta 1994). Based on these results, the possible intercellular communication during sexual reproduction of the *Closterium* was presented. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education (Japan) to H.S.

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EFFECT OF OLIGOGALACTURONIC ACIDS ON TRANSITION OF PLANTS FROM VEGETATIVE TO REPRODUCTIVE MORPHOGENESIS

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In a number of data on the interesting physiological characteristics of pectic cell wall fragments their activating and inhibiting influence on plant morphogenesis in vitro was shown. We investigated the influence of oligogalacturonic acids on plant morphogenesis with the application of new model system - intact plants in vivo. Stem apices of two photoperiodically sencitive plants: long-day *Rudbeckia bicolor Nutt.* and short-day *Perilla nankintnsis (Lour.) Decne.* growing under noninductive for flowering conditions - were dropped with the solutions of pentagalacturonic acid and oktagalacturonic acid. A part of plants was subjected simultaneously to a short photoperiodic induction. After droppind stem formation in *Rudbeckia* and flowers bud formation in *Perilla* were observed. Oktagalacturonic acid was found to be more morphogenetically aktive than pentagalacturonic acid when influencing *Rudbeckia*. In *Perilla* combination of oligogalacturonic acids with simultaneous influence of 5 days photoperiodic induction was found to be the most effective. The experiments conducted shoved the manifest morphogenic activity of oligogalacturonic acids during transition of plants from vegetation to flowering.

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SEASONAL ACTIVITY OF URIDINE 5'-DIPHOSPHOGLUCOSE : CONIFERYL ALCOHOL GLUCOSYL TRANSFERASE ACTIVITY LINKED TO CAMBIAL GROWTH IN JACKPINE

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Coniferin, the 4-*O*-β-D-glucopyranoside of *trans*-coniferyl alcohol, varies seasonally in perfect synchrony with seasonal diameter growth of conifers (Can. J. Bot. 67:2663, 1989; Forest Sci. 37:953, 1991). In this study, we investigated the activity of UDPG:coniferyl alcohol glucosyl transferase (CGAT), the enzyme catalyzing coniferin biosynthesis, in cambium and developing xylem of jackpine (*Pinus banksiana* Lamb.) over the annual cycle of growth and dormancy. Using coniferyl alcohol and UDPG as substrates, CAGT activity was quantified by reversed-phase high performance liquid chromatography (HPLC) with λ262 nm detection and peak area integration of coniferin. CAGT activity was also determined by incubating the enzyme and coniferyl alcohol with [^{14}C-glucose]-UDPG followed by HPLC, collection of the coniferin fraction, and counting of aliquots in triplicate by liquid scintillation spectroscopy. Similar CAGT activity data were obtained with both methods. CAGT occurred in the cytosol but activity could not be detected in the microsomal fraction. No CAGT activity was detected in the cambium in early spring, but activity appeared in synchrony with resumption of cambial cell-division activity. CAGT activity and endogenous coniferin both increased in association with elevated meristematic activity, then fell and dropped to non-detectability in early autumn at the time when cell-division activity had ceased and wood formation was still occurring. On a tissue weight basis, higher CAGT activity was present in developing xylem than in the cambium; however, CAGT activity disappeared in the lignifying tissue when cell divisions ceased, prior to the completion of lignification of the last differentiating cambial derivatives. Coniferin is considered to be a storage reservoir of coniferyl alcohol in support of lignification, but our data indicate that gene expression for CAGT may be linked to mitosis or cytokinesis.

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THE CONSTITUTION OF TWO LIGNANS BY INTERACTIONS OF A BASIC PEROXIDASE FROM MICROSOMES OF *ZEA MAYS L.* WITH FERULIC ACID AND CAFFEIC ACID

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The metabolism of cinnamic acids underlies in the early steps a strong enzymatical control. Isolated microsomes of green or elicited or etiolated seedlings of *Zea mays L.* contain a lot of these enzymes. Solubilised proteins were investigated in native anodal and cathodal electrophoresis. In our investigations we found 7 acid and 1 basic peroxidases. These groups of enzymes play an important role for the composition of the cell wall. The basic peroxidase is able to form dimeric products from cinnamic acids like ferulic or caffeic acid (FRIAS et al., 1991, GRÖGER et al., 1993, Turner et al., 1993). In the UV-spectra of products a bathochrome shift is specially noticed. This is a hint for a conjugated ring system. The identified products resulting from the enzymatical reaction are dimeric lignans with ß-ß-linkage of the propenoic acid side chain which was elucidated by HPLC, ESMS and ^{1}H-n.m.r.. H_2O_2 is a typical coenzyme of these kind of enzymes. In the native plant exist the hydrogen peroxide generating system from NADPH and oxygen (Gross, 1980, Gröger et al., 1994). The turnover of ferulic acid is 2,1 fold stronger in elicited or green plants in comparison to etiolated ones. In the case of the maize-sort "Bastion" was determined a specific catalytic activity of 40,66 μkat x mg^{-1} protein.
The mostly unknown effects of lignans and the easy inducibility of the responsible peroxidases raise the question for a possible role of these compounds in pathogen attack or signal transduction.

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INHIBITION OF PHENYLPROPANOID SYNTHESIS IN SOYBEAN CELLS DELAYS THE CESSATION OF CELL DIVISION ON CYTOKININ STARVATION BY SPARING POLYAMINES

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Effects on growth, mostly of an inhibitory nature, have been attributed to phenolic compounds *in vivo* and *in vitro.* Consequently, inhibition of their synthesis by L-α-aminooxy-β-phenylpropionic acid (L-AOPP), a competitive inhibitor of PAL, the enzyme controlling the first step in phenylpropanoid synthesis, was checked for stimulation of growth in soybean cell suspension cultures (*Glycine max*, cv. Acme). The promotive effect of L-AOPP, measured as an increase in cell number, was more clearly seen under growth limiting conditions of cytokinin starvation. At least one more cell division cycle was completed in the presence of L-AOPP before growth by division ceased and growth continued by expansion only. Phenolic acids conjugate, as is known, with polyamines, modulating the free levels of these plant growth substances. Thus, the effect of L-AOPP on the titres of free and conjugated polyamines (putrescine, spermidine and spermine) was investigated in the course of cytokinin starvation by HPLC. In the initial period especially (when polyamine synthesis would still have been active and before growth became restricted to cell expansion), an increased level of free putrescine was detected in the presence of L-AOPP relative to controls. This additional free putrescine, saved from conjugation by reduced availability of phenolic conjugation partners, temporarily delayed the decrease in free putrescine associated with the cessation of cell division. This indicates that an interaction between phenolic acids and polyamines is involved in the mechanism of growth promotion by L-AOPP.

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CULTURE FILTRATES OF LUTEOLIN-INDUCED *RHIZOBIUM MELILOTI* HAVE ATI-LIKE ACTIVITIES

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We have been studying the physiological conditions that lead to the production of an auxin transport inhibitor (ATI) by *Rhizobium meliloti.* Using a radioactive bioassay, we have shown that culture filtrates from *R. meliloti* induced with the flavonoid luteolin produce a factor that will compete with ^{3}H-*N*-1 (napthyl)phthalamic acid for its receptor on plant microsomal vesicles. The production of this activity is specific for luteolin and is independent of the luteolin-induced *nod* gene regulator, NodD. We have determined that ATI levels correlate with cell growth in minimal media. We show here that ATI production appears not to be a result of the catabolism of luteolin for nutritional needs because addition of carbon sources has no affect on ATI levels. The addition of nitrogen inhibits ATI production as well as growth. The production of this molecule also does not appear to be solely a by-product of starved cells, and *R. meliloti* will produce ATI even in chloramphenicol-treated cultures. A highly fluorescent compound with NPA-competing activity has been isolated from 10 liters of *R. meliloti* culture filtrate. The UV absorption spectrum indicates a maximum at 215-220 nm suggestive of a fatty acid. The specturm also lacks the characteristic flavonoid shoulder at 330-340 nm.

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EFFECTS OF SALICYLATE, ETHEPHON AND JASMONIC ACID ON THE ACTIVITLES OF PEROXIDASE AND CATALASE IN CUCUMBER

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Salicylic acid (SA), ethylene and jasmonic acid (JA) have been considered to be inducers of the resistance to pathogens in many plants. Peroxidase (POD) and catalase (CAT) are closely related with the production of active oxygen species which play important roles in the plant disease resistance. In this study, 50-d-old cucumber plants, grown in sterilized soil at 18℃ with a daily illumination of 12 hours, were used to study the effects of SA on CAT activity and the level of H_2O_2 and the effects of SA, ethephon (ETH) and JA, singly or in certain combination, on POD activity in the leaves. Results showed that significant increase in POD activity was found in the treated first true leaf within four days after spraying with SA. It is interesting to note that the untreated second leaf even showed greater increase in POD activity than the first leaf which received SA directly. All tested concentrations of SA, 0.5,1.0,2.5 and 5.0 mmol/L, gave similar effect on the POD activity with 2.5 mmol/L of SA more effective than others. ETH also induced an increase in POD activity in both treated leaf and untreated leaf 24 hours after spraying at the concentrations of 10^{-5},10^{-4},10^{-3} and 10^{-2}mol/L, among which the concentration of 10^{-3}mol/L gave the biggest effect. Whereas, JA only slightly increased POD activity in the treated first true leaf at the concentration of 0.05, 0.5, 5.0 and 50mmol/L. The combination of SA/ETH, SA/JA and JA/ETH showed additive effects on POD activity. The CAT activity and the level of H_2O_2 showed respectively a slight decrease and increase in the first true leaf 3, 6, 12 and 24 hours after incubation of the plants in 1/2 MS culture solution containing 1 mmol/L of SA. This work was supported by a grant from the NSF of the P. R. China.

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SALICYLATE ALSO PLAYS ROLES IN THE WOODY PLANTS—APPLE TREE

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Salicylic acid (SA) has been found to have important roles in flower induction of Lemna plants, heat production in Arum plants and the induction of the resistance to various pathogens in several plants, such as tobacco, cucumber, tomato and Arabidopsis. Our preliminary experiment showed that 5mmol/L of SA also significantly increased the resistance of Malus xiaojinchinesis, a kind of apple rootstock, to fungus pathogen, podospera lencotrica.. In this study one year old ` Golden Delicious´ apple trees were used to study the effects of various concentrations of SA on shoot growth, photosynthesis intensity and peroxidase (POD) activity which is considered to be associated with plant disease resistance. The results showed that the photosynthesis intensity underwent no change and no effect was found in the shoot growth in the same year after spraying with SA below 5mmol/L. Quaiacol was used to determine total soluble POD activity. Results showed that the greatest increase in POD activity apppeared in the treatment with 2.5mmol/L of SA, which was about 3-fold higher than that of the control, other tested concentrations, 0.25, 5.0 and 10.0mmol/L also increased the POD activity. The time course experiment showed that a great increase in POD activity was detected one and three days after spraying with SA, thereafter the increase declined, but maintained at least for seven days after the treatment. Polyacrylamide gel electrophoresis of POD isoenzyme showed that two new bands with Rf values of 0.53 and 0.56 appeared clearly in all test concentrations from one day to seven days after spraying. All these studies demostrated that woody plants, such as apple tree, are also sensitive to SA. This work was supported by a grant from the NSF of the P. R. China.

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THE TOBACCO ANIONIC PEROXIDASE: MOLECULAR CHARACTERIZATION OF AN AUXIN-REGULATED PROMOTER

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Auxin is known to alter the expression of many plant proteins. Protein profiles change significantly after exposure to auxin--the expression of some proteins is enhanced; others are suppressed. The genes for several of these auxin regulated proteins have been cloned. The majority of cloned genes are positively regulated by auxin and code for proteins of unknown function. To this list of auxin regulated genes we add the anionic peroxidase of tobacco (TobAnPOD). The anionic peroxidase is the predominant peroxidase isozyme in the aerial portions of tobacco. Transient expression assays with tobacco leaf protoplasts transformed with the TobAnPOD promoter (>3kb) fused to the GUS coding region and NOS terminator were used to screen for possible effectors of expression. Auxin reduced GUS expression significantly with half maximal suppression at 30 µM IAA or NAA. Full activity could be restored by the addition of the anti-auxin, PCIB. PCIB alone increased GUS expression above untreated controls. Ethylene, BA, GA, ABA, or heat shock had no effect on transient expression. 5' deletions of the TobAnPOD promoter/GUS fusion were transformed into protoplasts to identify possible regulatory regions. Both positive and negative regulatory regions were identified. Auxin suppression, however, was observed with all deletions, even when deleted to -60 bps. Sequencing the full promoter revealed several regions with homology to genes that are positively regulated by auxin. The tobacco anionic peroxidase is negatively regulated by auxin at the level of transcription, possibly by multiple regulatory regions within the promoter or by auxin responsive element(s) close to the TATA box or in the untranslated leader. The implications of auxin suppression of the tobacco anionic peroxidase is discussed in relation to plant growth.

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EXOGENOUS AUXIN REGULATES THE EXPRESSION OF ANIONIC PEROXIDASE IN LEAF AND LEAF PROTOPLAST OF TOBACCO

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Tobacco anionic peroxidase (TobAnPOD)is localized in cell wall and is believed to be involved in lignin biosynthesis. It is known that anionic peroxidases are also responsible in cell wall cross-linkage during primary growth. Treatments with various concentrations of IAA result in different effects on the gene. In protoplast isolated from leaf and transformed with TobAnPOD promoter fused with ß-glucuronidase (GUS) showed suppression of the gene expression at IAA concentration as low as 10 µM and half maximal suppression occurs at 30 µM. However, in leaves of whole plant transformed with the same gene chimera, 100 µM IAA suppresses this gene expression and 10 µM IAA induces its expression. Similar results were obtained with RNA hybridization, which indicates that the gene regulation by IAA is at the transcriptional level. More information of plant's RNA levels after treated with IAA will be obtained. Developmental studies of this gene in leaves displayed continuum of increasing activity from leaf emergence to maturity. The significance of these results to IAA regulation to plant growth and peroxidase's functions within will be discussed.

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MOLECULAR AND GENETIC STUDIES OF ADVENTITIOUS ROOT FORMATION IN *ARABIDOPSIS*

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Adventitious roots are those which form at an unusual location on the plant, such as stem or leaf tissue and have considerable agronomic significance in some species. We are studying adventitious root formation in *Arabidopsis thaliana* because of the genetic and molecular resources which this model system offers. We have developed a simple protocol by which 100% root formation can be induced on *Arabidopsis* stems by controlled exposure to indole butyric acid (IBA). We have previously observed more extensive IBA-induced root formation on a mutant *amt-1*, known to have elevated levels of tryptophan and IAA, suggesting that endogenous IAA may play a role in the plant's response to exogenous IBA or other rooting compounds. We have also observed a polarity in root formation on stem cuttings; all roots form at the basal end of the stem segments, suggesting that polar transport of endogenous IAA may be involved. In support of this idea, we find that root formation is inhibited by 2,3,5-triiodobenzoic acid (TIBA), an inhibitor of polar auxin transport. We are screening a variety of *Arabidopsis* mutants with characterized alterations in IAA physiology to investigate the roles of each of these parameters in the rooting process. Using differential display we have identified transcripts specifically expressed in the callus stage which precedes root emergence, as well as transcripts restricted to the rooting stage. We plan to characterize unique transcripts identified by this process and to study their expression and role in the rooting process.

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EARLY AUXIN-REGULATED GENE EXPRESSION IN TOBACCO.

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The phytohormone auxin is involved in the control of many aspects of plant growth and development. Auxin-mediated cell growth is a fast response, elongation occurring after a lag period of 12 to 20 minutes. Prior or concomitantly to cell elongation auxin triggers the rapid induction of specific mRNAs; the regulation of this process appears, at least partly, to be at the transcriptional level. We have cloned several auxin-regulated cDNAs from auxin-treated tobacco seedlings. Their sequence analysis revealed significant identities with previously described auxin-responsive genes characterized in soybean, mungbean, *Arabidopsis* or pea. They belong to the families of SAUR, Aux-ARG-IAAx or ADR genes. We have characterized the expression of these genes in response to auxin treatment in terms of dose-response, kinetics and specificity on 7 day-old tobacco seedlings. We have tried to establish a correlation between auxin-inducible transcript levels and endogenous auxin content. The transcript levels of four different auxin-inducible genes (2 different Aux, par A and B) were analysed in different tissues of mature tobacco plants and related to the endogenous content of free IAA. As model plants, we explored two tobacco genotypes differing in their IAA synthesis rate and steady-state level of free IAA, the wild-type tobacco SR1 and the derived transgenic line C, expressing the IAA biosynthesis genes iaaM and iaaH from the *Agrobacterium tumefaciens* Ti-plasmid. The data suggest a differential regulation of the different auxin-inducible genes by endogenous IAA. The effect of exogenous NAA treatments was also investigated on both genotypes. Previous results in our group demonstrated that another early response to auxin, the modification of the electrical potential of tobacco protoplasts, involves auxin perception at the plasma membrane.Work is in progress to determine if these plasma membrane receptors are able to trigger the regulation of particular auxin-responsive genes.

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EFFECT OF GA_3, 4-CL-IAA, AND SEEDS ON PROTEIN SYNTHESIS IN PEA PERICARP

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Our model system using pea fruit offers the unique feature of studying growth and development in a tissue that is responsive to both gibberellins (GAs) and auxin (4-Cl-IAA). Two-dimensional gel electrophoresis was used to characterize *de novo* synthesis of proteins during GA_3-, 4-Cl-IAA-, and seed-induced pea pericarp growth. Pea pericarp (2 DAA) were split down the dorsal suture either without disturbing the seeds (SP; control), or removing seeds (SPNS) 12 hours prior to hormone treatment to deplete the tissues of any seed stimulatory factors. GA_3, 4-Cl-IAA or GA_3 + 4-Cl-IAA ($5X10^{-5}$ M; 30 µl) were applied to the inner-wall of the pericarp attached to the plant. [^{35}S]-methionine was applied 4 hours prior to the end of the 6 hour hormone incubation period. Many polypeptides observed in the SPNS pericarp were not present in the SP pericarp or hormone-treated deseeded pericarp. Fourteen polypeptides present in the SP pericarp tissue (absent in deseeded pericarp) were also present in deseeded pericarp treated with GA_3 + 4-Cl-IAA or 4-Cl-IAA only; only 10 of these polypeptides were present in the deseeded pericarp treated with GA_3. These data show that exogenous 4-Cl-IAA + GA_3 and 4-Cl-IAA treatments affect protein synthesis in pea pericarp similarly to the seeds. However, differences in polypeptide patterns between GA_3 and 4-Cl-IAA (naturally occurring hormones in pea fruit) suggest they differentially affect growth and development of pea pericarp. Supported by NSERC grant OGP0138166

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REGULATION OF GENE TRANSCRIPTION BY ETHYLENE DURING TOMATO FRUIT RIPENING

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Ethylene activates transcription of a set of genes at the onset of tomato fruit ripening. We are studying the mechanism of ethylene control of transcription of two of these genes, E4 and E8. The expression of E4 is strictly dependent on the presence of ethylene, and E4 can be activated by ethylene in leaves as well as fruit. Analysis of the E8 promoter confirmed that E8 is controlled both by ethylene and by ethylene-independent fruit ripening developmental signals (Deikman et al., *Plant Physiol.* 100: 2013-2017, 1992). The combined evidence of cis-elements and interacting DNA-binding proteins suggests that activation of transcription of E4 and E8 by ethylene requires the action of two cis-elements. Deletion of E4 sequences from -140 to -85 eliminated ethylene responsive expression of a chimeric gene, and a linker-scanning mutation series of E4 sequences from -160 to -90 indicated that nucleotides between -140 and -131 are the most important for ethylene-responsive expression. The second cis-element required for ethylene responsive gene transcription spans the E4 TATA box. Mutation of the E4 TATA box to the TATA box for polygalacturonase, a gene that is expressed during fruit ripening but not in response to ethylene, destroyed ethylene-responsive transcription of the chimeric gene. The E4 TATA box interacts with a nuclear protein we previously showed also interacts with sequences upstream of the E8 gene (not overlapping the E8 TATA box), and this E8 binding site is 3' to sequences required for ethylene-regulated transcription of E8. We are now studying the function of this DNA-binding protein by examining the consequences of mutation of the E8 binding site on gene transcription.

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CLONING AND EXPRESSION ANALYSIS OF ONE GENOMIC AND THREE cDNA CLONES FOR TOMATO ABSCISSION POLYGALACTURONASES

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Leaf abscission was induced by exposing tomato explants to 25 µL/L ethylene for 72 h. A cDNA library was constructed using mRNA extracted from ethylene-induced leaf abscission zones. The library was screened with a cDNA clone for an avocado fruit-ripening polygalacturonase. A 1.2 kbp cDNA clone, TAPG1, was identified. Rescreening of the same cDNA library and a tomato genomic library with TAPG1 as a probe resulted in the identification of two more putative PG cDNA clones and one genomic clone, respectively. The three PG cDNA clones and the genomic clone share 80% to 90% identity at the amino acid level. Preliminary RNA blot experiments showed expression of the three PG cDNA clones in ethylene-treated leaf and flower abscission zones but not in petiole and fruit tissue. We are currently using RNase-protection and *in-situ* hybridization to define the tissue-specific and spatial expression patterns for each of these four PG genes.

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ETHYLENE-INDUCED CELL SEPARATION INVOLVES EXPRESSION OF DIFFERENT CELLULASES IN PEPPER PLANTS

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Cell separation is a general phenomenon which occurs at several moments throughout the life of a plant. It is caused by the activity of a number of cell wall hydrolases produced immediately before and during the phenomenon. In many cases, *de novo* synthesis of these enzymes is promoted by ethylene. In particular, this hormone induces an increase in cellulase (EC 3.2.1.4) activity during the main cell separation events which occur in pepper plants. RT-PCR experiments have allowed the cloning of three short DNA fragments encoding three different cellulases. cDNA libraries have been constructed by using poly A^+ RNA obtained from both leaf abscission zones and ripening fruits. Screening these libraries with the three cellulase cDNAs has yielded a number of positive clones. Sequence analysis has confirmed the presence of three different mRNAs coding for cellulase in pepper plants. Expression of the different cellulases has been studied following ethylene induction of cell separation events.

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ISOLATION OF CUCUMBER ACC SYNTHASE AND ACC OXIDASE GENES: THEIR ROLE IN SEX EXPRESSION

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Sex expression in the cucumber plant, *Cucumis sativus* is genetically controlled but can be modified by environmental conditions and plant hormone treatments. Hormonal regulation of sex expression is complex, with auxin and ethylene promoting feminization and gibberellins and ethylene inhibitors promoting masculinization. DNA was extracted from leaves of gynoecious and monoecious isogenic lines of cucumber (*Cucumis sativus* cv. Marketmore). An ACC synthase gene (CS-ACS1) and two distinct ACC oxidase genes (CS-ACO1/2) were successfully amplified from DNA by polymerase chain reaction (PCR), using degenerate primers. Phylogenetic analysis of the amino acid sequence deduced from the PCR product shows that CS-ACS1 belongs to a group of ACC synthases that have been demonstrated to be induced by auxin in vegetative tissues. Expression of these genes in apices of monoecious and gynoecious isogenic genetic lines and in response to treatments such as ethylene and auxin that induce female flower development is currently under study. Southern blot hybridization analysis of nuclear DNA was carried out in both monoecious and gynoecious lines.The hybridization pattern between the two sex lines was identical when pCS-ACO1/2 were used as the probe(s), while when probing with pCS-ACS1 strong hybridization to an additional fragment in the gynoecious line was apparent. Thus, a restriction fragment length polymorphism (RFLP) exists at the ACC synthase level in this genetic material.

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DIFFERENTIAL EXPRESSION OF 1-AMINOCYCLOPROPANE-1-CARBOXYLATE SYNTHASE ISOGENES IN MUNGBEAN HYPOCOTYLS

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We have studied expression patterns of ACC synthase isogenes in mungbean hypocotyls, using three different cDNAs; pVRACS1, pVRACS6 and pVRACS7. Auxin treatment increased the levels of all three cDNA transcripts with different kinetics and magnitudes. In the absence of IAA, transcripts of ACS6 and ACS7 were not detected, but ACS1 showed weak basal expression level. Expression of these genes were also regulated by kinetin, ethylene and cycloheximide, respectively. All three isogenes were induced by kinetin with different magnitudes. The kinetin effect was most effective on ACS1 expression. Ethylene supressed the auxin-induced accumulation of three ACS transcripts. Cobalt ion which supresses ACC oxidase activity, increased the level of ACC synthase transcripts. NBD did not affect the expression of auxin-induced ACC synthase genes. Cycloheximide treatment resulted in increased level of ACS1 transcripts, while it reduced auxin-induced accumulations of ACS6 and ACS7 transcripts. These results suggest the existence of an operation of different regulatory networks in the expression of these genes.

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CHARACTERIZATION OF AN AUXIN-INDUCIBLE 1-AMINOCYCLOPROPANE -1-CARBOXYLATE (ACC) SYNTHASE GENE, VR-ACS6, IN MUNGBEAN

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ACC synthase is the key enzyme in the biosynthetic pathway of the plant hormone ethylene. In mungbean (*Vigna radiata*), ACC synthase is known to be encoded by at least seven divergent genes. One of them, VR-ACS6, was specifically induced by auxin signal. The effect of auxin was very rapid, detected within 30 min after treatment. Ethylene and ABA supressed the auxin induced accumulation of VR-ACS6 transcripts, respectively, while kinetin increased it. We have isolated and structurally characterized the gene. VR-ACS6 consisted of three exons and two introns where the position of the first intron was conserved as in other ACC synthase isogenes. The promoter of VR-ACS6 contained potential *cis*-acting regulatory elements responsible for the hormonal responses. Two of the sequence motives [TG(G)GATTTT, GACCCAC] located within -91 to -52 of VR-ACS6 were thought to be the putative AuxRE. They were also found in the promoter regions of twin genes for auxin-type ACC synthase from winter squash. We are currently investigating functions of the DNA sequences with transient assay system using mungbean protoplasts.

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cDNA CLONING AND CHARACTERIZATION OF A GIBBERELLIN-RESPONSIVE GENE IN CUCUMBER HYPOCOTYLS

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A cDNA clone corresponding to a gibberellin (GA)-responsive gene (*CRG16*) was isolated from hypocotyls of cucumber (*Cucumis sativus* L. cv. Spacemaster 80) by differential screening. *CRG16* is thought to contain an open reading frame of 195 bp encoding a protein (CRG16) that is extremely hydrophobic and contains a proline-rich region. The amino acid sequence of CRG16 does not show significant homology with any entry in databases. Northern blot analysis revealed that the *CRG16* mRNA level in the hypocotyls began to increase 1-3 hours after GA4 treatment and was highest 6 hours after treatment. On the other hand, cucumber hypocotyl elongation began 4-6 hours after GA4 treatment and was still continuing 24 hours after treatment. The accumulation of *CRG16* mRNA was strongly promoted in the elongating region by GA4 treatment. These results indicate that the accumulation of *CRG16* mRNA is closely related to cucumber hypocotyl elongation promoted by GA4 treatment.

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GIBBERELLINS AND ALPHA-AMYLASE GENE EXPRESSION IN GERMINATING WHEAT GRAINS

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Evidence that gibberellins (GAs) are components of the embryo-produced stimulus that initiates α-amylase production in intact cereal grains is far from conclusive. The scutellum of wheat was identified as the major site of *de novo* GA biosynthesis by measuring ent-kaurene accumulation in grains treated with 2*S*,3*S*-paclobutrazol. Several GAs of the early 13-hydroxylation GA pathway accumulated in the embryo of untreated grains between 24 and 48 h. [^{14}C]-GA_{20} was metabolised to GA_1, GA_3, GA_8 and GA_{29}, showing the presence of both 2β- and 3β-GA hydroxylase activities in wheat embryos. Bioactive GA_1 and GA_3 also increased in the endosperm during this period, whereas abscisic acid contents of embryo and endosperm decreased rapidly. Treating grains with 2*S*,3*S*-paclobutrazol decreased GA_1 and GA_3 contents of scutella by 70-80% over 4 days without affecting α-amylase mRNA transcripts significantly. In contrast, a 50-80% decrease in the content of bioactive GAs in the endosperm was associated with a 70-80% reduction in transcripts for both α-amylase gene families in aleurones of paclobutrazol-treated grains. It was concluded that the initiation of α-amylase gene expression in aleurone was dependent on embryo-produced GAs, whereas that in scutella appeared to be independent of *de novo* GA biosynthesis.

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STRUCTURE OF A GIBBERELLIN (GA_3) RESPONSIVE GENE EXPRESSED IN SHOOT TISSUE OF DWARF *Pisum sativum* L.

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Cis-acting hormonal response elements are involved in the gibberellin (GA_3) induced gene expression in cereal grain germination (Skriver *et. al.* 1991, Rogers and Rogers 1992) and are putatively required for stem elongation in GA-induced gene expression in dwarf peas. In order to identify and characterize such regulatory elements a ^{32}P-labeled 901 base pair cDNA for a GA-regulated gene in pea stem was used to screen an EMBL-3 pea genomic library (Clontech). Southern analysis of restriction fragment digests of two positive clones was performed. These two clones represent slightly different fragments of the same genomic region. The sequence of the gene and associated 5' region are presented along with identification of GA response elements as ascertained by mobility shift and fingerprint assays. Promoter function is to be characterized by transient transfection of pea protoplasts by promoter truncation experiments using a GUS reporter system.

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MEMBERS OF A NEW FAMILY OF DNA-BINDING PROTEINS BIND TO A CONSERVED *CIS*-ELEMENT IN THE PROMOTERS OF α-*AMY2* GENES

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The promoters of wheat, barley and wild oat low pI α-amylase (α-*Amy2)* genes contain a number of conserved *cis*-elements. We have isolated two cDNAs encoding proteins (ABF1 and ABF2) that bind specifically to one of these elements, Box2, contained within the sequence ATTGACTTGACCGTCATCGG. The two proteins are unrelated to each other except for a conserved region of 56-58 amino acids that consists of 25 highly conserved amino acids followed by a putative zinc-finger motif, $C\text{-}X_{4\text{-}5}\text{-}C\text{-}X_{22\text{-}23}\text{-}H\text{-}X_1\text{-}H$. ABF1 contains two such regions, whereas ABF2 possesses only one but also contains a potential leucine zipper motif, suggesting that it could form homo- or heterodimers. ABF1 and ABF2 expressed in *E.coli* bound specifically to Box2 probes in gel retardation experiments; this binding was abolished by the transition-metal-chelating agent 1,10-*o*-phenanthroline and by EDTA. ABF1 and ABF2 appear to be representatives of two classes of a new family of plant sequence-specific DNA-binding proteins. Work is underway to establish the binding site characteristics of these two DNA-binding proteins and their involvement in α-*Amy2* gene expression.

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CDNA CLONING AND CHARACTERIZATION OF GIBBERELLIN-RESPONSIVE GENES IN PHOTOBLASTIC LETTUCE SEEDS

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The germination of photoblastic lettuce (*Lactuca sativa* L. cv. Grand Rapids) seeds is under phytochrome-mediated photocontrol. Both exogenously applied gibberellin A_1 (GA_1) and red light irradiation induce germination, and the endogenous level of GA_1 increases after red light irradiation. These facts indicate that GA plays an important role in the induction of germination. In order to accumulate information on genes which control lettuce seed germination, we have tried cDNA cloning of GA-responsive genes in photoblastic lettuce seeds. Two cDNA clones, cLRG5 and cLRG11, for GA-responsive genes were isolated from photoblastic lettuce seeds by differential screening. Northern blot analysis using inserts of these cDNA clones showed that the level of *LRG5* and *LRG11* mRNAs increased 10 h after GA treatment, and that red light irradiation also increased the mRNA level. Nucleotide sequencing showed that cLRG5 contained an open reading frame encoding a putative polypeptide consisting of 380 amino acids. The putative polypeptide showed a very high degree of homology with alcohol dehydrogenases from several species of higher plants. With regard to cLRG11, no homologus gene has been reported.

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ANALYSIS OF GIBBERELLIN-REGULATED GENE EXPRESSION IN ANTHERS OF THE GIBBERELLIN-DEFICIENT MUTANT OF TOMATO (*GIB-1*)

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The role of gibberellin (GA) in the anther development is studied the *gib-1* mutant of tomato. The GA-deficient mutant is both male and female sterile, but this phenotype is reversed by GA application. The pollen mother cells in the mutant are arrested in the G1 phase of premeiotic interphase. Jacobsen and Olszewski have isolated cDNAs corresponding to mRNAs that are regulated by GA treatment. Developmentally arrested anthers treated with GA3 were used after 48 h treatment in a differential screening. This screening resulted in the isolation of several clones which we have obtained and analysed. After Northernblot analysis the clones could be placed in two classes with respect to the kinetics of their expression patterns. The clones of class I increase in abundance 8 h after GA3 treatment, while class II consists of clones that are newly expressed 48 h post treatment. Accumulation of gene expression of class I was already found when arrested buds were treated with 0,5 ng GA3/bud. The clones were sequenced and further analysed for their gene expression in the wild type tomato. The clones will be used as early developmental markers for studying the role of GA in the anther development of tomato.

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GIBBERELLIN-INDUCED EXPRESSION OF HISTONE H3 IN THE GROWING INTERNODE OF DEEPWATER RICE

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Rapid growth of submerged deepwater rice is induced by the combined action of the phytohormones ethylene and gibberellin (GA). In isolated stem sections containing the highest internode, rapid growth can be induced by GA alone. Previous work in our laboratory has shown that only cells in the intercalary meristem respond to GA. We therefore focussed our attention on transcripts accumulating during GA-induced growth in the intercalary meristem. To attain this goal, we used the differential display method to identify differential expressed genes, following application of GA. The first gene identified encodes histone H3 and the onset of expression of this gene correlates with the onset of DNA synthesis in the intercalary meristem. However, GA also induces the expression of H3 in regions of the internode other than the intercalary meristem.

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STRUCTURE AND EXPRESSION OF A PEA INVERTASE GENE

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In order to determine the relationship among gibberellin (GA) stimulation, invertase gene expression and stem elongation in dwarf peas, a pea genomic library (Clontech Laboratories) was screened with a ^{32}P-labeled partial-length cDNA prepared by PCR from GA-treated dwarf pea cDNA (PcI-5). The two primers used in PCR correspond to the two conserved amino acid sequences (NWMNDPNG and MWECVDF) found in invertase sequences from pea and other species. Restriction enzyme mapping and Southern blot hybridization studies identified two positive clones with an identical 22 kb insert and a third, with a 14.5 kb sequence which is included in the other two. A 3.1kb fragment cut by EcoR I and Sal I from one of these clones has been completely sequenced by the dideoxynucleotide chain-termination method. Alignment of this sequence with PcI-5 and the invertase gene of *Arabidopsis thaliana* indicates this is the 5' end of a pea invertase gene with its first 3 exons. The organization of this partial gene is similar to that of invertase genes in *A. thaliana* and *Lycopersicon esculentum*. Exon 1 contains a hydrophobic region with all the characteristics of a signal peptide and its deduced amino acid sequence has 52% identity to the exon I of *A.thaliana*. Exon 2 is only 9 bp long and encodes part (DPN) of a highly conserved region found in all known invertase proteins (NDPNG). Two pyrimidine boxes (CCTTTT) and one variant (60% identity) of a GA response element (GARE) of the GA response complex in the barley alpha-amylase promoter were found in the 5' untranslated sequence. Expression of the invertase gene, as determined by both Northern blot hybridization and RT-PCR, is detected in root and young internode tissue. Kinetic studies of the invertase gene in response to GA are presented.

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REGULATION OF HYDROXYPYRUVATE REDUCTASE GENE EXPRESSION BY CYTOKININ

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To better understand the mechanism of cytokinin action, we have investigated the cytokinin-modulated expression of the hydroxypyruvate reductase (HPR) gene in the emerging cotyledons of pumpkin and cucumber seedlings. A pumpkin HPR cDNA was generated by RT-PCR and its nucleotide sequence was determined. An antisense HPR RNA was prepared for RNase protection analysis of HPR mRNA expression patterns in the cotyledons of dark-grown pumpkin seedlings. Treatment of the cotyledons with benzyladenine (BA) was shown to modulate HPR mRNA levels in a dose- and time-dependent manner. Similarly, nuclear run-on studies showed that the rate of transcription is also enhanced by BA treatment of the cotyledons. Band-shift analysis of the 2.2 kb region upstream of the cucumber hpr-A gene revealed a 97 bp restriction fragment (-382 to -285) showing unique cytokinin- or light-enhanced binding of nuclear proteins prepared from nuclei of etiolated cucumber cotyledons. DNase I footprints showed that the protected promoter region (-326 to -306) contains a nucleotide sequence similar to the as-1 motif found upstream of various light-inducible genes. Characterization of the band-shift complexes by two-dimensional band-shift analysis reveals several independent DNA-protein complexes, while *in situ* UV-crosslinking assay suggests a common core of proteins bound to the DNA (Supported by USDA grant 94-37304-1047).

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PROBING CYTOKININ ACTION - II

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We have been using the moss, *Physcomitrella patens*, as a model system to study cytokinin-mediated plant development. Most plant hormones cause rapid transcriptional changes in target cells, although none have been established for cytokinin. We have used a number of differential screening approaches, none of which resulted in the isolation of cDNAs corresponding to transcripts showing rapid changes in response to cytokinin. We concluded that cytokinin was not having an early effect (within 8 hours) on transcription. To identify a window of transcriptional changes induced by cytokinin, we isolated RNA from moss plants untreated and treated with cytokinin for between 8 and 72 hours, performed *in vitro* translations and analyzed the polypeptide products using 2-D gels. Changes in polypeptide spots were apparent between 12 and 24 hours of treatment with cytokinin. These data correlate with the observation that the cytokinin response is reversible within 24 hours. Since cytokinin promotes a change in meristem activity in moss development, we have isolated cDNAs showing high similarity to genes from higher plants that regulate meristem development. Moss cDNA homologues of the *floricaula (flo)* gene from *Anthirrinum*, and the corresponding *Arabidopsis* gene, *leafy*, have been isolated. Northern hybridisations showed that moss *flo* transcripts were present in moss plants only after 36 hours treatment with cytokinin. Thus, the transcriptional activation of the gene(s) is unlikely to be a primary effect of cytokinin. More likely is that the gene(s) are involved in downstream events in gametophore development. Further Northern analysis, together with the production of transgenic plants, carrying sense and antisense moss *flo* cDNAs, are being generated to examine the function of these genes. We have also isolated MADS-box genes from moss and preliminary data suggest that the genes are activated after 12 hours treatment with cytokinin. These genes may therefore be candidates for cytokinin-response genes in mosses. Further work is in progress to generate transgenic mosses carrying antisense MADS-box cDNA constructs.

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EFFECTS OF ALLIEN ipt GENE ON THE DISTRIBUTION OF PHYTOHORMONES IN TRANSGENIC TOBACCO PLANTS

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Expression of ipt gene for cytokinin synthesis and selection of cytokinin-transgenic plants at the stage of propagation by cutting lead to construct genetically determinated regenerants of normal phenotype (shoot-root). Cytokinins (Z,ZR,2iP, 2iPA) and free IAA were determined by HPLS and ELISA test in leaves and roots of normal (N) and transgenic (cyt-T) aseptically grown plants. In roots of N plants the level of endogenous cytokinins was higher than in leaves. Also the contents of 2iP and 2iPA were higher than that of Z and ZR; and 2iP+2iPA/Z+ZR ratio was constant. In roots of cyt-T plants the cytokinins level was about 25% of total one, and these cytokinins were 2iP and 2iPA, mainly. On the contrary, in leaves of cyt-T plants the level of cytokinins was significantly higher, and these cytokinins were Z and ZR. Expression of ipt gene increased also level of endogenous freeIAA in leaves, but not in roots. Thus, it is shown that there are some differences in the hormone distribution between leaves and roots as a result of expression of ipt gene.

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ISOLATION OF CYTOKININ INDUCED GENES IN *NICOTIANA TABACUM* PETIT HAVANA SR1 THIN CELL LAYER CULTURES.

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Using Thin Cell Layer (TCL) culture, a system was developed to study gene expression associated with the initiation of cytokinin induced vegetative bud formation in *Nicotiana tabacum* Petit Havana SR1. Reproducible shoot formation was obtained on Thin Cell Layers on liquid MS medium containing $3x10^{-6}$ M indole-3-butyric acid and 10^{-5} M kinetin. Callus formation was induced in the presence of $3x10^{-6}$ M indole-3-butyric acid and $5x10^{-7}$ M kinetin. mRNA expression in both cultures was examined by means of differential display PCR during a time course of 12 days. Genes showing preferential expression in the shoot induction culture were isolated as putative cytokinin induced genes involved in shoot formation. The poster will discuss the methodology and the characterization of the isolated genes.

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EVIDENCE FOR POST-TRANSCRIPTIONAL REGULATION OF THE *SESBANIA ROSTRATA* EARLY NODULIN GENE *SRENOD2* BY CYTOKININ

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The *SrEnod2* gene codes for a proline-rich protein which is expressed at early stages of *S. rostrata* root-and stem nodule development. Previously it has been shown by Dehio and de Bruijn (The Plant Journal 2(1), 117-128; 1992) that the *SrEnod2* message accumulates in roots, in a time and concentration dependent manner, in response to the exogenous application of cytokinin. A short (10 minutes) exposure of roots to cytokinin is sufficient to stimulate *SrEnod2* mRNA accumulation. In order to determine if *SrEnod2* mRNA accumulation in response to cytokinin is via transcriptional and or post-transcriptional processes, nuclear run-on assays were preformed. Two week old *S. rostrata* seedlings were treated with the cytokinin BAP for time periods of 0 (no BAP), 2, 4, and 6 hours, and nuclei/RNA were isolated. Radiolabelled transcripts from the nuclei at each time point were hybridized against an immobilized *SrEnod2* DNA probe on slot blots. No change in trancription of the *SrEnod2* gene occurred over the time course of BAP treatment, although *SrEnod2* mRNA accumulated to greater than 4 fold, indicating an involvement of post-trancriptional processes. The translational inhibitor, cycloheximide, was found to be capable of inhibiting *SrEnod2* mRNA accumulation by cytokinin. In addition, cycloheximide when applied after cytokinin treatment caused a rapid decay of *SrEnod2* mRNA. The protein phosphatase and kinase inhibitors, Okadaic acid and staurosporine, respectively, were applied exogenously to *S. rostrata* plants in order to determine if protein phosphatases and kinases are involved in mediating this signal transduction pathway. Okadiac acid completely abolished *SrEnod2* mRNA accumulation as well as basal levels of *SrEnod2* mRNA, whereas staurosporine inhibited only the response to cytokinin. Experiments determining *SrEnod2* mRNA levels in both nuclear and ribosomal fractions in response to cytokinin will be presented.

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PARALLEL EFFECTS OF HORMONES ON HYBRID PROLINE-RICH PROTEIN (HyPRP) GENE EXPRESSION AND HAUSTORIUM INDUCTION IN PARASITIC ANGIOSPERM *CUSCUTA REFLEXA* SUGGEST A NEXUS BETWEEN THE TWO PROCESSES INVOLVING CYTOKININ.

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Cytokinin (BA or iP) induced haustoria formation in *Cuscuta* vines *in vivo* or in vine segments *in vitro*; auxin (NAA) or gibberellin (GA_3) inhibited the induction. Subapical (2-6 cm from apex) segments, maximally responsive to BA, were committed to haustoria formation by 20 h of its treatment when NAA or GA_3 no longer inhibited. A *Cuscuta HyPRP* cDNA clone was isolated and identified by differentially screening a BA-induced haustorial cDNA library (cf *Gene 141* (1994) 207-210). A *Cuscuta extensin* cDNA clone was isolated from the same library using carrot *extensin* probe. *HyPRP* and *extensin* gene expression were compared by Northern analyses using stringent wash protocol and laser densitometry. The results showed: 1. *in vivo*, *HyPRP* mRNA level was highest in the same subapical region maximally responsive to BA *in vitro* in haustorium induction and fell steeply to negligible level in later growing and non-growing (15-24 cm) regions. Significantly, endogenous iP in the apical (0-5 cm) region was 5-10 times that in the next (5-10 cm) region. By contrast, *extensin* transcript level, low in the apical (0-6 cm) region, was maximal in the non-growing region; 2. *in vitro*, BA treatment maintained *HyPRP* transcript level upto 24 h when segments were committed to haustoria formation, while it fell in control segments. In the presence or absence of BA, NAA or GA_3 treatment reduced *HyPRP* mRNA level in 24 h and also inhibited haustoria formation. In contrast, BA reduced *extensin* mRNA in 24 h; NAA inhibited this reduction. The results suggest BA acts by stabilizing *HyPRP* transcripts while NAA and GA_3 control by inhibiting *HyPRP* gene transcription.

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THE EFFECT OF ABSCISIC ACID ON THE SYNTHESIS OF NUCLEIC ACIDS IN GERMINATING COTTON SEEDS

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The influence of abscisic acid on the content and synthesis of RNA and ribonucleoprotein psrticles (polysomes, ribosomes and informasomes) during early stages of the germination of cotton seeds (Gossypium hirsutum, 108-F variety) is studied. The effect of the hormone's action depends on the organ, concentration and duration of the ABA action. Synthesis of different RNA fractions and formation of ribosomes and polysomes is inhibited in the presence of ABA during seed germination. Degradation of polysomes takes place under the influence of ABA. It results in the increase of ribosomes' contents which do not participate in protein synthesis, in the shift of the qualitative state ofsynthesized RNA molecules and ribonucleoproteid particles. The study of ABA influence on the already synthesized RNA and RNP-particles has shown that redistribution of radioactivity by the fractions of RNP-particles which is expressed in the considerable reduction of the label in the polysome area and its increase in the ribosome and postribosome areas takes part. The analysis of the obtained data proved that the range of ABA action is sufficiently wide and its influence is manifested on the translation and transcription level, moreover, direct as well as electoral effect on the definite stages of metabolites' biosynthesis is observed.

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ABA-REGULATED GENE EXPRESSION IN GUARD CELLS OF *PISUM SATIVUM*

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Stomatal guard cells, being highly specialized sensory and effector cells, represent an excellent system with which to study the processes of signal-response coupling. The phytohormone abscisic acid (ABA) is one of the signals to which guard cells respond by initiating K^- flux across the plasma membrane, leading to stomatal closure. ABA is also known to induce the expression of a number of genes in various cell types. However, although guard cells are exposed to high concentrations of ABA via the transpiration stream and are clearly able to sense and respond to increased levels of ABA, it is not known whether such ABA inducible genes are also expressed in guard cells. Our aims are to investigate the expression of such genes by northern analysis, using as a probe the pea cDNA clone pPsB28, representing an ABA-inducible dehydrin gene (Robertson and Chandler (1992) Plant Mol. Biol. **19**: 1031-1044). Epidermis and mesophyll tissue were prepared from the *argenteum* mutant and isolated guard cells obtained by treatment of peels with cell wall-degrading enzymes. The ABA content of various tissues was determined by radioimmunoasssay. Our results demonstrate that mesophyll, epidermal and guard cells accumulate dehydrin mRNA when exposed to endogenous and exogenous ABA *in planta*, and that dehydrin gene expression is rapidly terminated on removal of the stimulus. Dehydrin expression was also detected in guard cells exposed to ABA *in vitro*, indicating that after enzyme isolation these cells are still competent to respond to ABA by altering their gene expression. We have examined *in vitro*, the dose response characteristics of ABA-induced dehydrin expression, responses to ABA treatments at high and low pH and the effects of osmotic stress (0.6M mannitol) in these tissues.

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JASMONATE AND ABA HAVE DIFFERENT SIGNALLING PATHWAYS IN BARLEY (HORDEUM VULGARE L.)

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We are interested in the recognition of Jasmonate and the subsequent signal transduction that leads to the activation of specific genes encoding jasmonate induced proteins (JIPs). So far, all known barley JIPs are induced by both, jasmonate and abscisic acid (ABA). The question whether ABA and jasmonate act through the same signalling pathway has not been answered conclusively (1). Using differential display (2) we have identified PCR fragments that hybridize only to RNA from jasmonate treated leaves but not to RNA from ABA treated leaves. Our results provide direct evidence that jasmonate responsive genes (JRGs) are not automatically responsive to ABA, suggesting that both hormones act via separate signalling pathways. This finding will be very important for the analysis of cis regulatory elements in jasmonate responsive genes since an interference with ABA response elements can be excluded.

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A 221-bp PROMOTER FRAGMENT OF THE TOMATO GENE LEUCINE AMINOPEPTIDASE IS CRUCIAL FOR WOUND- AND JA-RESPONSE

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By screening for genes that are induced upon wounding and by the plant hormone abscisic acid (ABA) in leaves of potato plants several cDNA clones were isolated, which showed homology to cysteine and cathepsin D protease inhibitors, and to bovine lens leucine aminopeptidase. The mRNAs corresponding to these genes were shown to accumulate systemically in leaves after mechanical wounding, and also to be strongly induced by jasmonic acid (JA). Genomic clones encoding leucine aminopeptidase (LAP) were isolated from a tomato genomic library using the potato cDNA as hybridization probe. Analysis of GUS activity resulting from expression of chimeric LAP promoter-GUS genes in transgenic potato plants revealed expression in leaves, after mechanical wounding or treatment with abscisic and jasmonic acids. Promoter sequences down to -309 with respect to the start codon were sufficient to confer increased gene expression upon wounding or JA-treatment. A DNA domain that mediates both wound- and JA-response was further localized to a 221-bp region between -309 and -88. This domain when fused to a cauliflower mosaic virus (CaMV) 35S truncated promoter makes the CaMV promoter responsive to MeJA. In vivo footprinting studies revealed protected DNA sequence motifs within the domain identified. Evidence for the relevance of these elements in mediating the response to JA/MeJA will be presented.

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WOUND- AND METHYL JASMONATE-INDUCIBLE TRANSCRIPTS CORRESPONDING TO PROTEINS ASSOCIATED TO MYROSINASE IN *BRASSICA NAPUS*

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Myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) consists of a group of isoenzymes capable of hydrolyzing the thioglucoside bond in glucosinolates; the resulting aglycone rearrange spontaneously and yield for instance isothiocyanates, organic thiocyanates, epithionitriles and nitriles. The exact outcome of the reaction depends on a number of factors such as type of glucosinolate, pH, Fe^{2+} and additional proteins. This system is found mainly in Brassicaceae and Capparaceae and is thought to function as a wound activated defense against generalized herbivores. Myrosinases in seeds of *Brassica napus* can be found both as free dimers and in large complexes, myrosinases associated with non-myrosinase proteins. This study is focused on the associated non-myrosinase proteins. Complexes have been purified from seeds by using antibodies directed against either the myrosinase or some of the associated proteins and by performing peptide sequencing we have been abel to clone transcripts corresponding to two different types of proteins, myrosinase associated- and myrosinase binding-protein (MyAP and MyBP, respectively). These clones belong to gene families, according to Southern analyses and at least some of the members are responsive to wounding and methyl jasmonate (MeJA). The level of transcripts corresponding to MyAP increase faster and are augmented to a greater extent in response to wounding than does transcripts corresponding to MyBP. MyAP exhibit similarity to a nodulin (enod8, *Medicago sativa*) and a to proline-rich protein (cex, *Brassica napus* and APG, *Arabidopsis thaliana*) and MyBP exhibit similarity to a D-galactose-specific lectin from *Artocarpus integer* and to a salt-induced protein from *Orysa sativa*. How these proteins relate functionally to the myrosinase-glucosinolate system or to the wound-response are currently not known.

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UPSTREAM ELEMENTS IN THE VspB PROMOTER MEDIATE RESPONSE TO MEJA, SUGAR AND PHOSPHATE

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The soybean vegetative storage protein genes VspA and VspB encode glycoprotein acid phosphatases. Vsp gene transcription can be synergistically activated by the plant growth regulator jasmonic acid or methyl jasmonate (MeJA) and soluble sugars. Sugar-mediated activation of Vsp expression can be inhibited by inorganic phosphate. Previous promoter deletion analysis showed that the region -585 to -403 with respect to the transcription initiation site in the VspB promoter could confer a response to MeJA and sugar. In this study, we carried out DNaseI footprinting experiments with soybean nuclear extracts to define the protein binding sites. Four sites named Box-I, II, III and IV were identified within this 180bp region. Competition DNA binding assays showed that oligos with Box-II sequences could specifically compete the low mobility band, while mutations in the Box-II sequence abolished the competition. DNaseI footprinting and competition DNA binding experiments with pea nuclear extracts generated similar results. Functional significance of these protein binding sites was evaluated by substitution mutation of the motifs in the promoter fused to GUS reporter gene, and subsequent transient gene expression assays using tobacco protoplasts. Results demonstrated that mutations in Box-II caused a sharp decrease in the phosphate response. Efforts are underway to isolate the transcription factors from soybean cDNA expression library.

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EFFECT OF GROWTH REGULATORS ON EXPRESSION OF ADULT PLANT LEAF RUST RESISTANCE GENES IN WHEAT.

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Growth regulators are known to affect many biological functions of plants including their response to pathogens. The effect of three plant growth regulators on expression of adult plant leaf rust resistance of an Indian cultivar Arjun was investigated. Twenty single seed descendent progenies, homozygous for adult plant resistance of cultivar Arjun were sown on three dates in a randomized block design with three replications to generate 120 day old, 100 day old, and 80 day old plants. These were simul-taneously sprayed with aqueous solutions of 100 ppm gibberellic acid, 50 ppm Kinetin and 30ppm abscisic acid . An epidemic of a mixture of variants 77-1 and 77-2 of race 77 able to knock down all known seedling resistance genes was created. The highest terminal disease severity on 80 day old untreated control plants was 30S. The disease severity on 120 day old untreated control plants which were in reproductive phase was 10S. Both kinetin and ABA treatment did not affect disease severity of plants at any developmental stage. The GA treatment increased highest terminal disease severity from 10S to 20S on 120 day old plants and from 30S to 60S on 80 day old plants. The average disease severity values for each treatment also showed similar trend. These observations suggest that GA application on young plants without an effective leaf rust resistance gene, enhances leaf rust development. However, with the onset of reproductive phase, the adult plant resistance starts expressing which shows very little change in its expression with growth regulator application.

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VISUALISATION OF A STEEP RADIAL CONCENTRATION GRADIENT OF IAA OVER THE VASCULAR CAMBIUM SUGGESTS A ROLE IN POSITIONAL SIGNALLING

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Radial concentration gradients of morphogenetic substances are likely to be involved in positional control during cambial growth and wood differentiation. The most potent morphogen in cambial growth is indole-3-acetic acid (IAA), which stimulates both cambial cell division and tracheary differentiation. We have visualised the radial distribution of IAA over the vascular cambium region in mature *Pinus sylvestris* (L) trees. Thirty µm consecutive tangential cryosections were obtained from the cambial region, including tissues between the mature phloem and xylem, and IAA was analysed in each section by a novel microscale technique employing GC-SRM-MS. In an active cambium, steep radial concentration gradients were found over the cambial meristem and its developing derivatives with peak concentrations of 3-6 µg g^{-1} FW within the cell division zone levelling down to 80 ng g^{-1} FW towards mature phloem and xylem. It was also found that the IAA concentration in dormant cambia obtained in mid January was similar to those in the active cambium. The demonstration of this morphogenetic gradient over the vascular meristem together with the observation of similar IAA levels in active and dormant cambia lead us to suggest that IAA acts on cambial growth through positional signalling. This concept states that IAA acts on cambial growth through the width of the radial gradient, determining the population of cells in different developmental states, rather than through changes in its concentrations.

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DETERMINATION OF THE ORIGIN OF VASCULAR CAMBIUM IAA BY ISOTOPE DILUTION AND MASS-SPECTROMETRY.

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Polar flow of IAA is known to maintains the structure and function of the vascular cambium, and manipulation of the level of IAA in the vascular region has been shown to affect most aspects of secondary cambial growth. Altered characteristics include; cell division, radial size and secondary wall thickness of the xylem cells and, therefore, all the key properties of wood. To understand the role of IAA in the regulation of cambial growth and wood formation, knowledge is required of IAA transport and metabolism and the resulting distribution pattern of IAA in the vascular cambium. On the basis of indirect evidence from debudding and defoliation experiments the apical shoot is generally believed to be the principal source for supplying IAA to the vascular cambium. Experiments with IAA transport inhibitors has demonstrated that the basipetal transport from the apical shoot is through the polar transport system, and not by mass flow in the phloem. However, it has repeatedly been suggested that biosynthesis of IAA also takes place within the cambial region itself. In this study, elongating current-year *Pinus sylvestris* (L.) shoot were replaced with a source of $^{13}C_6$-IAA in lanolin, in order to investigate how much of the IAA pool in the vascular cambium region that originated from the apical supply. After 2,4, and 6 days, IAA was extracted from different positions of the subjacent 1-year-old internodes. The dilution of endogenous IAA by polary transported $^{13}C_6$ IAA was determined by mass spectrometry and the amount of native ^{12}C and applied $^{13}C_6$-IAA was quantified, using D_5-IAA as internal standard. The resulting IAA concentration (^{12}C-+^{13}C IAA) in the $^{13}C_6$-IAA treated shoots was similar to that in control shoots. About 90% of the IAA pool in the upper part of the internode was in the form of $^{13}C_6$-IAA, therefore originated from the apical source. Further down the shoot, 80 % of the IAA pool was substituted with $^{13}C_6$-IAA. It can be concluded that the amount of IAA in the vascular cambium of the 1-year-old shoot is primarily determined by the supply of IAA transported from the current shoot. However, the results also indicate that a minor pool originates from elsewhere, possibly the mature needles. Work is in progress to investigate this possibility.

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EXOGENOUS SPERMIDINE EFFECT ON MAIZE ROOT GROWTH IN RELATION TO ITS ACTION ON ETHYLENE BIOSYNTHESIS

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Poliamines, such as spermidine, applied to 3 d-old maize seedlings through roots, induces several changes in metabolism and structure of the primary roots, namely, enhancement of K^+ uptake and efflux, increase of putrescine titer and a strong inhibition of root growth rate due to inhibition of both mitotic index and cell elongation. A possible involvement of ethylene in the inhibition of root growth has been suggested on the basis of a few considerations: 1) spermidine stimulates ethylene biosynthesis as reported in different plant system; 2) exogenous ethylene suppresses root elongation and increses radial expansion in maize; 3) ethylene and spermidine both share S-adenosylmethionine as a biosynthetic intermediate. In this context we examine the effect of exogenous spermidine on ethylene biosynthesis, 1-aminocyclopropane-1carboxylate (ACC) oxidase activity, and ACC level in the absence and presence of several inhibitors of ethylene biosynthesis in maize roots. Results indicate that pre-treatment of maize seedlings with spermidine induces an enhancement of ethylene production from roots compared to control tissues with no effect on ACC oxidase activity and ACC level accompanied by a strong inhibition of root growth; this inhibition is not reversed by addition of inhibitors of ethylene biosynthesis suggesting that spermidine inhibition of root growth is not mediated by stimulation of ethylene biosynthesis, probably due to more available S-adenosylmethionine. This could be a consequence of reduction of spermidine synthesis caused by increase in endogenous spermidine level, following exogenous spermidine absorption.

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CHANGES IN THE ENGENOUS ABA LEVELS OF CUCUMIS SATIVUS EXPLANTS IN VITRO IN THE PROCESS OF DE-DIFFERENTIATION

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The uptake of ^{3}H-ABA, endogenous level of (+)-S-ABA /ABA/ and its relationship to the de-differentiation of two tissues of *Cucumis sativus* L. were studied. ^{3}H-ABA accumulation is higher in hypocotyl than in cotyledons, but the endogenous level of ABA was detected to be higher in cotyledons. The most intensive callus induction occurred in the hypocotyl simultaneously with lower endogenous levels of ABA and, on the contrary, a higher level of ABA was found in cotyledons. In apical and basal parts of cotyledons, differences in the level of ABA were observed. In general, the uptake of ^{3}H-ABA was the highest in tissues with a lower level of ABA where de-differentiation was the most intensive. Comparison of the ABA level in cultures with a different regenerative ability suggests that in explants from cotyledons prior to callus induction the level remains at the original level. When callus is induced, the level of ABA decreases and in the case of shoot regeneration (and its respective photosynthetic activity), the level of ABA increases again. The results suggest that autotrophic processes could be related to the increase of the endogenous level of ABA.

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MINIASSAY, A USEFUL SYSTEM TO STUDY THE EFFECTS OF SEEDS, APPLIED PGRS AND OTHER EXTERNAL TREATMENTS ON THE PHYSIOLOGY OF POD FRUITS

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In order to study the regulation mechanism of pea pericarp growth, a micro assay system, referred to as the MiniAssay, has been developed. The MiniAsay allows a particular experimental treatment and its control to be conducted on a single pod. The method is to cut and split down both suture lines of a young pod (two to three days after anthesis), leaving the two pericarp halves fully separated from each other except at the peduncle juncture. The split pea pod is then enclosed with a clear plastic bag. The two separated pericarp halves bear the same genetic and physiological characteristics, but grow independently on the plant. This assures a direct comparison of a treatment applied to one pericarp half with another (usually, the control) applied to the other pericarp half, with very limited interference from random variables. Using the MiniAssay system, we found: 1) pea pericarps considerably declined the growth rate within 24 h of the seed removal; 2) 4-Cl IAA highly significantly promoted the growth of pods with a dose of 5 ng (can be further lowered) per pericarp-half applied at the deseeding time; 3) the dose required for exogenously applied GA_3 to recover the growth potential of a deseeded pericarp-half was about ten times of 4-Cl IAA; and 4) individual GAs exercised differential effects (activities and modes) on growth promotion of deseeded pea pericarps, their activities (as applied at 1 μg per pericarp-half) ranged (most active) GA_4, $GA_7 > GA_3 > GA_8 > GA_{20} > GA_{19}$ (inactive). We also found that GA metabolism in pea pericarps was affected by the presence of developing seeds, wounding, and applied 4-Cl IAA. The MiniAssay is relatively reliable and sensitive, and should be applicable to other legume fruits and for different research goals.

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EFFECT OF GA_3 ON MAIN RICE AND RATOONING RICE

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Main rice-ratooning rice is a main cropping system in some rice area of Asia, Africa and America. Problems with yield increase of main rice and ratooning rice are often due to small grain number per panicle, low grain weight of main rice and less ratooning seedlings per plant. Seed development and ratooning bud germination are related to hormone contents in their respective tissue. The objectives of this study were to determine (i) the effects of defferent concerntrations of GA_3 applied to main rice at 15 days after full heading on ratooning bud germination and yield of main rice and ratooning rice, and(ii) the effects ef GA_3 on hormone contents, leaf senescence and root vigour. The experiments were conducted in pots and field from 1990 to 1992. The cultivar used was hybrid rice Shanyou 63. GA_3 increased yield of main rice by raising grains per panicle and looo-grain weight. GA_3 promoted germination of ratooning buds and increased ratooning seedlings per plant by enhancing the percentage of effective ratooning buds at node five and six (from above to bottom). The yield of ratooning rice was also increased by GA_3 because the effective panicles per ha of ratooning rice increased. The effective panicles at node 2 and node 3, especially at node 5, of treated ratooning rice were much higher than that of control. GA_3 had little influence on grains per panicle and looo-grain weight of ratooning riee. CTK and IAA contents of ratooning buds at all nodes, especially at node 5, were increased by GA_3 . ABA content of ratooning buds at all nodes, especially at node 5, was reduced by GA_3 significantly. The green leaf area per plant, Chlorophyll content and net photosynthetice rate in flag leaf of treated main rice were much higher than these of control. GA_3 promoted ratooning buds rooting. The root-shoot ratio and root oxidizability of main rice were enhanced by GA_3 . The delay of leaf senescence and enhance of root vigour could be responsible for the yield increase of main rice and ratooning rice.

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CYTOKININ ACTS ON DIVISION THROUGH REGULATING DEPHOSPHORYLATION AND ACTIVATION OF $p34^{cdc2}$ MITOTIC KINASE AT THE G2/M CONTROL POINT.

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Cytokinin-induced activation of the cell cycle control protein $p34^{cdc2}$ was indicated by the effects of auxin-like compounds (NAA and 2,4-D) and cytokinin-like compounds (BAP and kinetin) on excised tobacco pith and on suspension cultured cells of *Nicotiana plumbaginifolia*[1]. Auxins provided without cytokinins induced synthesis of $p34^{cdc2}$ -like protein, which was detected with antibody specific for the PSTAIR sequence of 16 amino acids that is perfectly conserved in the cdc2 family[2], and was purified by affinity chromatography using the essential mitotic protein $p13^{suc1}$ of yeast[3]. Cytokinins did not induce synthesis of this protein, however the enzyme induced by auxin alone was catalytically inactive as H1 histone kinase and required the additional presence of cytokinin for its activation. Lack of cytokinin resulted in G2 phase arrest with inactive $p34^{cdc2}$ containing high levels of phophorylated tyrosine that could be removed *in vitro* by phosphatase enzymes with resulting activation as H1 histone kinase. *In vivo* the addition of cytokinin to suspension cultured cells resulted in rapid synchronous dephosphorylation and activation of $p34^{cdc2}$ kinase and consequent progress through division. It is concluded that cytokinin acted on division through promoting dephosphorylation of $p34^{cdc2}$ mitotic kinase. Direct interaction with cell cycle proteins was detected by reaction of the $p13^{suc1}$ -purified fraction with [2-^{3}H] 8-azido-BAP photoaffinity reagent. Reaction was cell cycle phase dependent and specific for cytokinin not purine, therefore indicating that cytokinin may influence division by acting directly on the protein catalysts.

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HORMONE METABOLISM ASSOCIATED WITH POD SHATTER IN OILSEED RAPE

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Loss of yield due to seed shedding by mature siliques or pods (pod shatter) is a universal problem with current oilseed rape varieties. Both ethylene production and β-glucanase activity seem to be associated with this process. In this contribution we report on the kinetics of the endogenous ACC, IAA (both free and conjugated) and free ABA in seeds, pods and dehiscence zone during pod development and maturation. Introducing AVG confirmed the close association of the endogenous ACC levels in the seeds with the observed ethylene outburst. Results obtained by 4-CPA application closely linked IAA metabolism in the dehiscence zone with the dissolution of the middle lamella leading to pod shatter.

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HIGH LEVELS OF GA_3 INCREASED BERRY SHATTERING OF SEEDLESS GRAPES

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The purpose of this study was to examine the suitable concentration of GA_3 which can produce high yields of Thompson seedless grapes with good quality and low percent of berries shattering. The cause of post harvest berry shattering associated with GA_3 application was considered. Although yield was increased and the size of berries was improved, there were some adverse effects as berry shattering was increased with the application of higher levels of GA_3. The increase in cluster length was parallel to the level of GA_3 applied. The level of 20 ppm before flowering + 40 ppm after fruit set was more effective in increasing the yield/vine, while 50 ppm was less effective and slightly reduced the yield. GA_3 increased the juice volume in the berries, but TSS was reduced. Berry shattering was more as the level of GA_3 increased upto 50 ppm. This colud be a result of lignin and cellulose content increase in the rachis and pedicels. The higher levels of GA_3 also induced more diameter and more number of secondry xylem cells along the radial rows. It is recommended that level of GA_3 should not exceed 10 ppm before flowering to increase the cluster length and not more than 40 ppm for increasing berry size. Within these levels increase in Thompson seedless grape yields could be achieved with less berry shattering during handling and storage.

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EVIDENCE FOR A ROLE OF ß-1,3-GLUCANASE IN DICOT SEED GERMINATION

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We investigated the function of class I vacuolar ß-1,3-glucanases in the germination of *Nicotiana tabacum* L. cv. "Havana 425" seeds. Rupture of the seed coat (testa) and rupture of the endosperm are separate events in tobacco seed germination well separated in time. Measurements of ß-1,3-glucanase activity, class I ß-1,3-glucanase antigen and mRNA, and expression of a ß-glucuronidase reporter gene regulated by the tobacco class I ß-1,3-glucanase B promoter showed that class I ß-1,3-glucanases are induced just prior to the onset of endosperm rupture but after the completion of seed-coat rupture. This induction was localized exclusively in the micropylar region of the endosperm where the radicle will penetrate. Furthermore, this induction is not a classical defence response, because chitinase activity is not induced. Treatment of seeds with 10^{-5} M abscisic acid (ABA) did not appreciably affect seed-coat rupture but greatly delayed subsequent endosperm rupture by >100 hrs and resulted in the formation of a novel structure consisting of the enlarging radicle with a sheath of greatly elongated endosperm tissue. Therefore, ABA appears to act primarily by increasing the mechanical resistance of the endosperm which then delays radicle emergence. ABA treatment markedly inhibited the rate of ß-1,3-glucanase accumulation but did not delay the onset of induction. Independent of the ABA concentration used, onset of endosperm rupture was correlated with a critical concentration of ß-1,3-glucanase at the site where rupture will occur. Our results provide strong evidence that class I ß-1,3-glucanases promote radicle penetration of the endosperm, which is a key limiting step in tobacco seed germination.

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CELL WALL METABOLISM OF PHYTOINHIBITORS AND WAY OUT FROM DORMANT STATE

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The aim of our study is to detect the relationship between phytoregulaitor rate and the activity of peroxydase located in different places. The seeds of Butomus umbellatus, being underwater in dormant state, are the object of our investigation.

The phytoregulaitors are isolated by thin-layer chromatography. The activity of phytoregulaitor fractions was detected by biotest with wheatcoleoptiles and peroxydase activity was detected by the reaction with guaiacol.

We have shown that the peroxydase activity associated with cell walls (PAACW) increased after the rise of cytoplasmic and membrane peroxydase activity. The phytoinhibitor activity decreased at the increase of PAACW.

We came to the conclusion that the peroxidase pool located in cell walls is produced by immobilization of its cytoplasmic and membrane isoform. The level of phytoinhibitors changed due to PAACW when the seeds went out from the dormant state.

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A NOVEL FLOODING-RESPONSIVE GENE HOMOLOGOUS TO THE PUTATIVE CELL-WALL LOOSENING XYLOGLUCAN *ENDO*-TRANSGLYCOSYLASE

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The vast majority of identified flooding-responsive genes encode enzymes of glucose-phosphate metabolism, which function to allow limited energy production in the face of limited oxygen supply. However, in addition to the induction of these genes, flooded plants undergo structural modifications such as formation of intercellular air spaces (aerenchyma) which may help in survival by facilitating gas exchange between submerged and aerated parts. These modifications require the activation of wall acting enzymes and are strongly linked to endogenous ethylene. However, these modifications have not been addressed at the molecular level. Here, we report the isolation of a complete cDNA (XET-H) and genomic sequence (*g1005*) encoding a gene that may be involved in flooding-induced cell-wall metabolism leading to structural modifications. The gene is homologous to xyloglucan *endo*-transglycosylase (XET), an enzyme that is proposed to play a role in wall loosening or degradation during cell expansion, seed germination and fruit softening. The deduced amino acid sequence of XET-H exhibits high homology to XET sequences from a range of species including soybean, wheat, *Vigna*, tomato and *Arabidopsis*. Stretches of homology are present along the entire length of the sequence, and the homology is particularly high in the middle and carboxy-terminal regions. For example, XET-H shares 67% identities and 81% positives with the wheat sequence over a 90 amino acid stretch. Furthermore, XET-H and all homologous sequences contain several identical stretches including a nine amino-acid stretch proposed to be the catalytic domain of *Bacillus subtilis* lichenases (a related set of glucanases). Thus, analysis of XET-H and the encoding gene will likely offer first molecular insights into flooding-induced structural modifications such as aerenchyma formation.

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ACTIVITY OF CELL WALL DEGRADING GLYCANASES IN METHYL JASMONATE-INDUCED LEAF ABSCISSION IN *KALANCHOE BLOSSFELDIANA*

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Many studies concerned the role of jasmonates in senescence of different organs, mostly leaves, but only a little attention has been paid to the role of the compounds in abscission of leaves and other organs. Methyl jasmonate (JA-Me) greatly promoted the leaf abscission in intact *Kalanchoe blossfeldiana* plants treated before flower bud initiation or during flowering. Spraying of plants with JA-Me at a conc. of 500 mg/l caused the leaf abscission in 100%, and at a conc. of 125 mg/l promoted leaf abscission in about 20%, during 2-3 days. JA-Me at a conc. of 800 mg/l greatly increased CO_2 evolution (about 10 times) and stimulated ethylene production. Pretreatments of plants with IAA, GA, BA, and ABA one day before treatment with JA-Me did not prevent leaf abscission. It was shown that JA-Me markedly increased the total activities of cellulase, polygalacturonase, pectinase, and xylanase in petioles, but did not affect activities of these enzymes in the blades. These results suggest that JA-Me promotes the degradation of cell wall polysaccharides in the abscission zone and this way induces leaf abscission in *Kalanchoe blossfeldiana.*

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MODIFICATION OF MOLECULAR-MASS OF CELL-WALL POLYMERS DURING CELLULAR AGEING AND GIBBERELLIN-REGULATED GROWTH OF PEA (*Pisum sativum L.*) ROOTS

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Root growth is under control of externally applied gibberellin(GA) in the presence of an inhibitor of GA biosynthesis. GA promotes the elongation of roots with slender elongation zone whereas cortical cells laterally expands without GA application. Earlier studies have shown that the changes in molecular-mass distribution of pectin(PC) and in sugar composition of hemicellulose(HC) are taking place during the GA-regulated growth of roots. In this paper, temperature-dependent disintegration of PC and changes in molecular mass of HC along root axis will be reported by comparing the effect of GA on the roots of dwarf (cv. Little Marvel) and normal (Alaska) peas.

Cell-wall PC extracted by CDTA at room temperature exhibited broad peak on sepharose 2B chromatogram with molecular mass (Mm) greater than 500 kDa. The highly integrated PC gradually disintegrated into smaller molecules with Mm 10-40 kDa by heating PC solution at 60-100 °C for 2 h. Thus, CDTA-extracted PC holds highly integrated structure which is susceptible to temperature higher than 60 °C. Glycosidic bonds may not be involved in the integration since no monomeric or oligomeric uronide was produced during the heating. The Mm distribution of this PC from GA-treated roots was larger than that of non-treated roots, suggesting that PC of elongated slender roots is more highly integrated than that of non-elongating thick roots. HC exhibited two peaks on sepharose 4B chromatogram; Mm of larger peak was greater than 500 kDa and that of smaller peak located between 10-40 kDa. The larger peak dominated in the apical part of the root, whereas smaller peak was dominant in the basal part. HC from GA-treated roots was more rich in larger peak than that of GA-starved roots. The GA effect was more remarkable in the dwarf pea than in the normal one. It was suggested that disintegration of cell-wall polymers are taking place during cellular ageing along root axis of GA-starved roots whereas highly integrated structure of PC and HC are maintained in young cells supplemented with GA. *Acknowledgment* : This research was partially supported by Nagoya City University's program for the Invitation of Visiting Scholars from Abroad.

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BRASSINOLIDE-INDUCED STEM ELONGATION

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Brassinolide (BL) stimulated elongation of etiolated squash (*Cucurbita maxima* Duch.) hypocotyl segments. Segments with removed outer tissues (epidermis and two or three cell layers of collenchyma) were still responsive to BL, while IAA did not stimulate elongation of peeled segments. BL changed mechanical properties of the cell wall of outer and inner tissues of the segments, indicating that BL caused elongation growth of the segments by altering cell wall properties of both outer and inner tissues. Analyses of the cell wall polysaccharides, such as monosaccharide composition, molecular weight distribution of pectins, hemicelluloses and xyloglucans, and synthesis of polysaccharides using ^{14}C-Glc, however, disclosed that neither synthesis nor breakdown of specific polysaccharide was stimulated by BL. BL-induced elongation of the segments was further promoted by the addition of K^+ ion. Na^+ was not substituted with K^+. Monensin and vanadate inhibited BL-induced elongation in the presence of KCl, while a K^+ channel blocker, tetraethylammonium, and a Na^+-K^+-Cl^- transporter inhibitor, furocemide, did not inhibit BR-induced elongation in the presence or absence of KCl. The results suggest that BL induces elongation growth by causing a subtle change in the cell wall polysaccharides and/or by the mechanism involved in K^+.

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HORMONAL REGULATION OF ENDO-ß-MANNANASE ACTIVITY DURING GERMINATION OF TOMATO SEEDS

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Endo-ß-mannanase (EC 3.2.1.78) activity appears to be involved in weakening of the endosperm cap opposing the radicle tip, resulting in radicle protrusion in tomato (*Lycopersicon esculentum* cv. Moneymaker) seeds. Tissueprints on activity gels with endosperm and embryo tissue from wild type seeds gave a good impression of the site and the amount of endo-ß-mannanase activity. Endosperm caps clearly showed high activity prior to germination. Embryos also contained high endo-ß-mannanase activity prior to radicle protrusion, supposedly present in the apoplast. Gibberellin deficient seeds from the *gib1* mutant were unable to germinate without exogenous gibberellins. Tissueprints from water imbibed seeds showed no activity in either endosperm or embryo. Addition of 10μM GA_{4+7} resulted in the usual germination capacity and restored activity in endosperm cap and embryo. ABA added together with GA_{4+7} maintained endo-ß-mannanase activity but inhibited germination completely. Wild type seeds imbibed in ABA did not germinate, but showed high activity in endosperm cap and embryo. The same was true for wild type seeds imbibed in high concentrations of Mg^{2+}, Ca^{2+}, Na^+ and K^+ salts. Wild type seeds that had lost germination capacity upon prolonged storage showed hardly any activity. Addition of GA_{4+7} restored both germination capacity and endo-ß-mannanase activity in embryo and endosperm cap prior to germination. Expression of endo-ß-mannanase and germination are both regulated by gibberellic acid. ABA and osmoticum inhibited germination, but sustained expression of endo-ß-mannanase. Although the enzyme seemed to be necessary for germination, its presence was not exclusively responsible for germination.

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ALDEHYDE OXIDATION-REDUCTION ACTIVITIES OF THE CELL WALL BINDING ENZYMES IN BARLEY COLEOPTILE

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To analyze the cause of lower level of indoleacetic acid (IAA) of a dwarf barley mutant (Uzu) coleoptile, the enzyme activities of IAA synthesis were detected and compared with those of a normal strain. The supernatant at 1,000 g centrifugation of homogenate of coleoptiles in both Uzu and normal strain exhibited similar activities of the conversions of indoleacetaldehyde (IAAld) and other IAA precursors to IAA. We compared the conversion rate of IAA precursors to IAA of Uzu with that of normal strain using the pellet fraction at 10,000 g centrifugation. The coleoptiles were homogenized in 50 mM Tris-HCl buffer, pH 7.2, containing 5 mM EDTA + 5 mM dithiothreitol + 5 mM $MgCl_2$ + 1 mM benzamidine + 0.1 mM AEBSF and centrifuged at 10,000 g. The pellet was resuspended in homogenizing buffer and used as cell-wall enzyme preparation. The cell-wall preparations had IAAld oxidation-reduction activities, whereas the preparations did not catalyze the conversion of the other precursors to IAA. The activity of the conversion of IAAld to IAA by oxidation was lower in Uzu than in normal strain, but the conversion of IAAld to tryptophol by reduction was higher in Uzu than in normal. Both activities were disappeared by boiling at 90°C. The activities were not solubilized by treatments of detergent (1% Nonidet P-40), 1 M Na Cl or 50 mM $MgCl_2$. Treatment of 3 M LiCl for the cell-wall preparation could not solubilize, and moreover lost those activities. The oxidative activity was not inhibited by L-ascorbic acid and was slightly increased by NAD, while the reductive activity was slightly increased by NADH. The results suggest that the both activities are bound to cell walls and the lower level of IAA in Uzu probably results from the reduced activity of the oxidative enzyme bound to cell walls.

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A POSSIBLE MECHANISM OF PROMOTING EFFECTS OF JASMONIC ACID AND METHYL JASMONATE ON THE ABSCISSION OF BEAN PETIOLE EXPLANTS IN RELATION TO CELL WALL POLYSACCHARIDES AND CELLULASE ACTIVITY

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Jasmonic acid (JA), methyl jasmonate (JA-Me) and related compounds are considered to be a putative plant hormone. The loss of chlorophyll and the formation of the abscission were extremely promoted by exogenous application of these compounds. In this study, we report a possible mechanism of promoting effects of JA and JA-Me on the formation of the abscission in bean (Phaseolus vulgaris L. cv. Masterpiece) petiole explants in relation to sugar metabolism of cell wall polysaccharides and cellulase activity.

JA and JA-Me at more than 1 μM promoted the abscission of bean petiole explants in the dark and the light. JA and JA-Me did not promote ethylene production in bean petiole explants in the light. JA-Me had no effect on neutral sugar compositions and the quantities of pectic and hemicellulosic polysaccharides of cell walls in the abscission zone. However, the contents of cellulosic polysaccharides were significantly reduced by JA-Me. In the abscission zone, JA-Me accelerated the decrease of the level of UDP-sugars prior to the formation of the abscission, which are important key substrates of the synthesis of cell wall polysaccharides. The total activities of cellulase in the pulvinus and the petiole were much higher than those in other parts of 10-day-old bean seedlings. Cellulase activities were inhibited by the addition of IAA or cycloheximide. JA significantly increased the total activities of cellulase in the pulvinus and the petiole. These results suggest that JA and JA-Me did not only promote the degradation of cell wall polysaccharides in the abscission zone, but also inhibited the synthesis of it, and promoted abscission.

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PROBING MICROTUBULE FUNCTION IN ROOT GRAVITROPISM USING CYTOSKELETAL INHIBITORS

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Cortical microtubules (MTs) are implicated in the control of plant cell elongation because of their proposed influence on cellulose microfibril deposition. Evidence that growth and MT orientation are correlated has come from our studies on graviresponding and auxin treated maize roots which show that growth inhibition is characterized by MT reorientation from transverse to longitudinal. However, it remains unclear whether MT reorientation is a cause or the consequence of altered growth rate or direction. To address this question, we tested the effects of various anti-MT drugs on curvature and growth of maize roots. 3-day-old, light-grown roots of maize (cv. Merit) were immersed in solutions of 40 μM taxol and 5 μM oryzalin for 3 h and then gravistimulated for 1 h, fixed in horizontal position in formaldehyde and processed for indirect immunofluorescence. Curvature and growth distribution were measured using a video digitizer. Taxol stabilized MTs and prevented the MTs of cells on the lower side to reorient while oryzalin caused MTs to depolymerize. Despite the effects of the drugs on MTs curvature was similar to controls. This indicates that MTs may not be required for the gravitropic response and MT reorientation could be a consequence of altered growth during gravitropism. However, 10-12 h after drug treatment, the roots no longer reacted to horizontal orientation. Swelling in the tip region of the roots was observed both in taxol and oryzalin treated roots but the morphology of the cells differed between the two treatments. Since previous work has shown that auxin causes substantial reorientation of MTs, it is possible that cytoskeletal inhibitors may affect hormone transport. We are currently investigating auxin transport in maize roots exposed to various cytoskeletal inhibitors.

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NPA BINDING ACTIVITY IS ASSOCIATED WITH THE CYTOSKELETON IN VITRO

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N-1-naphthylphthalamic acid (NPA) is a potent and specific inhibitor of auxin transport. A single binding site for this ligand is found associated with the plasma membrane of zucchini. It has been suggested that the NPA binding protein (NBP) is on the cytoplasmic face of the membrane. Treatment of zucchini plasma membranes with 0.05% Brij 58, which will invert plasma membranes so that the the cytoplasmic face of the membrane faces the external media, leads to a 150% increase in NPA binding activity. NPA binding activity is associated with membrane vesicles coated with filamentous cytoskeletal components during sucrose gradient density fractionation. Extraction of plasma membrane vesicles with Triton X-100 resulted in retention of the NPA binding activity in the detergent-insoluble cytoskeletal pellet. This detergent-insoluble pellet was shown to contain cytoskeletal elements by fluorescence microscopy with rhodamine phalloidin and immunoblot analysis with an actin monoclonal antibody. Treatment of the detergent insoluble pellet with KI released NPA binding activity from the pellet into the supernatant. KI treatment was shown to release actin and α-tubulin monomers by immunoblot. Dialysis to remove KI led to the repolymerization of cytoskeletal elements and movement of NPA binding activity, actin, and α-tubulin into an insoluble cytoskeletal pellet. NPA binding activity partitioned into the detergent-insoluble cytoskeletal pellet obtained from both zucchini and maize membranes and was released from these pellets by KI treatment. Treatment of a cytoskeletal pellet with cytochalasin B doubled NPA binding activity in the resulting supernatant and released both actin and tubulin monomers. Together these experiments suggest that NPA binding protein is on the cytoplasmic face of the plasma membrane and that it interacts with the cytoskeleton *in vitro*.

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HORMONAL INVOLVEMENT IN CYTOSKELETON CHANGES DURING POTATO TUBERIZATION

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Potato tuberization was analyzed at the anatomical and cellular levels during the earliest morphogenic changes associated with tuber formation. In vitro tuber initiation was correlated with a reorientation of cortical microtubules, which preceded visible swellings, radial cell expansion and, finally, cell division. The role of the relative amounts of kinetin and GA4/7 present in the changes of microtubule orientation patterns is discussed.

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ABSCISIC ACID MEDIATED FREEZE TOLERANCE IN PEA SEEDLING TISSUES

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The freeze tolerance of pea epicotyl tissue is dramatically increased by exposure to 2°C for 30 d. The role of ABA in increasing freeze tolerance was investigated using the cv. 'Alaska' and an ABA-deficient mutant *wil*. Freeze tolerance was expressed as LT_{50} after treatment with exogenous ABA. Endogenous ABA was measured in pea shoot tips, root tips, and epicotyl tissue after cold treatment, dehydration , deacclimation, and applications of exogenous ABA using indirect ELISA. Two-dimensional PAGE was used to identify proteins associated with increased freeze tolerance. The LT_{50} of root tissue was decreased 1°C by either cold treatment or 10^{-4} M ABA. The LT_{50} of shoot tips and epicotyl tissue was decreased from 2 to 4°C by 10^{-4} M ABA, while exposure to 2°C for 30 d decreased the LT_{50} from 8 to 12°C. Cold acclimation increased endogenous ABA levels in the shoot tips to 0.34 $\mu g\ g^{-1}$ dwt, four times the amount in warm-grown tissue, but less than half of ABA-treated tissue. A 24 kDa protein was produced in all tissues and all genotypes after cold treatment and when ABA was present. N-terminal sequencing of the 24 kDa protein was unsuccessful, indicating that the N-terminus may be naturally blocked. ABA and cold treatment both increased freeze tolerance, but although there were similarities between the two treatments, cold acclimation apparently causes additional changes that are not caused by ABA.

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REDUCED EXPORT OF ABA FROM LEAVES OF FLOODED TOMATO PLANTS PROMOTES STOMATAL CLOSURE

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Stomatal closure is an adaptive response to soil flooding that occurs within 4 h of inundation. During this time, a flood-induced disruption in root hydraulic conductivity generated a negative hydraulic message that lowered leaf water potential. We were able to discount the transient loss of shoot hydration as the instigator of stomatal closure. Increased concentrations of foliar abscisic acid (ABA) at the time of closure suggested chemical regulation. However, delivery of ABA in sap expressed from flooded roots at rates equivalent to those of whole-plant transpiration was reduced when stomata began to close. Results from petiole-girdling experiments suggested that the stomatal closure and increased foliar ABA resulted from reduced translocation out of flooded leaves. We conclude that stomatal closure is regulated by shoot-sourced ABA that accumulates due to the altered source-sink relationships in flooded plants.

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RFLP MAPPING OF A GENE REGULATING COLD- AND DROUGHT-INDUCED ABSCISIC ACID PRODUCTION ON CHROMOSOME 5A OF WHEAT

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Major genes influencing frost resistance (*Fr1*) and vernalization requirement (*Vrn1*) have been localised on the long arm of chromosome 5A. To map the location of these genes single chromosome recombinant lines were developed from the cross between substitution lines Chinese Spring (Cheyenne 5A) (CS(Ch 5A)) and CS(*Triticum spelta* 5A). The RFLP data show, although a close genetic linkage exist between the loci *Fr1* and *Vrn1*, they are nevertheless separable. The level of abscisic acid (ABA) in plants increases as a result of water deprivation or low temperatures. Exogenously applied ABA induces frost tolerance in several plant species previously grown in non-cold-acclimatization conditions. Our study on CS(Ch) substitution lines revealed that the 5A chromosome of the frost resistant Ch increased the ABA content in the frost sensitive CS background following cold treatment. The leaf ABA accumulation in several of the 5A recombinant lines subjected to polyethylene glycol induced water stress revealed a close genetic linkage between both *Fr1* and *Vrn1* genes and the gene regulating ABA production.

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ABSCISIC ACID AND POLYAMINES INTERACTION ON CHILLING TOLERANCE OF CUCUMBER COTYLEDONS

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The potential relationship between Abscisic acid (ABA) and polyamines (PA) in the chilling-induced tolerance of excised cucumber cotyledons (*Cucumber sativus*, cv. Sprint) has been investigated. Exogenous ABA (1 mM), paclobutrazol (PBZ, 100 µg/ml) or putrescine (Put, 1 mM) were applied 24 h before chilling exposure (2°C). Chilling injury was measured, for up 16 days, by the rate of ion leakage (IL). ABA, PBZ or Put reduced the IL by 64%, 50% and 38%, respectively. Higher levels of PA were induced by ABA than by PBZ. Application of difluoromethylarginine (DFMA), an inhibitor of PA biosynthesis, reduced the increases in PA without affecting the beneficial effect of ABA or PBZ. After 8 and 16 days at 2°C, the ABA content in cotyledons treated with Put were substantially higher than in those treated with PBZ. Fluridone, an inhibitor of carotenoids synthesis, was effective suppressing the ABA increase, and did not alter the rate of ion leakage of cotyledons treated with ABA or PBZ. These results indicate that the promotion of chilling tolerance in cucumber cotyledons by these regulators appears not to be mediated only by the stimulation of PA and ABA, and their role in this adaptative response may be through independent mechanisms.

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ABSCISIC ACID IS A CAUSATIVE FACTOR IN THE MODE OF ACTION OF THE AUXINIC HERBICIDE QUINMERAC IN CLEAVER

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Quinmerac (7-chloro-3-methyl-quinoline-8-carboxylic acid) is a new, highly selective herbicide which effectively controls important dicotyledonous weeds, such as cleaver (Galium aparine L.), in sugarbeet, oilseed rape and small grain cereals.
Studies using young Galium plants treated with quinmerac (10^{-6} to 10^{-5} M) hydroponically, showed that the mode of action of this herbicide is based on its auxin activity.
In the shoot tissue ethylene formation was stimulated through induction of 1-aminocyclopropane-1-carboxylic acid (ACC) synthesis within 3 h of treatment. This effect was accompanied by leaf epinasty and massive accumulations of abscisic acid (ABA) in root and even more in shoot tissue. Likewise, hydroponic application of 10^{-4} M 3-indoleacetic acid increased ACC and ABA levels and induced phenotypical alterations in plants similar to those caused by quinmerac. As physiological consequences, ABA accumulation resulted in reductions in stomatal aperture, water consumption, photosynthetic CO_2 uptake and root and particularly shoot fresh weight. Additional treatment with the ACC synthesis inhibitor PACME decreased quinmerac-induced leaf epinasty, ABA accumulation and reduction of shoot growth. On the other hand, morphological symptoms, such as stem epinasty and local necrosis on the stems, appeared to be directly controlled by the auxin activity of quinmerac itself. In sugarbeet, oilseed rape and wheat corresponding effects were not observed. It is concluded that different tissue/target sensitivity contributes to the mechanism of quinmerac selectivity.

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DEHYDRIN SYNTHESIS IN SEEDS OF DESICCATION TOLERANT AND RECALCITRANT SPECIES: INDUCTION BY ABA AND WATER STRESS

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We are interested in determining differences between desiccation-tolerant and desiccation-intolerant species, with respect to the tissue-specificity and stress-inducibility of dehydrin protein synthesis. During development of castor bean seeds (which acquire desiccation tolerance around mid-development), there were substantial changes in the pattern of dehydrin synthesis within the endosperm. In particular, new polypeptides with Mr~28-30 kD were detected during late development and were also induced in seeds subjected to premature desiccation. These polypeptides persisted following germination/growth of mature seeds, but did not increase in seedlings in response to stress treatments. Castor bean seedlings treated with ABA synthesized a 48-kD dehydrin in endosperm and cotyledons; polypeptides with Mr~51 and 32 KD were induced in the radicle. Water stress and desiccation induced 48-kD dehydrins in endosperm, similar to the ABA treatment. A 43-kD protein was induced in response to high osmolarity, while high salt and low temperature induced 43-kD and 36-kD proteins. Similar stress-specific differences in dehydrin synthesis were also found in the cotyledons and radicles of castor bean seedlings, although the ABA-inducible dehydrins were consistently induced. As part of our comparative anatysis, dehydrin synthesis was also analyzed in two recalcitrant species. Two dehydrins (~40 kD) were detected in the cotyledons and axes of mature *Castarea* seeds; an additional 31-kD polypeptide was also present in the cotyledons. *Artocarpus heterophyllus* axes contained two high MW dehydrins (~50 kD), while in cotyledons, dehydrins were absent. The stress- and ABA-inducibility of dehydrin synthesis in different tissues of the two recalcitrant species will be presented.

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CHARACTERIZATION OF PKABAg1, A NEW MEMBER OF THE PKABA1 PROTEIN KINASE SUBFAMILY FROM WHEAT

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A protein kinase genomic clone (PKABAg1) has been isolated using a protein kinase cDNA clone (PKABA1) representing a dehydration- and ABA-responsive mRNA. PKABAg1 is 7 kb in length and has 650 bp of promoter region, nine exons, two large introns, and six smaller introns. PKABAg1 has a putative abscisic acid response element (ABRE) containing the hexamer motif GACGTG. The gene is transcriptionally active as determined by RT-PCR and hybridizes to the same ABA-induced transcripts as PKABA1. PKABAg1 encodes a predicted polypeptide with all twelve conserved catalytic subdomains characteristic of serine/threonine protein kinases and an aspartic acid amino acid stretch near the carboxyl-terminus. Comparison of the deduced amino acid sequences indicates that PKABAg1 has 97% similarity (95% identity) to PKABAg1, but has diverged considerably in the last two exons. PKABAg1 has highest sequence similarity to other plant protein kinases with carboxyl-terminal amino acid stretches, and moderate similarity to plant SNF1 protein kinases. Most protein kinases are regulated by post-translational modifications such as phosphorylation. Accumulation of the PKABA1 transcripts in response to ABA and environmental stress suggests an additional regulatory control point at the transcriptional level. Our identification of PKABAg1 will serve as the future basis for determining structural features of PKABAg1 that confer ABA- and environmental stress-responsiveness. Supported in part by USDA-NRICGP No. 94-37100-0313.

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A TOMATO *LEA* GENE CONFERS SALT AND FREEZING TOLERANCE WHEN OVEREXPRESSED IN *SACCHAROMYCES CEREVISIAE*

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During periods of water deficit, plants accumulate late embryogenesis abundant (LEA) proteins, which are thought to protect cells from stress resulting from dehydration. To study the function of LE25, a LEA protein from tomato, *le25* was overexpressed in *Saccharomyces cerevisiae* under the control of the *GAL1* promoter. *S. cerevisiae* cells expressing LE25 had improved stress tolerance to salt and low temperature stress. Growth of the transformant was improved compared to the control strain in a medium containing 1.5 M NaCl. While the control strain had a long lag phase of 40 h, cells overproducing LE25 had a lag phase of only 10 h. However, there was no growth improvement in response to 1.5 M sorbitol. The transformant also had an increased survival rate after freezing stress, but not after high temperature stress. These results together with the predicted secondary structure indicate a possible function for LE25 as an ion scavenger which can mitigate initial salt shock.

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RESPONSE OF ABA IN RICE (*Oryza Sativa* L.) LEAVES TO WATER STRESS

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The rice seeds of IR661 (Indica), Yanjing 2 (Japonica) and Shanyou 63 (hybrid combination) were swon and grown in nursery trays. A water stress treatment was carried out at the leaf age 5 by holding water for 120 h. Leaf water potential (LWP) was determined with chamber pressure, and abscisic acid (ABA) in rice leaves was measured with radioimmunoassay. The level of ABA in the leaves little changed when LWP was > -0. 02 MPa, and increased quickly when LWP was lower than the value. It could be considered that the critical LWP for ABA obivious accumulation in rice seedling leaves was about -0. 02 MPa. The accumulation of ABA in the leaves under different water stress status (LWP < -0. 02 MPa) varied in the 3 cultivars. Drought-sensitive cultivar Yanjing 2 had the highest ABA accumulation in the leaves among the 3 cultivars under light water stress (LWP > -0. 04 MPa). However, Less drought-sensitive cultivar IR661 had the highest accumulation of ABA in the leaves and Yanjing 2 had the lowest under heavy water stress (LWP < -0. 05 MPa). it was implied that the cultivar with higher ABA content in the leaves under heavy water stress had stronger resistance to drought.

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FIELD PERFORMANCE OF MAIZE PROGENIES DIVERGENTLY SELECTED FOR LEAF ABSCISIC ACID CONCENTRATION

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The concentration of abscisic acid (ABA) in leaves of drought-stressed plants has been shown to be related to the agronomic performance of small grain cereals. Genotypes obtained by means of divergent selection from a common population segregating for ABA concentration provide a proper means to investigate the relationship between this trait and other characteristics. In this experiment, we analyzed the field performance of maize genotypes selected for low or high ABA concentration in the leaf. The genotypes were divergently selected from the F2 populations of IABO78 x Os420 and B88 x Mo17. In 1993, 30 F3 progenies derived from the F2 plants selected in each direction were tested in a replicated (four reps) field trial with irrigation volumes corresponding to 50% of the evapotranspiration, after accounting for rainfall. Leaf samples for ABA concentration were collected at two growth stages before anthesis. ABA concentration was determined with an ABA-specific monoclonal antibody. In both crosses, the group including the 30 F3 progenies selected for high ABA concentration showed ABA values significantly higher than the group selected for low ABA. Differences were more marked in the samples collected at the second sampling. Selection for ABA significantly affected several traits and caused contrasting effects in the two crosses. In both crosses, progenies selected for high ABA showed a shorter silk delay, a larger leaf area, a lower plant height, and a higher kernel weight. As to grain yield, kernel no./plant, pollen shed date and drought stress index, a different trend was detected in the two crosses. In B88 x Mo17, the high-ABA progenies showed a higher yield (+26%) and kernel no./plant (+25%) than low-ABA progenies, while in IABO78 x Os420 the high-ABA group showed a lower grain yield (-27%), a lower kernel number/plant (-39%), shed pollen later (+1.5 d) and were more sensitive to drought stress (+13%). The contrasting results obtained in the two crosses may indicate different roles of ABA, i.e. as an indicator of the level of drought stress in IABO78 x Os420 and as a biochemical signal capable of limiting the negative effects of drought in B88 x Mo17. These results indicate the importance of verifying in different crosses the effects associated to the selection of a physiological trait.

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IDENTIFICATION OF ABSCISIC ACID-INDUCED GENES OF *ARABIDOPSIS THALIANA* BY T-DNA TAGGING AND *IN VIVO* GENE FUSION

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During the past decade several molecular techniques have been employed for cloning of plant genes that are expressed under specific conditions, e. g. in response to environmental stress such as low temperature (LT) and drought or to plant hormones such as abscisic acid (ABA). We have employed T-DNA tagging with a promoter probe to identify ABA-induced genes of *Arabidopsis thaliana* that might have a role in tolerance of plants against both freezing and desiccation. We have generated a library of 1500 *A. thaliana* plants tagged by two T-DNA tagging vectors pMHA2 and pΔgusBin19. Both vectors harbor a promoterless *gus (uidA)* as a reporter gene. Characterization of T_2 progeny of these T-DNA tagged transgenic lines by *in situ* staining of the leaf tissues for GUS activity indicated the presence of *in vivo* gene fusions to constitutively expressed genes of *A. thaliana* in 17.3% of the lines (260/1500). A total of 1500 T_2 lines was tested for ABA- and 1200 lines for LT-induced GUS activity, and two of these exhibited expression of the *gus* reporter gene in response to these stimuli. In one of the lines (274) GUS activity was predominantly induced by LT, whereas in the other line (317) it was mainly responsive to ABA. The induction properties of these fusions were verified by fluorometric assay of the GUS activity and by northern analysis of the corresponding transcripts. Currently we are analysing whether individual/s of these lines exhibit an altered phenotype for drought or freezing stress tolerance. We also attempt direct cloning of the inducible gene fusions by inverse PCR. These results will be discussed. We gratefully acknowledge grants from the Nilsson-Ehle Foundation and the Royal Swedish Academy of Agriculture and Forestry.

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THE PHYTOHORMONES AND DROUGTH RESISTANCE IN POLYPLOID PLANTS

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The influence of soil drougth on water-retaining capacity, heat resistance, content of water, ABA, cytokinins and gibberellins was studied in greenhouse experiments with di- and tetraploid plants of plum (*Prunus salicina* Lindl.). Under drougth the increase in water content and high water holding capacity of leaves was found in tetraploids. During water deficit accumulation of ABA began later in autotetraploids, than in diploid plants, but it was considerable. There was an increase of gibberellin level in tetraploids under repeated drougth. The cytokinin content rose essential in tetraploid plants under drougth. The phytohormones are supposed to be involved in the regulation of plant responses to drougth and promoted the recovery of growth after stress.
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THE EFFECT OF ABA, CHILLING, AND DESICCATION ON GERMINATION OF *QUERCUS SUBER* SOMATIC EMBRYOS

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Somatic embryos of *Quercus suber* (cork oak) initiated from leaves of mature trees were unpredictable in their maturation and germination. In order to improve germination several treatments were tried including chilling, desiccation and the addition of ABA. Desiccation appeared to substitute somewhat for chilling. Significantly improved germination was induced by adding ABA to the medium for 2 weeks prior to drying and chilling. The concentrations of endogenous IAA and ABA were determined in untreated embryos selected by maturation state. There appeared to be significantly greater amounts of IAA in immature compared to mature embryos. The effect of chilling and drying on IAA levels was somewhat variable although an overall trend towards a decrease in values was observed. ABA levels increased in mature compared to immature embryos. The levels decreased significantly when the embryos were chilled and desiccated for one week. Additional chilling did not cause further decrease in ABA levels. These results suggest that inhibition of germination in oak somatic embryos may be due to an inhibitory level of ABA and that chilling and desiccation act to decrease the levels of ABA as occurs naturally in many woody seeds.

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CHANGES IN THE LEVEL OF ENDOGENOUS ABSCISIC ACID AND CYTOKININS, PROTEIN SYNTHESIS IN THE ROOT MERISTEMS OF ZEA MAYS, L UNDER THE INFLUENCE OF LOW TEMPERATURE, ABA AND 6-BAP

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Plant meristems fulfil such exeptional in the plant life role that in the present work for the lack of data on this question we have specifically asked whether root meristems take part in the plants response to stress conditions and whether phytohormones contribute to the physiological response of tissues to low temperature stress. Biological activity of endogenous abscisic acid and cytokinins in the root meristems of 2-days seedlings of Zea mays, L was investigated by bioassay methods after 24h. of low temperature (8 ± 1^0C) treatment. The influence of low temperature (8 ± 1^0C), abscisic acid (ABA $10^{-5}M$), and 6-benzylaminopurine (6-BAP $10^{-11}M$) was carried out by registration of ^{14}C-leu accumulation from the incubated medium into the proteins of roots meristems tissues of 2-days Zea mays seedligs. The plants were subjected to 2,4,24 h. of above-said factors. It was shown that at the simultaneous increasing of the free ABA content the amount of free cytokinins decreased. Various durations of low temperature treatment decreases the ^{14}C-leu insertion into the tissues proteins. No protective effects of 6-BAP and ABA on protein synthesis under hypoterm conditions have been observed. Possible influence of low temperature, ABA, 6-BAP, cytokinins in connection with their protective function on ^{14}C-leu penetration into the root meristems is discussed.

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WATER STATUS AND ABA CONTENT OF FLORAL TISSUES IN DROUGHT STRESSED WHEAT

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Chemical signals from roots, such as ABA, might affect seed set on wheat plants growing in dry soil during floral development. To test this possibility, we grew uni-culm plants in a controlled environment and withheld water from pollen mother cell meiosis to late boot stage--a period of high sensitivity to drought and exogenous ABA. The water potential (Ψ_w) of glumes decreased in concert with leaf Ψ_w, but ovary and anther Ψ_w were buffered from changes in leaf Ψ_w. Free ABA content of flag leaves increased ≈20-fold as turgor declined. ABA content in floral tissues increased ≈10-fold, compared to controls, even though turgor remained at or above control levels in all floral tissues examined. The drought treatment decreased seed set ≈17%. In half the droughted plants, we pressurized the roots and soil as the soil dried to maintain leaf Ψ_w at control levels. Seed set on (droughted) pressurized plants was not different from that on well-watered unpressurized plants even though soil moisture in droughted and (droughted) pressurized treatments decreased to a similar extent, and ABA content of leaves and floral tissues of (droughted) pressurized plants increased 2- to 3-fold over control levels. These results indicate that a favorable shoot water status is critical for high seed set in wheat. ABA (or a precursor of ABA) produced by roots in dry soil may ultimately accumulate in floral tissues. However, this chemical signal apparently has little effect on seed set.

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WILTING AND ABA REGULATED GENE EXPRESSION IN *ARABIDOPSIS THALIANA*

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Reduced turgor induces a number of ameliorative responses in plants, a key one being increased biosynthesis of ABA. We have used the ABA-deficient *aba* and ABA-insensitive *abi* mutants of *Arabidopsis thaliana* to analyse wilting and ABA induced gene expression. We have shown that both up-regulation of the expression of a clone encoding a potential thiol protease, and down-regulation of *rbcS*, are induced separately by turgor loss and ABA. We have also constructed cDNA libraries from wilted Arabidopsis shoots. These have been screened differentially, using cDNA probes representing wilted wild type and turgid *aba* shoots, or with a subtracted cDNA probe enriched for wilt-inducible sequences. This was produced by subtractive hybridisation with PCR-generated biotinylated cDNA prepared from turgid tissue, followed by avidin-bead separation. A large number of clones were identified and their wilt-inducibility confirmed by diagnostic RNA dot blot analysis. The sequences of some of these have been determined and their expression correlated with ABA biosynthesis in wilted and turgid ABA-deficient and insensitive genotypes. One clone, 43b, is homologous to that encoding indole-acetonitrilase (Bartling *et al* 1992 Eur J Biochem **205**,417), an enzyme of auxin biosynthesis in Arabidopsis. Accumulation of mRNA for this clone is strongly induced by water loss but not by ABA. Sequencing of another clone, T4, has so far revealed homology to tryptophan monooxygenase from *Agrobacterium tumefaciens*. Expression of the gene corresponding to this clone is also strongly induced by wilting but not ABA. Partial sequencing of other clones has identified both genes previously reported by other groups (e.g. *kin2*, Kurkela & Borg-Franck 1992 PMB **19**,689) and others which so far have no homology with sequences deposited in the data bases.

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EXUDATION RATE AND HYDRAULIC CONDUCTIVITY OF MAIZE ROOTS ARE ENHANCED BY SOIL DRYING AND ABA TREATMENT

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When young maize seedlings were fed with ABA into their root systems, exudation rate of detopped plants was more sensitively enhanced than stomatal resistance in the shoots. Enhanced root exudation was also observed when mildly droughted plants were re-watered. To compare the mechanisms involved in this response under these two treatments, we examined solute transport in the exudates and *in situ* whole root hydraulic conductivity following ABA feeding to the roots and soil drying-rewatering treatment. Increases of both solute concentration and delivery rate in the exudates of pre-droughted plants appeared capable to account for about half of the enhanced exudation rate. The ABA-fed plants, however, only showed marginal increase of solute delivery rate and in fact somewhat reduced concentration of solutes in their exudates. *In situ* root hydraulic conductance, assessed with a pressure-flow method on the undisturbed whole root systemm at a water content of field capacity, was greatly increased by both of these treatments. Results suggest that drought-induced ABA in the roots may improve the biophysical property involved in the water transport into roots, and raise root pressure when droughted plants are rewatered.

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ETHYLENE AND SHOOT ELONGATION: A COMPARATIVE STUDY OF TWO *RUMEX* SPECIES FROM CONTRASTING HABITATS

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Terrestrial plants growing in river forelands have to cope with periods of submergence. Survival of *Rumex* species depends on restoration of leaf-air contact. This can be accomplished by enhanced shoot elongation, an adaptation induced by ethylene. *R. palustris*, a flood-resistant species, which is frequently flooded in its natural habitat and is capable of enhanced shoot elongation under water, is compared with *R. acetosella*, a non-elongating flood-sensitive species from dry field sites. The ethylene release rate of *R. palustris* plants was ca. 7 nL g^{-1}DW h^{-1} under both drained and submerged conditions. Submergence inhibited ethylene release of *R. acetosella* plants from ca. 15 to 1 nL g^{-1}DW h^{-1}. Nevertheless, during 3 days of submergence ethylene concentrations of 5-10 μL L^{-1} were found in both species. Thereafter, *R. palustris* plants maintained a concentration of ca. 5 μL L^{-1}, whereas in *R. acetosella* it was as high as 20 μL L^{-1} after 6 days. These data were obtained using a continuous flow system in line with a photoacoustic set-up. From these results and data on (M)ACC concentrations, *in vivo* activities of ACC-synthase, malonyltransferase and ACC-oxidase were calculated. Upon submergence the activities of ACC-synthase and malonyltransferase were stimulated in both species. In *R. palustris* plants these activities decreased after 8 hours of submergence, probably due to negative regulation by ethylene itself. Since both species initially have an equally high ethylene concentration under water, it is clear that the elongation response depends on sensitivity towards this hormone. *R. palustris* plants can reach the water surface through rapid growth and may also survive submergence for a longer time by the ability to keep its ethylene concentration relatively low in the long-term, two traits that *R. acetosella* does not possess.

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INVOLVEMENT OF ETHYLENE IN HEAT-INDUCED ELONGATION OF LATERAL BRANCHES

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Shoot tips and nodal segments of *Hancornia speciosa* (a tropical fruit tree) cultivated *in vitro* at 31°C usually show initiation of lateral bud growth; further elongation of these buds is arrested. However, at 35°C formation and elongation of branches of second, third and even the fourth order is stimulated. Ethylene is commonly involved in plant responses to high temperature. In order to explore a possible involvement of ethylene on the heat-induced elongation of the lateral branches, we followed the time course of ethylene evolution in shoots growing *in vitro* at 31 and 35°C. We also studied the effect of inhibitors of ethylene action (silver thiosulphate, STS) and biosynthesis [(Aminooxy) acetic acid, AOA] on lateral branch elongation. Initially, Explants growing at both 35°C and 31°C increased their rates of ethylene evolution. However, seven days after the start of the experiment ("day zero"), ethylene evolution from explants growing at 35°C declined. Explants at 31°C continued to increase ethylene release until day 14, when ethylene evolution started to decline. STS (0 to 1 μM) increased the number and length of lateral branches in shoots growing at 35°C, while 10 μM STS stimulated branching at 31 and 35°C. AOA at 1μM enhanced the effect of high temperature on the stimulation of branch elongation. Further studies are underway to elucidate how changes in temperature affect ethylene metabolism and how ethylene may eventually change the dynamics of other growth regulators under environments conducive to lateral branch development in *H. speciosa*.

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ETHYLENE RELEASE AND SUBSEQUENT LEAF ABSCISSION IN WATER STRESSED SEEDLINGS OF CITRUS REQUIRE PREVIOUS ABA ACCUMULATION IN ROOTS

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We have previously shown that ethylene controls leaf abscission in drought-stressed seedlings of citrus. During water stress, ACC largely accumulates in roots and when the plants are rehydrated ACC is soon afterward translocated to the aerial parts. This ACC transported from roots to shoots is rapidly converted to ethylene, which in turn causes leaf abscission (Plant Physiol. 1992, 100: 131-137). In the present work, we have studied the effect of norflurazone, an inhibitor of carotenoid biosynthesis and therefore indirectly of ABA, on ethylene and ABA levels as related to leaf abscission. In addition to ACC, ABA also increased in both water-stressed roots (20-fold) and xylem sap of rehydrated plants. Norflurazone treatments did not modify water potential, stomatal resistance and ABA levels in leaves of drought-stressed plants. However, this inhibitor reduced both ABA accumulation in roots (4-fold) and ABA transport through the xylem fluid of these plants. Furthermore, norflurazone inhibited the ethylene raise which normally occurred after rehydration in drought-stressed plants and consequently arrested also leaf abscission. The results show that water stress promotes both ABA and ACC accumulation in citrus seedlings roots. The data also indicate that the inhibition of the ABA accumulation in roots reduces the ethylene increase observed in leaves as well as final leaf abscission. Thus, the ACC accumulation which takes place in roots in response to water stress is probably mediated by a previous increase in root ABA.

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ETHYLENE AND THE CONTROL OF ASCORBATE PEROXIDASE ACTIVITY IN PINE

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Ethylene is one of the factors implicated in the control of plant stress responses. We have shown recently that ethylene biosynthesis can be induced in Scots pine (*Pinus sylvestris* L.) needles by detachment and treatment with H_2O_2 and $Na_2S_2O_5$ (Ievinsh & Tillberg 1994). In H_2O_2-treated needles a 500-fold increase in 1-aminocyclopropane-1-carboxylic acid (ACC) accompanied by more than a 200-fold enhancement of ethylene evolution was evident. The purpose of the present work was to study ascorbate peroxidase (APX) responses in chemically stressed pine needles and to evaluate a possible role of ethylene in regulation of APX activity during stress. APX activity was induced in detached and intact pine needles in response to H_2O_2 and $Na_2S_2O_5$ treatment though the protein synthesis *de novo*. Both ACC and exogenous ethylene (ethephon) caused an activation of APX which was effectively suppressed by inhibitors of protein synthesis. Chemical stress-dependent increase in APX activity was abolished by inhibitors of both ethylene biosynthesis (aminooxyacetic acid) and action (silver thiosulfate). From the presented results it appears that both H_2O_2 and ethylene are involved in the control of APX induction in stressed pine needles, ethylene being rather a modulator than an inductor of APX response.

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GROWING OUT OF STRESS: SUBMERGENCE, ETHYLENE AND GIBBERELLIN IN *RUMEX* SPECIES WITH CONTRASTING FLOODING RESISTANCE.

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River floodplains serve worldwide as important domains for agriculture and nature preservation, yet are under frequent constrain of flooding. Physiological adaptations of plants towards flooding were studied in the flooding tolerant *Rumex palustris* and the non-flooding tolerant *Rumex acetosa*. For *R. palustris*, enhanced petiole elongation under submerged conditions was found to be mediated by a rise of endogenous ethylene, while for *R. acetosa* submergence and high ethylene limited growth. Both submergence and ethylene treatment enhanced responsiveness to exogenous GA_3. For *R. acetosa*, a contrasting effect of submergence and ethylene on GA responsiveness was found. Analysis of endogenous GAs in *R. palustris* and *R. acetosa* by ELISA and GC-MS analysis showed the presence of GA_1, GA_4, GA_9, GA_{19}, GA_{20}, GA_{24} and GA_{53}. Results with GC-SIM analysis of *Rumex palustris* petiole tissue of submerged and ethylene treated plants showed an enhancement of the 13-OH and non 13-OH pathway compared to drained, aerated plants. In contrast, for *R. acetosa* no substantial differences in levels of GAs could be detected between drained, submergence or ethylene treatment. Results indicate that in the genus *Rumex* flooding tolerance involves a change in the ethylene transduction pathway from growth inhibition (*R. acetosa* response) to growth stimulation *via* enhancement of GA responsiveness and concentration (*R palustris* response).

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ETHYLENE PRODUCTION IN PISTIL OF TULIPS IN RELATION TO EXOGENOUS AUXIN TREATMENT AND WOUNDING

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It is well known that IAA stimulates ethylene production in many plant organs by inducing the synthesis of ACC. Also wounding increases ethylene production through *de novo* synthesis of ACC synthase. It was shown that pistil in tulips as a main source of auxin produce low level of ethylene but ACC oxidase activity is relatively high. Application of IAA on stigma of intact tulips greatly stimulated the growth of pistil and stem elongation and strongly delayed their senescence but ethylene production was not affected. On the other hand wounding (slicing) of tulip pistil greatly induced ethylene production. We suggest that in the tulip pistil exists any mechanism preventing auxin-induced ethylene production by blocking of ACC biosynthesis. It should be mentioned that ethylene induces flower bud blasting in tulips. It is well known also that primary structure of wound-induced ACC synthesis is different from that the auxin-induced the enzyme in the same species, and implies that there are two different genes for ACC synthase, one for wound induction and the other for auxin induction (Nakagawa et al., 1988).

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THE ROLE OF ETHYLENE AND SALYCILIC ACID IN GROWTH RESPONSES OF *Lupinus albus* UNDER HYPOXIA

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The temporal aspects of root growth under effects of oxygen defficiency, aminoxyacetic acid (AOA), and salycilic acid (SA) treatment in seedlings of *Lupinus albus* were investigated in relation to the ethylene production, quantified by gas chromatography. Seedlings grown under root hypoxia showed a significant decrease in relative growth rate; the ethylene production was also influenced negatively and its biosynthetic inhibition caused by AOA was accompanied by a decrease of root growth. SA produced opposite effects.Results are discussed in terms of the known effects of hypoxia on the endogenous levels of phenolic substances and the possible influence of SA on the responses of plants to stress conditions.

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OZONE ACTIVATION OF ETHYLENE BIOSYNTHESIS IN TOMATO PROCEEDS VIA DIFFERENTIAL ACCUMULATION OF TRANSCRIPTS FOR 1-AMINOCYCLOPROPANE-1-CARBOXYLATE SYNTHASE GENES.

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Stress ethylene emission is positively correlated with the ozone sensitivity of various plant species. It has been postulated that ethylene is involved in ozone damage in plants (reviewed: Kangasjärvi et al., Plant Cell Environ 17, 783 (1994). We show that ozone exposure of seven-week old tomato plants for 5 h lead to leaf injury when 90 nl/l ozone was exceeded. ACC concentrations were markedly but transiently elevated (up to 20-fold) within 2 h at 170 and 250 nl/l ozone, while 90 nl/l ozone did not influence the ACC levels. ACC synthase activity had a time course identical to ACC concentrations, with maximum activity at 2 h (25-fold increase at both high ozone concentrations) and exhibited no increase at 90 nl/l ozone level. The genetic regulation of SAM- (precursor of ACC), ACC- and ethylene synthesis was studied from leaf polyA$^+$ RNA using three gene-specific probes for SAM synthetase, four gene-specific probes for ACC synthase, and pTOM13 for ACC oxidase. The rapid changes in ACC synthase activity and ACC levels were mirrored in the gene expression, but transcripts for only one of the four ACC synthase genes studied, ACS2, were detectable. The ACS2 mRNA levels were extremely low before ozone exposure, increased slightly at one hour, peaked at two hours and were already decreasing by the end of the 5-hour exposure. Similarly, mRNA levels of one SAM synthetase gene increased at two hours. ACC oxidase displayed the fastest response; the mRNA levels for TOM13 increased several fold already at 30 minutes decreasing thereafter. This implies immediate increase in conversion of ACC to ethylene, which might activate ACS2. However, when plants were exposed to ethylene (300 nl/l and 600 nl/l) to see if only ethylene can increase ACC synthase activity in the leaves, no changes were detected.

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AN ETHYLENE-MEDIATED SHIFT IN SENSITIVITY TO AUXIN REGULATES THE HYPOXIA-INDUCED FORMATION OF ADVENTITIOUS ROOTS IN *RUMEX PALUSTRIS*

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Hypoxia stress induces the development of large numbers of aerenchymatous adventitious roots in the wetland species *Rumex palustris* Sm.. Similar roots develop when aerated plants are treated with either auxin or ethylene. We investigated if these two plant hormones play a role in the process of hypoxia-induced adventitious rooting.

Auxin levels in the root-forming zone of plants treated with a hypoxic agar solution did not differ from those in aerated plants. Ethylene concentrations in the hypoxic root systems, however, increased largely because of the slow diffusion of ethylene in agar. Furthermore, inhibition of ethylene production in hypoxia-treated plants with AVG caused a decrease in the number of adventitious roots. The effect of AVG could be counteracted by application of ACC. This indicates that increasing ethylene concentrations in hypoxia-treated plants trigger the formation of adventitious roots. On the other hand, inhibition of auxin transport in the shoot of hypoxia-treated plants caused a severe decrease in both auxin levels and root numbers. In addition, inhibition of ethylene production after application of auxin did not prevent formation of adventitious roots.

According to this evidence we can conclude that although auxin levels do not change during hypoxia of the root system, the present auxin concentration is a prerequisite for the development of adventitious roots. Lower concentrations result in a inhibition of root formation. Ethylene triggers the rooting process by increasing the sensitivity of the root-forming tissue to auxin.

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ETHYLENE BIOSYNTHESIS IN DAMAGED AND UNDAMAGED NORWAY SPRUCE TREES

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Contents of different components of the ethylene biosynthesis pathway are measured in differently damaged Norway Spruce trees. The needle material origins from 13 different representative sites in southern Germany, which have been monitored optically during the last ten years. The levels of free 1-aminocyclopropane-1-carboxylic acid (ACC), ist malonyl conjugate (MACC), ACC-synthase-activity and the emission of ethylene show big differences between damaged and undamaged trees. In damaged trees all parameters of the ethylene-biosynthesis pathway are significantelly increased. We could also proof that these parameters show diurnal variations and these variations are much more intensive in the needles of damaged trees. This implies, that damaged trees respond more sensitively to the enviormental conditions. In view of the fact, that ozone is the main air pollutant in most of the areas where the needles were harvested, the increasing concentrations of ACC, MACC and increasing ACC-synthase activity in the needles of damaged trees could be the result of the high stress. At the moment the genes of ACC-synthase from *picea abies* are sequenced by our group.

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INTERACTION OF INDOLYLACETIC ACID, INDOLYACETYLASPARTATE AND ETHYLENE IN REGULATION OF GROWTH PROCESSES IN STEM CUTTINGS

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Indolylacetic acid (IAA) at concentrations routinely used for rooting of stem cuttings strongly enhances ethylene biosynthesis, and at the same time it is rapidly conjugated to indolylacetylaspartate (IAAsp). On stem cuttings from 9 - 14 days old bean plants (*Phaseolus vulgaris L.*) as the model, we studied the role of these three moieties in induction of growth changes that usually occur after IAA treatment of cuttings. Of these changes, the bases swelling and leaves abscission are the most apparent. Ethrel as a source of ethylene was used. The bases of cuttings were treated with 10^{-4} and 2×10^{-4} M IAA and IAAsp and 1,2 - $3,6 \times 10^{-4}$ M Ethrel. IAA and IAAsp were applied after a period of Ethrel action or at the same time with Ethrel. IAAsp and in particular IAA increased the swelling of the bases of bean cuttings. Ethrel, if applied alone did not stimulate swelling in most cases, but if applied together with IAA and IAAsp it acted synergistically. Higher Ethrel concentrations caused abscission of cotyledons and leaves on cuttings. IAAsp significantly prevented this abscission. IAA was less effective in this, and it even enhanced drop of leaves and cotyledons at special conditions. IAA appears to act indirectly in prevention of organ abscission, only after its conversion to IAAsp.

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EFFECT OF METHYL JASMONATE ON THE BIOCHEMICAL CHANGES IN PEANUT SEEDLINGS DURING DROUGHT

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Peanut(Arachis hypogaea L.) seedlings were grown in Knop solution containing methyl jasmonate (MJ) at different conc. Three-leaf-seedlings were transferred to polyethylene glycol(MW 6000) solution to investigate the drought resistance by MJ. The higher relative water content and lower permeability in leaves could be seen while seedlings were treated with MJ. This chemical increased the levels of ABA and proline as well as peroxidase activity. After MJ treatment, the activities of superoxide dismutase(SOD) and catalase (CAT) in seedlings were found to be maintained at higher level than that of the control. Some new SOD isoenzymic bands were formed after MJ application. Ascorbic acid content was higher in MJ treated seedlings than that in the control. The level of malondialdehyde would be decreased while seedlings were treated with MJ during drought. Therefore, MJ could result in increasing the level of ABA, proline and protective enzymes, such as SOD and CAT, and eventually protected the integrity of plasmamembrane to avoid drought damage. The conc of 200 mg/L MJ may be an optimal cono for peanut to resist drought.

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IAA INTENSIFIES SALT STRESS-INDUCED PROLINE ACCUMULATION AND FREE RADICAL GENERATION IN BRASSICA JUNCEA

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Investigations were carried out to study the effect of auxins on proline accumulation and free radical generation in the shoots of *Brassica juncea* exposed to salt stress. Seedlings were raised in mineral growth medium supplemented with IAA at concentrations ranging from 0 to 10^{-4}M, in presence and absence of 200 mM NaCl. IAA (at concentrations above 10^{-7}M) suppressed the growth of control seedlings and intensified growth retardation in stressed seedlings. The presence of IAA at and below 10^{-7}M did not bring about any significant alteration in the level of proline and malondialdehyde (a cytotoxic product of lipid peroxidation and indicator of the extent of free radical generation). The presence of IAA at higher concentrations (at and above 10^{-6}M) caused significant increase in the level of proline both in presence and absence of NaCl. The level of malondialdehyde also increased with increase in the concentration of IAA. These results suggest that auxins i) intensify salt stress-induced growth retardation and ii) promote proline accumulation as well as free radical generation in *Brassica juncea*.

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INVESTIGATIONS ON THE CONTENT OF INDOLE-3-ACETIC-ACID IN SPRUCE NEEDLES OF HEALTHY AND DAMAGED TREES OF VARIOUS SITES.

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Since the end of the seventies, damage to forest trees has appeared in Central Europe, which is now designated as "novel forest decline". Although early discussions on forest damage had brought up the speculation that changes in hormones are also responsible for the symptoms of new forest damage, there are less or no definite statements on alterations in the hormone system of damaged trees.

Based on our research work concerning the effects of phytohormones on the photosynthetic apparatus, the nitrogen metabolism and the transpiration rate, we started a survey on the concentration of indole-3-acetic acid (IAA) in spruce trees of various sites. The concentrations of the hormone were specified with a radioimmunoassay after the needle extract had been purified in several steps. The content of IAA in needles of damaged trees was distinctly lower than the content in needles of healthy trees. We could proof an annual rythm of the IAA content with the maximum of concentration in june. A distinct diurnal course with highest level of concentration during the early afternoon was also observed.

IAA produced by leaves delays abscission and is involved in the regulation of cambial activity and xylem development. Therefore the decreased IAA concentrations in the case of damage could be responsible for the incomplete connection of the needles to the secondary xylem or for a blockade in the transport systems.The decreased IAA concentrations at damage would thus indirectly lead to a premature loss of needles.

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ENVIRONMENTAL CONTROLS ON THE EARLY GROWTH AND DEVELOPMENT OF THE RED MANGROVE, RHIZOPHORA MANGLE L.: RELATIONSHIP TO ENDOGENOUS GIBBERELLINS

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Mangroves are woody, halophytic angiosperms that dominate the intertidal zones of tropical and sub-tropical coastlines. These plants are rather unique in that they are able to tolerate conditions of extremely high salinity and irradiance. Survival under these conditions is generally accompanied by significant reductions in height growth, implicating gibberellins (GAs) may linked to this response. However the physiological mechanisms behind phenological transformations in mangroves are unclear. Furthermore, there remains considerable ambiguity concerning environmentally-controlled development during the dispersal stage of the life cycle when post-abscised, viviparous seedlings may endure lengthy periods of flotation before establishment. In preliminary experiments, applications of gibberellic acid accelerated stem elongation in *Rhizophora mangle* L. Subsequently, floating seedlings of this species were subjected to a variety of salinity and light treatments to examine specific aspects of early development in relation to levels of endogenous GAs. Stem, leaf, and root growth were enhanced in low salinity and low UVb/PAR treatments. Semi-quantitative analysis of GAs by enzyme-linked immunoabsorbant assays (ELISA) indicate that 1) the GA_1 biosynthetic pathway is dominant in *R. mangle* under optimal conditions, and 2) concentrations of GAs, in particular GA_1 and GA_{19}, correlate very well with phenotypic growth patterns among treatments. In an ecological context, the regulation of early shoot development by gibberellins may influence the success of early establishment and thus the recruitment and distribution of this species under variable salinity and light conditions.

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HEAT STRESS AND MAIZE KERNEL GROWTH AND DEVELOPMENT: ROLE OF CYTOKININ OXIDASE

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Heat stress during endosperm cell division in maize kernels (*Zea mays* L.) disrupts growth and reduces grain yield. Our previous work has shown that kernels exposed to short-term heat stress have no detectable levels of zeatin (Z) and the zeatin riboside (ZR) peak is reduced by 70%. No Z or ZR is detected in long-term heat-stressed kernels. The oxidation of Z and ZR by cytokinin oxidase is the major metabolic fate of cytokinins in developing maize kernels. Therefore, our objective was to investigate the role of cytokinin oxidase in this heat-induced reduction of kernel cytokinins. Maize kernels were grown *in vitro* at 25°C continuous (control) or transferred to 35°C, at 5 days after pollination (DAP), for either 4 days (short term) or 8 days (long term). Heat stress reduced kernel weight and increased abortion. Short-term heat stress reduced endosperm cell number by 87% and starch granule number by 58% of that of the control, and long-term heat stress reduced endosperm cell and starch granule numbers by about 95%. Cytokinin oxidase activity was determined for control and heat-stressed kernels between 6 DAP and physiological maturity. In control kernels, cytokinin oxidase activity showed a steady increase starting at 9 DAP and peaked at about 15 DAP. Enzyme activity in kernels exposed to short-term heat stress was higher but maintained a developmental pattern similar to that of the control. Long-term heat stress increased cytokinin oxidase activity compared to either the short-term or the control. The present study suggests that the increase in cytokinin oxidase activity may be the principal mechanism by which heat stress induces a reduction of kernel cytokinin levels. However, this does not exclude the possibility that heat stress also affects cytokinin synthesis. This work was supported by USDA National Research Initiative grant #92-37100-7440.

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EFFECT OF FUSICOCCIN ON EARLY STEPS OF BARLEY AND RICE GERMINATION AT HIGH SALINITY

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Seed treatment with fusicoccin (FC) lowers the Na^+ and proline content, raises the K^+ content, and enhances plant growth and yield under high salinity. The work was aimed at studying the effect of FC on the mitotic activity of barley and rice embryo roots at high NaCl concentrations. The seeds were soaked in water with FC, and placed in Petri dishes on paper wet with water or 0.1–0.3 M NaCl. The cell number was monitored with acetocarmine-stained microscopic preparations. FC attenuated the mitotic asynchronicity induced by high NaCl concentrations. With barley, the first dividing cells appeared in 16 h on water and in 18, 24, 30 h on 0.1, 0.2, 0.3 M NaCl, respectively; FC caused no effect in control but reduced the respective times to 17, 21, and 25 h on salt solutions. Similar effects were observed with rice. Thus, FC enhanced the mitotic activity of barley and rice embryo roots under high salinity.

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HORMONES AS STRESS FACTORS

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Hormones are highly specific endogenous regulators of physiological functions of plants and animals. At the same time it was shown that on some model systems a physiological reaction can be induced by exogenous hormones as well as by nonspecific stress factors (high temperature, high salinity, and so on). Such a situation is well known in animal embryology (processes of mesoderm induction) and in plant morphogenesis (rhizogenesis and xylogenesis). One can suppose that the hormone-like action of stress factors is based on induction of hormone synthesis; however, in many cases it was not experimentally confirmed. An alternative is that hormones can mimic the action of nonspecific stress factors, and that the hormone action includes both specific and nonspecific components. This seems quite logical. Switchover of cell or organ operation under hormone action imply negotiation of some "buffer" or "homeostatic" barriers (these barriers underlie the stable performance of biological systems). Under stress, many processes are uncoupled, permitting easy switchover. Reasoning from this, we undertook a literature search for hormones of animal and plant origin that can substitute for stress factors in some model systems (cell and tissue cultures), and vice versa. It is shown that the action of stress factors as well as hormones in such systems is pleiotropic: stimulation of cell divisionand differentiation, and apoptosis. Conditions were defined that determine triggering between different types of physiological responses to stress factors and hormones. The main means by which multicellular organisms can use nonspecific stress reactions for physiological regulation are discussed.

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PHYTOHORMONES AND LOW TEMPERATURE RESISTANCE OF WINTER VEGETATIVE PLANTS OF CENTRAL ASIA.

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A number of crop plants show their ability to interrapted vegetation during winter half-year in the conditions of Central Asia. For better understanding of the mechanism of plant adaptation to low temperatures and the influence of phytohormones during stress we have studied their contents, correlation and activity applying the methods described by V.I. Kefeli et al. (1973) with winter-vegetating rye (Secale cereale) Vakhshskaja-116 variety. It was shown that the activity of auxines and cytokinins sharply falls and that of abscisic acid increases during fall in temperature in the leaves and tillering nodes. The activity of gibberelline-like substances reduces at the beginning of winter and it do not change and even sometimes increases during short periods of fall in temperature. Intensive growth of plants early in spring correlates with high contents of auxines and gibberelline-like substances and free forms of cytokinins but abscisic acid does not dissappear. The activity of cytokinins and gibberelline-like substances fall during the period of the formation of generative organs. As to the activity of abscisic acid, it remains considerably high.

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MOLECULAR MECHANISMS OF GREEN SEED PROBLEM IN RAPESEED: FREEZE STRESS INDUCED DEVELOPMENTAL CHANGES IN CYTOKININ AND CHLOROPHYLL LEVELS AND CAB GENE EXPRESSION

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Mild freeze stress during seed maturation in rapeseed (*Brassica napus*) induces retention of some chlorophyll in the mature seed, thus causing "green seed" problem. This was accompanied by a rapid moisture loss by the frozen seed. Cytokinin bases and ribosides were analyzed by high performance liquid chromatography, *Amaranthus* betacyanin bioassay and enzyme-linked immunosorbent assay. Transient increases in cytokinins, especially zeatin were observed during the first two days after the freeze stress. Similarly, a transient increase in seed chlorophyll levels was evident in some experiments. The cab mRNA levels declined during development of both normal and frozen seeds. We suggest that freeze-induced rapid desiccation as well as increase in cytokinin levels in seed may be important factors in the "green seed" problem in rapeseed. We would like to thank NSERC for a strategic grant to BRG and A. Johnson-Flanagan of University of Alberta, Edmonton, Canada.

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CHARACTERIZATION OF WHEAT AND *ARABIDOPSIS* L-ISOASPARTYL METHYLTRANSFERASES THAT MAY BE INVOLVED IN THE REPAIR OF DAMAGED PROTEINS IN AGING SEEDS AND ENVIRONMENTALLY STRESSED SEEDLINGS.

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The accumulation of spontaneously damaged proteins in a quiescent seed or in a plant exposed to a variety of environmental stresses may be detrimental to the development of that plant. Thus, the recognition and repair of such altered proteins may be important in limiting protein deterioration in plant cells. One enzyme that appears to participate in this process is the L-isoaspartyl protein methyltransferase (EC 2.1.1.77). This enzyme has been proposed to be involved in the repair of spontaneously damaged proteins by facilitating the conversion of abnormal L-isoaspartyl residues to normal L-aspartyl residues. Based on the abundance of this enzyme in the seeds of a variety of plants and its unique substrate specificity, it has been hypothesized that it functions to prevent the accumulation of abnormal aspartyl residues in the proteins of aging seeds that can limit the viability of the embryo or its chances for germination. Our studies show that the expression and enzymatic activity of the methyltransferase in the winter wheat, *Triticum aestivum* is seed-specific. We show, however, that the methyltransferase mRNA and enzyme activity can be induced in vegetative tissue in response to ABA, dehydration and salt stress. Interestingly, we found that the activity of the methyltransferase in *Arabidopsis thaliana* is also seed-specific; however, this gene is constitutively expressed at very low levels in vegetative tissues and at higher levels in developing siliques. Thus, the regulation of the *Arabidopsis* methyltransferase appears to be different than that observed for the wheat methyltransferase. The identification of sequence motifs in the promoter of the *Arabidopsis* gene homologous to motifs in other plant promoters shown to confer ABA-responsiveness indicates that the *Arabidopsis* gene may also be ABA-responsive. The ability of a plant to regulate methyltransferase activity in its seeds and vegetative tissues in response to desiccation, aging, and environmental stress may allow the plant to efficiently repair protein damage associated with these physiological changes.

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PHYTOHORMONES AND RESISTANCE OF WHEAT TO GLYPHOSATE.

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The role of phytohormones (PHG)in the resistance of wheat plants to herbicide glyphosate has been studied. The effects of the preliminary treatment of wheat seeds with exogenous PHG such as abscisic acid (ABA), indole-3-acetic acid (IAA), gibberellic acid (GA) and 6-benzylaminopurine (6-BAP) on the resistance of adult plants to glyphosate have been investigated. Degree of plant damage by herbicide was estimated by visual evaluation. Besides, the influence of the treatment of wheat seedlings with glyphosate on the accumulation and formation of native PHG (ABA, IAA, and cytokinins) in apical leaves has been also studied. These experiments were carried out in laboratory conditions. The main goal of this work was to study the possible protective role of phytohormones against the damaging action of herbicides (glyphosate) on the wheat plants. It was found that IAA was able to increase the degree of plant damage, while ABA and synthetic analog of cytokinins 6-BAP decreased the biological damage of plants by this herbicide. Basing on the results obtained by HPLC-method, it has been revealed that the effect of glyphosate on wheat caused the considerable decreasing of IAA accumulation in plant tissues. Exogenous ABA was able to inhibit the destruction processes in plants. In particular, ABA and 6-BAP have increased the biosynthesis of chlorophyll in leaves. It has been also noted, that the damage effect of glyphosate on the adult wheat plants may be controlled by the treatment of the plant growth development substances. It has been assumed that the some plant hormones (ABA, cytokinins) could serve the antidotes against herbicides.

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ROOT ELONGATION IN VARIOUS AGRONOMIC CROPS BY THE PLANT GROWTH PROMOTING RHIZOBACTERIUM *Pseudomonas putida* GR12-2 AND ITS MUTANTS

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Seeds of canola, lettuce, tomato, barley, wheat and oats were inoculated with either the plant growth promoting rhizobacterium (PGPR), *Pseudomonas putida* GR12-2 or its mutants, *P. putida* GR12-2/*aux*-1 (auxin overproducing mutant) and *P. putida* GR12-2/*acd*68 (1-aminocyclopropane-1-carboxylate deaminase deficient mutant) alone and in conjunction with an inhibitor of ethylene biosynthesis, S-(E)-2-amino-4-(2-aminoethoxy)-3-butanoic acid (AVG). Variations in root length were compared by means of a growth pouch assay technique conducted under gnotobiotic conditions. All tested plants responded to all of the treatments in a similar manner. The root lengths increased significantly when seeds were treated with *P. putida* GR12-2 and/or AVG in comparison with a $MgSO_4$ control treatment. AVG plus the bacterial treatments had a synergistic effect in that the effects of the treatments were additive. These observations suggest that promotion of root growth by *P. putida* GR12-2 may be mediated through inhibition of ethylene production and/or ethylene effects.

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THE CHEMICAL TREATMENT TO INDUCE FLOWERING OF ASPARAGUS ON SOMATIC EMBRYOS.

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Flowering of *Asparagus officinalis* L. occurs normally two or three years after seed germination, and it is very rare to see a seedling flower. Since we found that treatment with carbamates and thiocarbamates induced flowering in seedlings, we investigated their effects on somatic embryos of asparagus. Somatic embryos were induced in auxin free medium, and were transferred to MS agar (2.5% agar) medium containing chemicals for 20 days. Embryos were transferred to MS liquid medium containing the chemicals for 8 days. Some chemicals induced flowering which formed flowers more than 70% of seedlings by seed treatments, 20% of the regenerated plants from the somatic embryos. The flowers were formed at the shoot tips and contained stamens. After flowering, the plants generally continued to grow normally and produced new shoots without flowers.

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A HISTORY OF THE FLOWERING HORMONE

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Is the history of 'florigen' (so-called) an embarassment to plant science or an exemplar of scientific research at its very best? I explore the half-century-old problem of the flowering stimulus to articulate general problem-solving strategies in science. In particular, how do researchers address major unsolved problems and introduce different investigative technologies? In pursuing a larger work-in-progress, I will invite conference participants to speculate on the future of the floral stimulus and "cast their ballot" on their preferred theoretical interpretations.

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REGULATION OF FLOWERING AND GIBBERELLIN METABOLISM IN PHYTOCHROME MUTANTS OF THE SHORT DAY MONOCOT SORGHUM BICOLOR

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Four genes are known in *Sorghum bicolor* which alter the critical night length needed to promote floral initiation. The ma_3^R allele causes markedly early floral initiation, promotion of shoot growth (both leaf blades and leaf sheaths) and reduction in tillering. Plants homozygous for this allele are deficient in an immuno-detected, PHYB-like phytochrome. Mutant ma_3^R plants are not altered in the timing of circadian peaks of Lhcb and Rbcs mRNAs, but extending the photoperiod beyond 14 h does delay floral initiation for up to 2 weeks. Genotypes carrying Ma_3 or ma_3 exhibit mid-day peaks in GA_{12}, GA_{53}, GA_{20} and GA_1 concentrations. Mutant ma_3^R plants are altered in rhythms of GA_{20} and GA_1 with highest levels occurring near dawn. Heterozygosity of ma_3^R with either ma_3 or Ma_3 restores near wild type shoot growth, tillering and flower initiation. Phytochrome and gibberellin contents are reduced but are more like wild type in heterozygous plants. GA biosynthesis inhibitors reduce shoot growth and delay floral initiation in both ma_3^R and non-ma_3^R genotypes. GA_3 applied as a soil drench restores shoot growth, hastens floral initiation and increases GA_1 content. At moderate levels, an inhibitor of $GA_{20} \rightarrow GA_1$ conversion inhibits floral initiation in a non-ma_3^R genotype but not in the ma_3^R genotype. GA_1 is lower in the non-ma_3^R genotype. Work to isolate and sequence phytochrome genes and ma_3^R is underway, and studies to determine whether altering photoperiods will change the daily pattern of GA levels as well as floral initiation are in progress. Our working model is that maturity gene 3 codes for phytochrome B, that ma_3^R is a deletion mutant, that PHYB regulates circadian production of GA_{20} and GA_1, and that GA_1 is essential for both shoot growth and floral initiation.

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TRANSITION TO FLOWERING OF MANGO DOES NOT REQUIRE SHIFT IN GENE EXPRESSION OF APICAL MERISTEM

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Classic descriptions of flowering involve an irreversible commitment of the apical meristem from that of production of proteins responsible for foliar development to those giving rise to floral structures. Evidence strongly indicates that this concept does not apply to tropical fruit species which undergo periodic flushes of vegetative or reproductive growth. Mango flowering occurs when growth is initiated during inductive cool temperatures. Vegetative growth results when buds are initiated under warm conditions. Pre-formed target tissues, specifically dormant lateral primordia and leaf bracts, appear to respond to a putative florigenic promoter and inhibitor, respectively, to give rise to inforescences or vegetative flushes. Results of experiments on mango are presented which demonstrate that floral induction can be readily influenced by changing conditions present during bud development. Newly initiated vegetative shoots can, thus, be influenced to make a transition to formation of inflorescences, and the reciprocal can be also accomplished. These transitions can be explained independent of direct involvement by the apical meristem. The presence of vestigial leaf structures in inflorescences reinforce the concept that apical meristems simply give rise to axillary target tissues which respond along a predetermined path once initiated.

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INFLUENCE OF GIBBERELLIN BIOSYNTHESIS INHIBITORS ON STEM ELONGATION AND FLORAL INITIATION ON IN VITRO CHICORY ROOT EXPLANTS UNDER DARK AND LIGHT CONDITIONS

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Introduction: Under continuous light, both stem elongation and floral initiation are observed on in vitro chicory root explants (Cichorium intybus L.). Photoperiods of less than 16 hours cause only vegetative growth, while under continuous dark stem elongation without leaf expansion or flower bud formation occurs. Under short-day conditions, stem elongation but no flower buds can be induced by GA_3. By adding GA biosynthesis inhibitors to the nutrient medium, the possible rôle of gibberellins could be further studied. **Methods:** Chicory roots of the variety Flash, vernalized for 28 weeks at + 1 °C were used. All gibberellin biosynthesis inhibitors: chlormequat chloride, mepiquat chloride, tetcyclacis, paclobutrazol, BAS 111.. W, LAB 198 999 and daminozide were added to sterile medium before solidification. The in vitro cultures were evaluated after 8 weeks growth. **Results:** The GA biosynthesis inhibitors were chosen as they belong to different groups inhibiting GA metabolism at distinct steps. Paclobutrazol (= compound with nitrogen - containing heterocycle) and compounds belonging to the cyclohexanetriones clearly reduced flowering-stem growth under light conditions and vegetative-stem growth under dark conditions. Under light conditions, flower bud initiation was not affected. **Conclusion:** Stem elongation does not seem to be a prerequisite for initiation of flower buds. There is strong evidence for GA_1 being synthesized during the in vitro culture period under photoperiodic control. GA_1 influences stem length, but not the initiation of flower buds. This system could be very useful for further GA metabolic studies in chicory.

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HORMONAL CONTROL OF SEXUAL EXPRESSION IN CUCUMBER

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The sex of a plant is revealed by the flowers it produces. There are three different functional flower types - male, female and hermaphrodite, and any or all of these may be present on an individual. Monoecious cucumber genotypes produce both male and female flowers, the positions and proportions of which can be modified by environmental conditions (long days and high temperatures enhancing males and inhibiting females) and applied plant growth regulators (PGRs: ethylene, auxin and abscisic acid promoting females, gibberellins promoting males). Previous crude assays of whole shoots or apices have sometimes shown correlations between endogenous hormone levels and effects of applied PGRs on sex. However, there is no data on flower buds themselves and the primary control mechanisms in sex determination still remain unknown. Therefore we have been measuring early changes in endogenous hormone levels in developing flower buds to determine if they reflect the applied PGR effects, e.g. whether male flower buds contain more GAs than female. Monoecious cucumber buds develop as hermaphrodites until approximately 1 mm long, then switch into unisexual development. Although modified by the environment, a reliable male-female pattern exists. From this, we have developed a method of accurately predicting where each flower type will be produced, enabling sampling prior to unisexual differentiation. Visibly post-differentiated buds were also collected along with subtending leaves from pre-differentiated buds. HPLC, RIA and GC-MS analysis indicate differences in hormone levels in pre-differentiated buds, despite their morphological similarity. In addition, we have demonstrated that paclobutrazol, a gibberellin biosynthesis inhibitor, enhances female development but only at doses much higher than those which inhibit stem elongation. Overall, the results suggest that there is a "female suppressing" rather than "male promoting" signal, which may explain the relatively low number of female flowers and the spaces between them.

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THE USE OF GROWTH RETARDANT PACLOBUTRAZOL TO REGULATE FLOWERING OF MANGO (*Mangifera indica* L.)

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Lack of profuse and regular flowering is considered as a major problem in mango cultivation in Malaysia. As indication by numerous reports, flowering and fruiting can be regulated by using growth retardant paclobutrazol. In relation to that, several experiments have been conducted to determine the appropriate methods, concentration and timing of paclobutrazol application on flowering of some mango cultivars. It appeared that paclobutrazol was very effective chemical for regulation of flowering. Both soil application and foliar spray could be used to induced flowering. However, factors such as age of the tree, canopy size, flushing conditions and weather conditions should be considered before applying the chemical in order to obtain better results.

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FLORAL TRANSITION IN THE LONG-DAY PLANT *SINAPIS ALBA* IN RELATION TO PUTRESCINE FLUXES.

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Sinapis alba L. was induced to flower by either a long day or a displaced short day. Following collection of leaf (phloem) and root (xylem) exudates from induced and non-induced plants, polyamines in the exudates were extracted, separated and analyzed quantitatively. The titers of free and conjugated putrescine, the major polyamine fractions in all samples, increased early and markedly during the floral transition, coinciding closely with movement of the floral stimulus out of the induced leaf. By contrast, putrescine titer in the root exudate did not increase. A spray of DFMO, an irreversible inhibitor of the putrescine-biosynthetic enzyme ornithine decarboxylase, at the 8th h of the long day considerably reduced the titer of free and conjugated putrescine in leaf exudate, and at the same time, markedly decreased the flowering response of induced plants. This effect of DFMO on flowering was substantially reversed by a simultaneous application of putrescine to the roots. DFMO sprayed on induced plants also suppressed early activation of the indices of both mitosis and DNA synthesis in the shoot apical meristem. These results support the view that the extra putrescine synthesized in induced leaves is a necessary component of the floral stimulus in *Sinapis*.

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EFFECTS OF BIALAPHOS ON THE CONSTITUENTS OF MALE-STERILE RICE PLANTS

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Bialaphos induced 94.8 % sterility at a concentration of 0.1 mM at the meiosis stage. The numeber of seeds, ear length and culm length in rice plants treated with bialaphos were almost the same as those of control. Rice plants treated with bialaphos have no crop injuries. All the stamens became degeneration but the shape of the pistils were the same as that of control in the observation of the glumous flowers treated with bialaphos under a microscope. The results suggested that the growth of the pistils treated with bialaphos was normal but that of the stamens alone was inhibited. The accumulation of ammonia as a result of glutamine synthetase inhibition induced by bialaphos is the direct factor causing rice sterility, since the degree of sterility increased proportionately with the endgeneous ammonia content. The accumulation of protein nitrogen and sugar caused in the sterile panicles. The decrease of free amino acids content caused in the sterile anthers, and the decrease of starch showed in the sterile pollen stained with iodine-potassium iodide. The results of the constituents of male-sterile rice plants suggested that the sterility induced by the decrease of metabolic activity of amino acid, protein, and carbohydrate in the rice plants.

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GIBBERELLIN RESTORES THE FERTILITY OF PETUNIA MALE GAMETOPHYTE

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The regulatory mechanisms of the gametophyte - sporophyte interactions during floral organogenesis were studied on the experimental system including three clones of petunia: (a) fertile - with functioning male and female gametophytes, (b) self - incompatible - with S-gene of incompatibility, expression of which inhibits pollen tube growth in sporophyte pistil tissues, and (c) clone with male sterility. The plants of these three clones were enriched with phytogormones by spraying the leaves during 24 days with 10 mg/l GA3. In all clones, a vigorous growth of vegetative and reproductive shoots was recorded in two weeks after treatments. The striking effect was exerted by gibberellin on the sterile petunia clone: contrary to the absence of seeds in untreated plants, gibberellin induced an active seed production (70-80% of control plants). Therefore, the elevation of GA level in plants of sterile clone favored the development of male gametophyte and successful pollination and fertilization.

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CLASSES OF FLOWER-INDUCING COMPOUNDS IN ASPARAGUS SEEDLINGS COMMONLY BIND TO MICROSOMAL CYT. *P*-450

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Introduction: The flower inducing compounds so far developed have been found to inhibit a cyt. *P*-450 enzyme, *t*-cinnamic acid 4-hydroxylase (C4H), in the microsomes, and this has led us to find that piperonyl butoxide, a brosd-spectrum *P*-450 inhibitor, also induces flowering. Cyt. *P*-450 has been thus postulated to be a possible triggering site for flowering.

Methods: To induce flowering, seeds of *Asparagus officinalis* were incubated with a test solution for 10 days at 25 °C. The binding of a compound to microsomal *P*-450 was followed spectrophotometrically.

Results and Conclusions: Amidines were prepared to develop a new, structurally different classe of flower inducing compounds. Some of them induced flowering as effective as the previous potent compounds, but did not inhibit C4H. However, when incubated with microsomal cyt. P-450, they produced type I spectra as well as other classes of compounds. When NADPH was generated in the mixtures, all classes of compounds gave type III spectra, indicating that they all form a reactive, high affinity species to produce a complex with *P*-450. The results suggested that the amidines inhibit cyt. *P*-450 other than C4H, and the others inhibit cyt. *P*-450 besides C4H. The target species of the flower inducing compounds was thought to be cyt. *P*-450.

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EAR INITIATION IN MAIZE (*ZEA MAYS* L.): EFFECTS OF ENVIRONMENTAL FACTORS AND GROWTH REGULATORS.

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In certain inbred genotypes of *Zea mays* L. which are used for seed production in western Europe, a reproductive failure affecting ear initiation has been observed, causing the topmost ear to be substituted at maturity by a sterile, leaf-like, structure. The occurrence of this ear failure is dependent on year, location, and sowing date, indicating that it is induced by environmental factors. Ear abortion has been induced in controlled environment by a chilling treatment, given just before tassel initiation. The same stress treatment after or before tassel initiation has either no effect or induces an acclimation response. SEM study of the abnormal axillary buds indicates that ear failure results from the early collapse of the axillary meristem, just after its emergence on the axill, and followed by the elongation of the prophyll. Exogenous applications of an auxin (2,4-D) or a cytokinin (benzyladenine), can respectively mimic or reverse abortion caused by the stress treatment. Benzyladenine also greatly promotes ear initiation in non-stress conditions. HPLC/RIA determination of the endogenous content of growth regulators indicates that chilling induces a change in the hormonal balance of the plant with a marked decrease in cytokinins. In contrast, acclimated plants exhibit a modified cytokinin complement. It is suggested that cytokinins are a determinant factor of ear initiation in maize.

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RESPONSE OF BROADCAST RICE GROWN AT MULTIPLE SOWING RATES TO FOLIAR APPLICATION OF GIBBERELLIC ACID (GA_3)

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To achieve good yields in broadcast cultivation of rice, Malaysian farmers cultivate at high sowing rates which require an increase of input demand like fertilizers and pest/disease management. To generate basic data on such crop performance a trial was conducted in the second (rainy) season at sowing rates of 80, 100 120, and 200 kg seeds per ha. using two current Indica varieties, MR84 and MR151. Evaluation of their varietal traits to growth and yield was tested with foliar application of GA_3 at 40 ppm, applied at active tillering and panicle initiation stages. Results showed that sowing rates affected tiller number only at the active tillering stage. At heading MR151 had more tillers and a lower leaf area than MR84; but not subsequently. GA_3 enhanced plant height after one or two applications, with the difference persistent to harvesting. Yield of brown rice was higher in MR151 than MR84, both were reduced by GA_3 applications. Panicle length was not affected by any treatments. MR84 had a higher spikelet sterility than MR151. Spikelet number per panicle was increased by GA applications in all treatments. Crop performance relative to the micro-environmental conditions and GA_3 applications were discussed.

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HORMONES AND LIGHT CONDITIONS IN THE CONTROL OF FLOWERING IN BIENNIAL *BRASSICA NAPUS* CV. WW1033.

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Flowering of the biennial *Brassica napus* cv. WW1033 can be induced or manipulated by vernalization, day-length extension, modification of red or far-red light composition and exogenous application of gibberellins (GAs) known to be highly florigenic. The possible involvement of endogenous GAs in flowering of cold-requiring biennial cvs. of canola, such as WW1033 by manipulation of light conditions including photoperiod and composition and vernalization using 3°C, 8h day for 6 weeks were investigated. Photoperiod prior to vernalization had no effect on post vernalization flowering, but long-days (LD, 16h) post-vernalization were required for flowering. Devernalization (no flowering) occurred in plants which were tranferred directly from 3°C to 20°C conditions, whereas a one week transition from 3°C to 20°C was required for 100% flowering. The GA and kaurenoid levels in tissue are being determined throughout the vernalization treatment. In the absence of vernalization, flowering can be induced with a continuous light (CL) treatment, but a 16h photoperiod is not inductive. Modification of light composition while maintaining uniform PAR and 16h photoperiod resulted in different growth patterns in young seedlings and differences in floral induction behaviour. Modification of the red:far-red (655 to 665:725 to 735 nm) from fluorescent lights (4.77), Metal Halide (MH)(2.44), MH+Incandescent (2.31), MH+Far-Red (2.07) and greenhouse conditions (1.23) resulted in hypocotyl elongation in 8 day old seedlings of 7.5 (fluor), 1.8 (MH), 3.5 (MH+inc), 4.5 (MH+FR) times that of green house-grown plants but only the MH+FR treatment gave floral induction. GA levels in tissue are being assessed for the above light conditions and will be presented. The experimental use of this cultivar, which can be induced to flower under a range of physiologically relevant conditions, may lead to a better understanding of floral induction.

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EFFECT OF NUTRITION, GA INHIBITORS AND C-16,17-DIHYDRO-GA5 ON CRANBERRY FLORAL AND VEGETATIVE BUD INDUCTION

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The cranberry (*Vaccinium macrocarpon* Ait.) is an evergreen, woody perennial that produces two shoot types: horizontal runners and vertical uprights from lateral buds of runners. In the field, floral and vegetative bud induction occurs in terminal apices during long days around full bloom. Cranberry plants remain vegetative under greenhouse conditions; however, vegetative and, to a lesser extent, floral buds can be induced with paclobutrazol (McArthur and Eaton, 1989; Sci Hortic 38:131). Three cultivars of cranberry were grown with differential mineral nutrition for 14 d and then treated with 2 mg of either paclobutrazol or trinexapac-ethyl (an acylcyclohexanedione). Stem elongation was inhibited by both chemicals; however, paclobutrazol was more effective and it alone decreased runner numbers. Paclobutrazol greatly promoted bud formation, and only it induced floral buds. Some runners of GA-inhibitor-treated plants overcame the effect of these chemicals and, especially for plants with a high level of mineral nutrition, showed vigorous growth even though other shoots on the same plant were still inhibited and set dormant buds. In a second experiment, plants were first treated with 0 or 4 mg of paclobutrazol and two days later each of four shoots per plant were treated with 0, 1, 3, 9 or 27 ug of endo-C-16,17-dihydro-GA5 (A florigenic compound kindly provided by Professor Richard P. Pharis [Evans et al, 1993; Planta 193:107]). Initially the GA5 stimulated stem elongation and delayed paclobutrazol-induced bud formation. However, a slight promotive influence on floral induction was detected when buds were examined several months later. The cranberry appears to have merit as a useful model plant for studying floral bud induction of woody perennials.

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DOUBLE MUTANTS FOR INFLORESCENCE MERISTEM IDENTITY GENES IN PEA

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Wild-type (WT) pea (*Pisum sativum* L.) flowers on second order inflorescences generated by I_2 meristems arising in the leaf axils. The first order inflorescences show an indeterminate growth habit and the I_1 meristem never gives rise directly to a floral (F) meristem. Lateral inflorescences are leafless and show a limited growth habit; they bear a small number of flowers, usually one or two before the I_2 meristem terminates as a hairy, rudimentary stub. The recessive non-allelic mutants *veg1* and *veg2* wholly prevent flower formation; all inflorescences appear as leafy shoots. In the less severe mutant *veg2^{inc}*, the leafy lateral inflorescences belatedly bear flowers but the I_2 meristem retains the unlimited growth habit of *veg2* plants. In the *det* mutant, the I_1 meristem behaves like an I_2 meristem, terminating in a stub after producing a small number of reproductive nodes (usually 1-3). The *veg1 det* double mutant does produce flowers and it expresses the *det* habit. We report here that double mutants *veg2 det* and *veg2^{inc} det* have the same phenotype as the single mutants *veg2* and *veg2^{inc}*, respectively. Thus in both cases the mutant allele at the *Veg2* locus is epistatic to *det*. In contrast to the situation with mutations which affect synthesis of the floral stimulus (*gi*) or inhibitor (*sn, dne, ppd*) in pea, we found no evidence of a graft-transmissible effect between WT and *veg2^{inc}* plants. These results are consistent with the view that the genes *Det* and *Veg2* confer identity on the I_1 and I_2 meristems, respectively. In the double mutant *veg2^{inc} det*, the I_1 meristem identifies as an I_2 meristem and expresses the unlimited growth habit of a *veg2^{inc}* lateral inflorescence. SEM analysis has shown no evidence of an I_2 stub forming on *veg2* or *veg2^{inc}* single mutants. Aberrant floral morphology is not uncommon in *det* and *veg2^{inc}* plants, implying that expression of the *Det* and *Veg2* WT genes is needed for normal functioning of floral meristems.

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STATE OF WATER AND ION HOMEOSTASIS IN RELATION TO FLOWER INDUCTION OF PHOTOPERIODIC PLANTS BY PHOTOPERIOD AND FLORAL STIMULUS

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Photoperiodic induction of flowering is a complex process of the signal formation which is displayed in changes of the apical morphogenesis. The purpose of this work was to study a number of the plant communication systems (phytohormones, state of water, ion homeostasis) during the flowering induction. Data on the purification of the floral stimulus by means of HPLC were presented and it was shown that the extract from the short-day flowering plants had a florigenic activity. Using the methods of continuous proton magnetic resonance, NMR spin-echo four fraction of the proton relaxation belonging to water contained in apices of tobacco plants induced for flowering were observed (T2=0,387-0,425 ms; T2= 3,7-3,9; T2=10,4-13,4 ms; T2=20,1-23,2 ms). Tissues of organs in vegetative plant were found to have only one component of water with relaxation time characteristic (T2) within the range of 10,4-13,4 ms. In apices of short-day plant Chenopodium rubrum induced for flowering two fraction of water with the time of relaxation T2=175 ms, T2=340 ms (1 short-day induction) and three fraction of water T2=165 ms; T2=180 ms; T2=350 ms in case of flowering induction by floral stimulus were detected. Vegetating plant tissues found to have only one fraction of water with proton relaxation time T2=280 ms. Induction of flowering by photoperiod or floral stimulus involves a sharp increase in the activity of ions (Ca, K) and decrease in that of (Na) in apices of plants. A posibility of functional relation between various plant regulation system during the flowering induction is discussed.

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SPECIFICATION OF MERISTEM IDENTITY IN PEA IS DISTINCT FROM REGULATION OF DETERMINANCY

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The *pim* (proliferating inflorescence meristem) mutant of pea (*Pisum sativum*) alters floral meristem (F) identity, but has no effect on either the indeterminate first-order inflorescence meristem (I_1) or the second-order inflorescence meristem (I_2) which exhibits limited growth. F meristems of *pim* are transformed into inflorescence meristems that branch extensively before producing aberrant, frequently petalless, flowers with additional F meristems in sepal axils. Both wild-type and *pim* I_2 meristems initiate a minimal number of F meristems before terminating as a hairy stub. Thus I_2 meristems have determinate growth, though not in the strict botanical sense. A recessive modifier gene extends *pim* branching and indeterminancy, but alone has no effect on phenotype. *pim* and *pim*, modifier double mutants affect the determinancy and identity of third-order F meristems, but not I_2 meristems. Indeterminate I_1 meristems terminate as hairy I_2 stubs in *det* (determinate) plants, but F meristems develop normally. Double mutants of *pim* and *det* have extensively branched inflorescences like *pim* and the I_1 terminates as a hairy stub like *det*. Thus, *det* and *pim* do not interact and are expressed independently (in an additive manner) in the double mutant. This supports our model that *Det* and *Pim* assign I_1 and F meristem identity, respectively, rather than more globally regulating determinancy. Supported by USDA and NSF.

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A GENETIC AND MOLECULAR ANALYSIS OF FLOWER INITIATION IN *ARABIDOPSIS*.

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At least 14 loci have been identified of which the mutants delay flowering in long days in the *Arabidopsis thaliana* ecotype Landsberg *erecta* (L*er*) "wild type". The epistatic relations among 10 of these genes, as well as their response to vernalization, daylength and gibberellin application have been determined. For the cloning of the late flowering locus *fwa*, by means of chromosome walking, the close linkage between *fwa* and *abi1* was used to localize the *fwa* gene on a YAC-contig isolated in that region. Two embryo-lethal mutants *emb20* and *emb35* flanking the *fwa* locus were used to select for recombinants on both sides of the locus. Since *fwa* is a partially dominant mutation and therefore possibly a gain of function mutation, we constructed genomic libraries of both mutant alleles to allow screening for the genomic fragments containing the mutant gene. These constructs will be used to introduce by transformation the mutant alleles in wildtype Landsberg *erecta* plants. Furthermore, all three alleles (wildtype and mutants) will be sequenced and the expected differences between the wildtype and the mutant alleles will indicate that the gene we isolated is *fwa*. Irradiation induced mutagenesis of the *fwa* mutant containing flanking mutant loci as markers resulted in the identification of several early mutants which either may be defective in the *fwa* locus or may represent intergenic suppressors.

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NEW PEA MUTANTS WITH ALTERED SENSITIVITY TO PHOTOPERIOD

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As long-day plants, wild type (WT) pea (*Pisum sativum* L.) plants grown in short photoperiods (SD) flower late relative to plants grown in long photoperiods (LD). This delay in flowering is associated with a profound phenotypic syndrome indicative of vigorous vegetative growth. This syndrome is characterised by shorter internodes, increased branching, elongated peduncles, and retardation of flower development, seed growth and whole-plant senescence. The "SD" syndrome, including the photoperiodic effect on flower induction, is known to be controlled by a graft-transmissible substance, which is referred to as a "flower inhibitor" but in the broader context is better thought of as a "juvenility factor" which promotes vegetative over reproductive growth. Light reduces the production of this inhibitor, and this is thought to be the main mechanism by which LD induce early flowering. Mutants *sn*, *dne* and *ppd1* are all deficient in this inhibitor. As a result they are early-flowering and day-neutral, lacking the capacity for delay of flowering in SD, and show strong orientation toward reproductive growth. These genes are proposed to be involved in the synthesis of the floral inhibitor. We have recently isolated two new mutants which show profound alterations in sensitivity to photoperiod. The recessive *fun* (*f*ar-red *un*responsive) mutant was isolated in screens under far-red light. Mutant seedlings are completely insensitive to far-red light, and appear to specifically lack phytochrome A (phyA) responses. The *fun* mutation causes delayed flower initiation and development in LD, and has pleiotropic effects which closely phenocopy a WT plant grown in extreme SD. These results suggest that phyA may be the main photoreceptor for sensing of day length. In addition, they imply that phyA controls flowering by down-regulating synthesis of the graft-transmissible flower inhibitor, and thus place downstream of phyA action all the flowering genes which affect synthesis of, or sensitivity to, the flower inhibitor. *Ppd2* is a dominant mutation conferring an early day-neutral phenotype which is essentially identical to that of the recessive *sn*, *dne* and *ppd1* mutants. We are currently exploring whether this mutation acts at the level of light perception, down-regulation of floral inhibitor synthesis, or perception of the floral inhibitor signal.

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STUDIES ON ZEARALNONE-BINDING PROTEIN IN *TRITICUM AESTIVUM* L. DURING VERNALIZATION

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Zearalenone (ZEN) is a new plant growth substance that was identified by Li and Meng in 1980's. It was found that ZEN was a key substance controlling plant flowering. To understand the regulating mechanism of ZEN on plant development, we investigated the specific ZEN-binding protein in winter wheat (*Triticum Aestivum* L. cv. Yan Da 1817) by means of Radio Ligand Binding Assay and biochemical technique. The results showed that the young seedlings of winter wheat contained the specific binding sites for ZEN. The analysis on the ZEN-binding sites indicated that the specific binding sites were sensitive to proteinase, heat and urea, so it was believed to be proteinaceous. Optimum pH for binding activity was 7—8. The ZEN derivatives, such as α-zearalanol and β-zearalanol, and animal estrogen 17β-estradiol competed the binding sites with ZEN. Whereas, other plant hormones could not displace ^{3}H-ZEN from its binding sites. Saturation analysis of ZEN-binding protein (ZBP) showed that the binding for ZEN was saturable. Scatchard analysis revealed the presence of a single type of binding sites. The dissociation constant for ZEN (Kd) was calculated to be 1.9×10^{-7}mol $\cdot$ L^{-1}, and the number of binding sites to be 6.2 pmol $\cdot$ mg^{-1} of soluble protein. ZBP precipitatd in 30% saturated ammonium sulfate. 3-to 4-fold purification of ZBP was achieved by 30% saturated ammonium sulfate precipitation. Sucrose-density gradient centrifugation analysis of ZBP showed that there were two peaks of radioactivity. One of the peaks corresponding to sedimentation coefficient of 4-5S was completely displaced by 500-fold excess of unlabeled ZEN and was believed to be the specific binding sites. The other peak was hardly displaced by 500-fold excess of unlabled ZEN, probably represent nonspecific binding fraction. This work was supported by National Natural Foundation of China.

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VERNALIZATION AND GIBBERELLIN PHYSIOLOGY OF WINTER CANOLA

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Winter canola (*Brassica napus* cv. Crystal) requires vernalization (chilling) to induce bolting and flowering. Since gibberellins (GAs) are involved in the regulation of stem elongation and reproductive development in many plants, the role of GAs in events induced by vernalization was investigated. Exogenous GA induced stem elongation but not flowering in nonvernalized plants, whereas plant growth retardants that block GA biosynthesis prevented elongation and flowering in vernalized plants. Endogenous GAs were extracted from 10 week vernalized and nonvernalized shoot tips harvested at 0-, 8- and 18-days post vernalization (DPV) and quantified using [$^2H_2$] internal standards and GC-selected ion monitoring. Generally, GA1,3,8,19 and 20 concentrations were higher in the vernalized relative to nonvernalized shoot tips at all time harvests. Since all of the monitored GAs were elevated, thermoregulation apparently occurs prior to GA_{19} biosynthesis. [^{3}H]Gibberellin A20 or [^{3}H]GA_1 was applied to vernalized and nonvernalized shoot tips at 5-, 11- and 14-DPV and following 24 h of incubation, shoot tips were harvested. Analyses by HPLC indicated that vernalization increased conversion of [^{3}H]GA_{20} to a [^{3}H]GA_1-like metabolite and reduced conversion of [^{3}H]GA_1 or [^{3}H]GA_{20} to polar ^{3}H metabolites, putative glucosyl conjugates. Collectively, these studies indicate a role for GA content and metabolism in the regulation of vernalization induced stem elongation with GAs serving as probable regulatory intermediaries between chilling treatment and subsequent stem growth.

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DEVELOPMENT OF A MODEL SYSTEM FOR STUDYING PHOTOPERIODISM IN TREES

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In temperate-zone tree species, short days typically induce vegetative bud set, the initiation of cold-acclimation, and the onset of dormancy. These responses, however, often differ between northern and southern populations of the same species. In the northern hemisphere, for example, trees from southern locations typically require shorter days to induce bud set than do northern trees. Although evidence from a number of tree species suggests that phytochrome plays an important role in photoperiodic perception, and that changes in gibberellin metabolism are probably involved in controlling the cessation of internode elongation, little else is known about the signaling pathway for photoperiodic responses in trees. The long-term goals of our research are to characterize the early events in short-day-induced bud set and to determine the genetic basis of photoperiodic ecotypes in trees. We are using black cottonwood (*Populus trichocarpa*) to develop a model system for addressing these questions. First, we characterized the photoperiodic responses of a northern and southern ecotype of black cottonwood by growing trees under a range of photoperiods. Compared to the southern ecotype, the northern ecotype had a longer critical photoperiod and greater photoperiodic sensitivity. Using night breaks with red and far-red light, we subsequently demonstrated that short-day-induced bud set is under phytochrome control. Currently, we are cloning and characterizing the phytochrome gene family from black cottonwood. This research should provide information on which phytochrome genes play important roles in dormancy induction and will serve as a foundation for investigating whether phytochrome signaling pathways differ among photoperiodic ecotypes.

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THE *det*1 AND *det*2 MUTANTS OF *ARABIDOPSIS* GENETICALLY SEPARATE THE GROWTH OF COTYLEDON PETIOLES AND BLADES

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The induction of cotyledon expansion and inhibition of hypocotyl elongation by bright light is one of the most dramatic responses in the developing *Arabidopsis* seedling. Genetic analysis of seedling photomorphogenesis has resulted in the identification of mutants which do not need a light cue to develop. The *det*1 and *det*2 mutants were the first of this class to be isolated based on their abnormally short hypocotyl phenotype in dark grown seedlings. Using image analysis and camera lucida, we have shown that the *det*2 mutation genetically separates development of the cotyledon blade and petiole since only the petiole (and not the blade) of this mutant elongates constitutively in the dark. These results place *det*2 into a subset of constitutive photomorphogenetic mutants affecting only a portion of the deetiolation responses to light. Physiological analysis of the *det*2 mutant suggests that this mutant is altered in its ability to utilize apoplastic sucrose. Unlike *det*2, both the cotyledon blades and petioles of the *det*1 mutant exhibit constitutive growth in the dark. The addition of exogenous cytokinins to wild type plants is not capable of inducing a *det*1 phenocopy in the developing cotyledons. Epidermal cell expansion completely accounts for the constitutive growth of *det*1 cotyledon blades. The *det*1 cotyledons lose the majority of their constitutive blade expansion (relative to the wild type) when they are excised from the rest of the seedling, suggesting that the *det*1 cotyledon phenotype results from interactions with other tissues in the developing seedling. Supported by N.I.H. grant #2T32HDO7183 to M.M.N. and N.S.F. grant #MCB-9220110 to E.V..

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LIGHT STIMULATES GERMINATION OF AUSTRALIAN ASTERACEAE VIA PHYTOCHROME AND GIBERELLIC ACID METABOLISM

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Seed of several Australian everlasting daisies (Asteraceae, Tribe Inulae) are stimulated to germinate by light. The effect of light on gibberellin (GA) metabolism was investigated in these seeds. White light increased seed germination in *Craspedia* sp., *Erymophyllum ramosum* and *Rhodanthe chlorocephala* subsp. *splendida* compared with darkness. In these taxa, red light (R, 640 nm) stimulated and far red light (FR, 720 nm) inhibited germination, indicating the involvement of phytochrome. Paclobutrazol, a GA biosynthesis inhibitor, reduced light-stimulated germination and this was overcome by exogenous GA_3. GA_3 and GA_1 promoted germination in the dark, but GA_{19} and GA_{20}, which are precursors to GA_1, generally did not. GAs were applied to paclobutrazol-treated seeds which were incubated under either R or FR. Very few paclobutrazol-treated seeds germinated without GAs under FR and exposure to R only marginally improved germination. Application of GA_{19} or GA_{20} to paclobutrazol-treated seeds, increased germination in *Rhodanthe chlorocephala* subsp. *splendida*, under R with no germination under FR. This trend was observed in *Craspedia* sp., but not in *Erymophyllum ramosum* or *Rhodanthe floribunda*. GA_1 substantially increased germination but light quality had no effect. It is suggested that R light increased germination in GA_{19}- and GA_{20}-treated seeds by phytochrome-stimulation of their metabolism to GA_1, which was the most active GA in seed germination. This research was funded by the Australian Research Council.

403 THE EFFECT OF LIGHT INTENSITY ON GROWTH AND PHOTOSYNTHESIS OF RED CLOVER WITH NITRATE OMITTED

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Scientific information about influence of light intensity and change of nitrogen nutration to pure symbiotic nitrogen fixation on plant-host is scanty. Red clover (*Trifolium pratense*) was cultivated on Knopp solution with aeration during 4 weeks. Then one party of plants transferred on the solution without nitrate. Growth was continued at light intensities: 30, 70 and 100W.m^{-2}. Dry weight was determined after liophylisation, soluble sugars in 80% ethanol extract and starch after hydrolysis with amylase using phenol-sulfuric acid reagent; chlorophyll content in acetone extract, and protein in buffer extract with dye were measured spectrophotometrically; root respiration - amperometrically and molecular nitrogen fixation by acetylene reduction(AR). Growth of the control plants correlated with light intensities, increasing during 8 weeks. Without nitrate growth was much slower and after 8 weeks was 63%, 29% and only 15% of controls at 30, 70, and 100W.m^{-2}. In these plants it was found less chlorophyll and soluble protein. There was not noticeable difference in soluble sugar content but starch content was higher in plants deprived of nitrate especially grown at the highest light. Root respiration per 1g of dry weight (DW) did not show great difference. But AR in absence of nitrate was much higher than on nitrate. After 8 weeks growth AR was maximal without nitrate at 30W.m^{-2} both as calculated per 1 g of DW as per one plant. High velocity of AR while low growth and biochemical signs of nitrogen starvation by diazotrophy are discussed. It is proposed that sink-source relations were broken when growth at diazotrophy. Decrease of shoot/root ratio and leaf surface suggest the change of hormonal state of plant in absence of nitrate.

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PHOTOCONTROL OF GIBBERELLIN PHYSIOLOGY IN *BRASSICA*

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Growth, gibberellin (GA) content and metabolism were studied in light and dark-grown seedlings of *Brassica rapa* and *B. napus* (oilseed rape or canola). Dark-induced shoot elongation was observed in both GA-deficient (*rosette*) and GA-insensitive (*Dwarf1*) dwarf mutants as well as in normal genotypes. Concentrations of endogenous $GA_{1,3,8,19 \text{ and } 20}$, as measured by GC-selected ion monitoring with $[^2H_2]$GAs, were relatively unchanged in whole seedlings grown in the light or dark but localized concentrations were approximately doubled in stems of dark-grown plants. Light intensity was varied in another study involving five light intensity treatments. Height and GA concentration were closely correlated, with both being progressively reduced with increasing light intensity. Metabolism of $[^3H]GA_1$ and $[^3H]GA_{20}$ also varied with light treatment. There were increased proportions of ethyl acetate-soluble $[^3H]$ metabolites (free GAs) in the low light intensity-treated plants compared to elevated proportions of putative glucosyl conjugates in higher light intensity treated plants. GA metabolism in a light-grown phytochrome B deficient mutant (*elongated internode*) was somewhat similar to that of dark-grown normal plants. These results demonstrate that light condition influences GA metabolism and content and also suggest photocontrol of GA sensitivity and possibly GA translocation.

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GIBBERELLIN STATUS OF *lip1*, A MUTANT OF *PISUM SATIVUM* WHICH DISPLAYS LIGHT-INDEPENDENT PHOTOMORPHOGENESIS.

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The *lip1* mutant of *Pisum sativum* L (garden pea), which is thought to be a phytochrome A mutant, shows light-independent photomorphogenesis (Frances et al.1992, Plant Cell 4: 1519). In complete darkness it shows many features of de-etiolation including short internodes, enhanced leaf expansion and pigment production. In light it has a semi-dwarf phenotype. The response of dark- and light-grown *lip1* seedlings to applied gibberellins (GAs) has been examined and compared with wildtype cv. Alaska (*Lip1*). Neither GA_1 nor GA_{20} could restore internode elongation in the *lip1* mutant to that of wildtype seedlings, suggesting that this mutant is probably not GA-deficient. Extraction and quantitation of endogenous GAs in dark- and light-grown seedlings using dideuterated internal standards confirmed that reduced internode elongation in *lip1* seedlings is not a consequence of GA-deficiency. Seedlings of *lip1* contained comparable levels of GA_1 to wildtype seedlings, However, dark-grown *lip1* seedlings contained lower levels of GA_{19} and higher levels of GA_{20} than dark-grown wildtype seedlings. Thus $[GA_{19}] : [GA_{20}]$ for dark-grown *lip1* seedlings was 0.38, whereas for dark-grown wildtype seedlings the ratio was 2.1. The possiblity that phytochrome A may exert some regulatory control on GA metabolism will be discussed.

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LIGHT-STIMULATED GROWTH OF LEAF CELLS

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Dicotyledonous leaves, unlike stems, are stimulated to grow by bright light acting through phytochrome, a separate blue-light system, and photosynthesis. Light-induced apoplast acidification, wall loosening, and ion uptake are preceded by a transient depolarization of the plasmamembrane in response to light. The nature of this early cellular response to light has been investigated separately in epidermal and mesophyll cells of the argenteum mutant of pea (*Pisum sativum*), and in phytochrome deficient cotyledons of *Arabidopsis* mutants. The bulk of the membrane depolarization observed in mesophyll cells is most likely a result of chloride efflux passing through ATP-modulated Cl^-channels, and is partially dependent on photosynthesis. Light stimulates channel activity on cell-attached patches of mesophyll protoplasts; excision of light-sensitive patches yields the above characterized Cl^-channel. On the other hand, the transient depolarization of epidermal cells is more likely due to calcium influx, and is not dependent on photosynthesis. In *Arabidopsis* cotyledons, light-induced depolarization is evident in photosynthetic cotyledons of wildtype seedlings, is diminished in the absence of PHYB, and absent in non-photosynthetic tissue. Ion substitution studies indicate that PHYB affects calcium, not chloride flux. Previous work showed that PHYB is essential for the full growth response of cotyledon cells to light. These results imply that PHYB is necessary for initial membrane transport responses of growing cells to light, and that there is an interaction between phytochrome and photosynthetic activity of these cells. Supported by NSF grants DCB-8903744 and MCB-9220110 to E.V.

407 THE INTERACTION OF LIGHT AND ABSCISIC ACID IN THE REGULATION OF PLANT GENE EXPRESSION.

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We have found that the concentration of the plant hormone abscisic acid (ABA) increases substantially when Lemna gibba or Arabidopsis plants are placed into the dark. In addition, we observe that ABA concentrations can be negatively regulated by the action of the photoreceptor phytochrome in Lemna gibba. In order to test whether the observed changes in ABA concentration in response to red light could be physiologically relevant, we tested a wheat Em promoter (which has been extensively studied for its responsiveness to ABA) using a transient particle bombardment assay in Lemna gibba. We were able to demonstrate negative regulation of the Em promoter by phytochrome action. In this case it is likely that the effect of phytochrome is mediated by changes in ABA levels. We confirm by RNA blot analysis that Lhcb and rbcS mRNA levels which are positively regulated by phytochrome can be negatively regulated by exogenous ABA. Finally, we tested the Lemna Lhcb2*1 promoter in the transient assay. The wild type promoter construct demonstrated positive regulation by phytochrome action; this phytochrome-responsiveness was still apparent in the presence of a high concentration of exogenous ABA. Furthermore, when a 2 bp mutation was introduced into the Lhcb2*1 promoter such that phytochrome-responsiveness was lost, we were still able to detect a response to ABA application. We conclude that in this case, unlike the Em promoter, separable promoter elements must exist to mediate responses to phytochrome and ABA action. Our results demonstrate that it is essential to take into account that dark treatments and the phytochrome system can affect ABA levels when interpreting studies of light-regulated genes.

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RECENT ADVANCES IN THE GENETIC CONTROL OF PHOTOMORPHOGENESIS IN PEA

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We have recently isolated and characterized several new photomorphogenic mutants of pea (*Pisum sativum* L.). These define five complementation groups. Mutants at the *lv* locus show reduced response to red (R) and white (W) light, and are deficient in responses attributed to phytochrome B (phyB). We have demonstrated that the majority of *lv* alleles are deficient in a phyB-like apoprotein. Light-grown *pcd1* plants have yellow-green foliage with a reduced chlorophyll (Chl) content and an abnormally high Chl*a*:Chl*b* ratio. Etiolated seedlings of the *pcd1* (*p*hytochrome *c*hromophore *d*eficient) mutant are completely insensitive to far-red (FR) light, show reduced responses to R and W, and have no spectrophotometrically detectable phytochrome. PhyA apoprotein is present at wild-type (WT) level in etiolated *pcd1* seedlings, but is not depleted by R treatment. Crude phytochrome preparations from etiolated *pcd1* tissue also lack spectral activity, but can be assembled with phycocyanobilin (PCB), an analogue of the endogenous phytochrome chromophore phytochromobilin (PΦB), to yield a difference spectrum characteristic of an apophytochrome-PCB adduct. These results indicate that the *pcd1* phenotype results from a deficiency in the phytochrome chromophore. Furthermore, etioplast preparations from *pcd1* seedlings can metabolise biliverdin but not heme to PΦB, suggesting that *pcd1* plants cannot convert heme to biliverdin. Like *pcd1*, seedlings of the *fun1* (*f*ar-red *un*responsive) mutant are also completely blind to FR light. However *fun1* seedlings show normal responses to R and W. We will also report on our current investigations into the phytochrome status and photophysiology of *fun1* and two other non-allelic mutants showing reduced response to FR.

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GIBBERELLINS, PHOTOPERIOD AND PETIOLE GROWTH IN STRAWBERRY

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Vegetative and reproductive development of strawberry is sensitive to photoperiod. Consequences include seasonal variations in total leaf canopy and fruit production. Gibberellins (GAs) are implicated as mediators of photoperiod responses and as regulators of stem elongation. However, most studies have monitored final plant dimensions, rather than the dynamic processes that lead to this state. We report here on the kinetics of strawberry petiole elongation, as influenced by photoperiod and GA content. The most rapid response to a long day → short day (LD→SD) transition was a 20% reduction in petiole elongation rate within 2 days. Maximal growth rate was at day 8, and was reduced by 30% in SD. Application of the GA biosynthesis inhibitor, paclobutrazol, reduced petiole elongation within one day under both LD and SD conditions. By day 8, growth rate was reduced 45% by paclobutrazol and 25% by SD. Cell length but not cell number was reduced by daylength and paclobutrazol. Endogenous GAs identified by GC/MS were GA_1, GA_4, GA_8, GA_{19}, GA_{20}, GA_{29} and GA_{34}. No differences were detected in concentrations between LD and SD at day 1 or day 4. At day 8, GA_1, GA_8, GA_{29} and GA_{34} concentrations were reduced in SD. Paclobutrazol reduced GA concentrations to low levels in both LD and SD treatments. In vivo feeds of [^{2}H] and [^{3}H]-GAs to intact petioles indicated GA metabolism was little affected by daylength. Metabolism of GA_{19} was slow, but GA_{20} was metabolised to GA_1, GA_8 and GA_{29}. GA_1 was metabolised to GA_8, possibly to a greater degree in SD. These results indicate operation of the early-13-hydroxylation pathway. However, rapid growth reduction by SD occurred prior to detection of, or in the absence of, differences in GA concentrations. We therefore conclude that part of the photoperiod response is mediated independently of GA concentration. It may be that sensitivity to GAs is affected, and/or that non-GA factors are operating.

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GRAVITY CONTROL OF LEAF ORIENTATION IN IPOMOEA NIL.

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When the potted Ipomoea nil plant is turned upside down, the leaves invert themselves in the light or dark within 6 to 12 hours so that the topside of each leaf is oriented upwards. The blade generally twists down to the right or, under some conditions, will "somersault". This phenomenon, as observed with an AG-6040 Panosonic time-lapse video system, appears to involve both growth-induced twisting (gravistrophism) and gravicurvature of the petiole. Blade removal eliminates the twisting response. No motor cells or pulvini have been observed in these leaves. Preliminary treatments with the auxin transport inhibitor, N-1-naphthylphthalamic acid, suggest a causal role for asymmetrical distribution of auxin (originating from the blade) in this petiole response.

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THE ROLE OF GRAVITY IN THE FORMATION AND MAINTENANCE OF HYPOCOTYL HOOKS

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Shoots showing a hooked form are found commonly in nature. In addition to the hypocotyl hooks formed by most dicotyledonous seedlings when grown in darkness which are discussed here, there are many flower stalks which show the same form (e.g. poppy (*Papaver*), coltsfoot (*Tussilogo farfara*) and *Cyclamen*) and also moss sporophytes and developing fern fronds. The environmental factor producing the hook is gravity, with the most apical tissue being orientated downwards (positive gravitropism) and the more basal regions oriented upwards (negative gravitropism). However, as new cells are produced behind the apex, the cells which were originally orientated downwards switch their orientation to upwards. We have termed this change in behaviour *gravitropic sign reversal* (Myers et al., 1994). By growing epigeal-germinating seeds held by their seed coat, hookless seedlings can be produced. If these are then placed horizontally, it is possible to follow hook formation and the growth rate changes which accompany it. Pre-treatment of hookless seedlings with red light before gravity stimulation removes the downward curvature of the apical cells but the upward orientation is retained, giving a seedling with no hook. Theories of gravitropic control will be discussed in relation to gravitropic sign reversal.

A.B. Myers, R.D. Firn and J. Digby (1994) Gravitropic sign reversal - a fundamental feature of the gravitropic perception or response mechanism in some plant organs. J. Exptl. Botany 45 77-83.

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GRAVICURVATURE IN THE *diageotropica* MUTANT OF TOMATO IS INHIBITED BY IAA, AUXIN TRANSPORT INHIBITORS, AND CYTOKININ

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The tomato mutant *diageotropica* (*dgt*) is insensitive to indoleacetic acid (IAA, the naturally-occurring auxin) in hypocotyl elongation assays. We have shown that intact, light-grown *dgt* seedlings can respond properly to gravity, although more slowly than wild type seedlings. In order to test whether the *dgt* gravity response is auxin-mediated, we measured gravicurvature of seedlings in solutions of IAA and 2,3,5-triiodoacetic acid (TIBA, an inhibitor of auxin transport). IAA and TIBA inhibit gravicurvature in both *dgt* and wild type plants. However, the *dgt* response is less sensitive to inhibition by TIBA. Since intact *dgt* seedlings do not elongate in response to auxin, this indicates that a different mechanism governs elongation and gravicurvature in *dgt*.

Since a number of *dgt* phenotypic traits can be phenocopied by exogenous cytokinin application in wild type plants, we measured the gravitropic response of intact seedlings in solutions of the cytokinins 6-benzyladenine, zeatin, isopentenyladenine, and kinetin. High concentrations of all cytokinins tested inhibited gravicurvature in both wild type and *dgt*. However, the *dgt* gravity response appears to be more sensitive to cytokinin inhibition than the wild type response. The differential effectiveness of the cytokinins tested will be discussed.

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NATURALLLY-OCCURRING INHIBITORS OF GIBBERELLIN-INDUCED GROWTH
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While many naturally-occurring substances which promote plant growth are known, little information is available on inhibitors of growth. We have used pea plants (Pisum satinum var. Little Marvel) as a source of extracts which are inhibitory to gibberellin-induced growth. Seedling shoots were extracted three times with methanol, and the extract concentrated in a rotary evaporator. The concentrated extract was purified by adsorption on a Sep-Pak Plus C18 Environmental Cartridge which was eluted with washes of 20, 40, 60 and 80% methanol in water. The eluates were mixed with gibberellin A_3 so that each plant received .05ug GA_3. The eluates from the 40 and 60% methanol eluates reduced the amount of growth produced by GA_3 alone. The extract alone applied to pea seedlings does not affect their growth nor injure the seedlings. The growth reduction can be prevented by adding an increased amount of GA_3 similar to the interaction observed in competitive inhibition.

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6-DEOXOTYPHASTEROL AND 3-DEHYDRO-6-DEOXOTEASTERONE, POSSIBLE PRECURSORS TO BRASSINOSTEROIDS IN POLLEN OF *CUPRESSUS ARIZONICA*

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Two new congeners of brassinosteroids, (22*R*, 23*R*, 24*S*)-22,23-dihydroxy-24-methyl-5α-cholestan-3α-ol and (22*R*, 23*R*, 24*S*)-22,23-dihydroxy-24-methyl-5α-cholestan-3-one, termed 6-deoxotyphasterol and 3-dehydro-6-deoxoteasterone, respectively, occur in relatively large amounts in mature pollen of *Cupressus arizonica*, together with many known brassinosteroids. GC-MS, NMR spectroscopy, and reductions were used to identify the new compounds. 6-Deoxotyphasterol, which is weakly bioactive, and 3-dehydro-6-deoxoteasterone may be precursors to the more oxygenated and more active brassinosteroids. It will be of interest to test their conversion to such compounds, as the steps before teasterone in the biosynthetic pathway(s) to brassinosteroids are as yet hypothetical.

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EFFECTS OF A SEAWEED EXTRACT AND AVG ON SQUARER ABSCISSION AND YIELD IN COTTON

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Cotton culture in China is characterized by high plant density and high N input which often result in too much vegetative growth and a high percentage of square or fruit abscission during early fruit development. Mepiquat chloride is effective in regulating vegetative growth, but not in preventing square abscission. Since ethylene is involved in regulating square abscission in cotton, AVG and 'Antidrop', a novel product formulated with seaweed extract which has been found to counteract the effect of ethylene, were used in a series of field and greenhouse experiments from 1992 to 1994 to test their effectiveness in inhibiting square abscission in cotton. Infusion of ethephon or ACC into pedicels resulted in squarer abscission within 24 and 48 hr, respectively, in the greenhouse. 'Antidrop' counteracted the effects of ethephon and ACC by 36% and 58% when applied together, but AVG was not effective. When applied alone, both AVG and 'Antidrop' significantly inhibited squarer abscission either by infusion into pedicels or by spraying onto whole plant. AVG (200 ppm) and 'Antidrop' (100 ppm) were applied twice in the field, once at pre-bloom and again 25 days later. Boll set was increased 28% by AVG and 31% by 'Antidrop' treatment. 'Antidrop' significantly promoted boll enlargement and fiber elongation, which gave a 19% increase in lint yield compared to the 9% increase produced by AVG treatment. The carbohydrate content and dry matter in 'Antidrop' treated squares and young fruit were significantly higher than in those treated with AVG, which showed that 'Antidrop' also promoted assimilate partitioning to the fruit.

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CHARACTERIZATION OF A POSSIBLE TUBER-INDUCING FACTOR(S) IN A CONTINUOUS USE HYDROPONIC SYSTEM.

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The feasibility of using plants for Bioregenerative Life Support Systems (BLSS) for long-duration space missions is being determined by NASA. A "continuous" planting and harvesting scheme is desirable in order to minimize the fluctuations in gas exchange, transpiration, and edible biomass that occur with "batch" planting schemes. Potato (*Solanum tuberosum L.* cv. Norland) is currently being grown in an atmospherically closed, controlled environment chamber using recirculating nutrient film hydroponics. Harvesting and replanting one quarter of the crop continuously on a 26-day cycle is being compared to 104-day batch production plantings. Both plant height and canopy size are smaller in the "aged" solution relative to planting in "fresh" nutrient solution. Continuous production on "aged" solution results in earlier tuber initiation (~ 7 days), reduced plant height, and a smaller plant canopy than the "batch" treatment. Hastened tuber initiation and reduction in plant size had minimal effects on quantum efficiency or harvest index of the plants grown in continuous production. In a separate study, the possible tuber inducing factor(s) overcame non-inductive environmental conditions, and could be removed from the nutrient solution by filtering through activated charcoal. Our results suggest that the observed developmental and morphological changes observed may be advantageous to BLSS.

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PHYSIOLOGICAL EFFECTS OF 5-AMINOLEVULINIC ACID IN VEGETABLE CROPS

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The role of 5-aminolevulinic acid (5-ALA) as a precursor of chlorophyll or a herbicide is well documented. In present report, to elucidate physiological effects of 5-ALA, this compound at low concentration (50, 100, 300 ppm) was applied to foliage or soil. 5-ALA increased fresh yield of spinach, and this increase was more marked in the case of fall cropping than in the case of spring one. The optimum concentration for this compound was 50 ppm. 5-ALA decreased the contents of nitrate nitrogen, total nitrogen and the ratio of nitrate nitrogen per total nitrogen, and this decreasing rate was marked in foliage treated with 50 ppm. The content of oxalic acid was also decreased by application of 5-ALA. However, no the optimum concentration was found. 5-ALA altered the content of fructan as nonstructural storage carbohydrates. The maximum value was found when 300 ppm solution was applied to soil. From these results presented here the authors wish to emphasize that at low concentration (50-300 ppm) 5-ALA are very useful for the production of harmless vegetables or the accumulation of fructan as a new carbohydrate sink in crops. The physiological mechanism of 5-ALA will be discussed.

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HORMONE FLUCTUATIONS AFTER POLLINATION IN *THEOBROMA CACAO* L.

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Theobroma cacao has a self-incompatibility (SI) system that has been described as late acting (ovarian) with both sporophytic and gametophytic control (Cope, 1962). However, early recognition of self-pollen may occur at the stigma (Aneja et al., 1994). Incompatible pollination causes abscission of the flower. In order to determine the timing of the SI response we measured pollen tube growth rates, ethylene production and endogenous levels of free IAA and ABA (GC-SIM-MS) after compatible and incompatible pollinations. Pollen tubes grew at the same rate after compatible and incompatible pollination (038 mm/h) and reached the ovary within 8 h. Penetration of all ovules occurred within 16 h. Ethylene levels in unpollinated and incompatibly pollinated flowers peaked 24 h after pollination but remained at base levels in compatibly pollinated flowers for 48 h. Levels of ABA increased significantly from 1.91 µg/100 mg DW to 3.54 µg/100 mg DW in unpollinated and incompatibly pollinated flowers . IAA increased most dramatically from 26.3 ng/100 mg DW to 65.1 ng/100 mg DW 24 h after compatible pollination. Ratios of the tested phytohormones showed no difference between unpollinated or incompatibly pollinated flowers but significant differences in compatibly pollinated flowers. These data suggest that there is no initial inhibition of pollen germination at the stigma and that the hormonal response takes place after pollen tubes have reached the ovary. We also infer that the incompatibility system of *Theobroma cacao* is based on the acceptance of compatible pollen and the "non-recognition" of incompatible pollen, i.e., incompatibly pollinated flowers behaved as though they were not pollinated.

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APICAL DOMINANCE AND GRAFT-TRANSMISSIBLE FACTORS IN *Pisum sativum L.*

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Four non-allelic mutants with increased branching, *rms1*, *rms2*, *rms3* and *rms4*, have been utilised to investigate the control of apical dominance in the garden pea, *Pisum sativum* L. Graftings studies have provided very strong evidence in support of the suggestion that three of these mutations (*rms1*, *rms2* and *rms3*) act to promote branching by altering the level of hormone-like substances which are produced in the rootstock and shoot. However, while the *rms1* and *rms2* mutations appear to alter the level of the same substance, the action of the *rms3* mutation appears to be independent of the alleles at the *Rms1* and *Rms2* loci. In contrast, the *rms4* mutation does not appear to control the level of a graft-transmissible substance. Quantifications (GC-MS-SIM analysis using D5-IAA) of the endogenous IAA level in mutant and wild-type shoot tissue indicated that the increased branching in each of the mutant shoots was not attributable to a reduced level of endogenous IAA. Rather, the *rms1*, *rms2* and *rms3* plants contained an elevated level of IAA in comparison with wild-type plants, prior to, during, and after bud release.

The level of endogenous cytokinins is also reported and discussed in relation to the action of these genes and the possibility that a novel substance may be important in the control of apical dominance in pea.

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THE RELATION BETWEEN BUD DEVELOPMENT AND CYTOKININ LEVELS IN NORWAY SPRUCE, *PICEA ABIES* (L.) KARST.

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The development of buds in annual shoots from the uppermost whorl of 15- to 20-year old Norway spruce trees were followed by scanning electron microscopy and their cytokinin content were monitored from June through October. Three classes of buds clearly different in size were collected every second week: apical buds, uppermost whorl buds, and lower lateral buds. Zeatin riboside (ZR) and isopentenyladenosine (iPA) were measured with ELISA after the purification of extracts with immunoaffinity chromatography and HPLC. The level of ZR, but not that of iPA, showed a positive correlation with bud size. ZR levels increased slowly in all buds until early September. Later in September and in October, there was a pronounced increase of ZR in apical buds, whereas ZR decreased slightly in whorl and lateral buds. The level of iPA showed a pronounced peak in July but decreased to low values from the middle of August. We suggest that there is a connection between bud development and ZR level. By its role in bud development, ZR may also be of importance in apical control as the annual shoot extension in spruce is predetermined during the bud development. The high ZR levels in apical buds in October are unexpected as the buds are dormant during this period.

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ENDOGENOUS CYTOKININS IN ROSE TISSUES

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In rose combination plants, bud break of the scion is influenced by the genotype of the rootstock, probably caused by differences in its cytokinin production. Indications for this are obtained by exogenous application of BA via flower tubes to decapitated plants, which resulted in more bottom breaks. In order to correlate data of growth and development of combination plants with the cytokinin production of the rootstock, plants are harvested periodically. Z, ZR, iPA and iPAR are quantified in the xylem exudate by means of HPLC separation and ELISA. Further insight in the cytokinins present in rose tissues is necessary to determine the 'key' cytokinins to define the quality of a rootstock. Therefore Madelon/Multic combination plants were grown and harvested in three developmental stages: (1) young plant, flower starts to open, axillary shoots start to grow, (2) plant with axillary shoots with visible flowers and swelling buds of the bottom breaks, (3) plant with 8-10 cm bottom breaks. In each stage upper, middle and lower leaves, stems, axillary shoots, bottom breaks, roots and xylem exudate were collected. Endogenous cytokinins were determined by immunoaffinity co-purification and HPLC with UV-spectrum detection, and GC-MS.

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CONCENTRATION OF ENDOGENOUS INDOLE-3-ACETIC ACID AND OXYGEN DURING THE EARLYWOOD-LATEWOOD TRANSITION IN *PICEA ABIES* STEMS

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Indole-3-acetic acid (IAA) is suggested to be the main regulator of cambial activity in forest trees, but there is evidence that ethylene, as well as cytokinins, gibberellins and abscisic acid also play a role. The concentration of oxygen decreases during the growing season, attaining a minimum well below 5% at about the time of the earlywood-latewood transition. This low oxygen level might inhibit the action of IAA and the synthesis of ethylene, which could explain the reduced radial expansion of cambial derivatives during latewood formation. This was investigated in potted *Picea abies* trees subjected to different combinations of temperature and photoperiod in controlled environment chambers to manipulate the earlywood-latewood transition and the development of cambial dormancy. Regardless of treatment, the IAA content, measured by combined gas chromatography-mass spectrometry (GC-MS), remained constant in both the cambium and the differentiating xylem. However, the level of oxygen in the cambial region, also measured by GC-MS, was higher in trees that produced latewood than in trees that did not. Thus, latewood formation cannot be attributed to a reduction in the levels of IAA or oxygen.

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INTERACTIONS BETWEEN TEMPERATURE MANIPULATIONS AND GA IN CONTROLLING STEM ELONGATION IN PEAS.

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Wildtype pea plants cv. Torsdag grown under a low day/high night temperature regime (negative DIFference between day and night temperature) developed shorter internodes and shorter epidermis cells than plants grown under an opposite temperature regime, high day/low night temperature (positive DIF). Plants grown under negative DIF had lower relative growth rate and lower net assimilation rate than plants grown under positive DIF. Under positive DIF, plants allocated relatively more assimilates to the stem than to the leaves, while the opposite was the case under negative DIF. The photosynthetic capacity was highest in plants grown under negative DIF, and these plants also had a higher content of total soluble proteins and a lower content of total chlorophylls than plants grown under positive DIF. Stem elongation of the gibberellin (GA) deficient dwarfs le and ls and the GA insensitive dwarf lkb was affected by DIF-treatments in a similar pattern as in the wildtype, although, the response was weaker. However, the GA insensitive slender mutants (la cry^s) did not respond to DIF. DIF treatments had a minor effect on the stem elongation of the very short dwarf mutant na, but growth stimulation induced by exogenous GA_1 was significantly enhanced by positive compared to negative DIF, indicating a higher sensitivity to GA_1 in plants grown under positive than under negative DIF. The relative response between positive and negative DIF decreased at a high GA_1 dose. This is consistent with the lack of DIF response in the slender mutant where the GA response is saturated. Effects of different temperature regimes on levels of endogenous GAs are currently being investigated.

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MAJOR CYTOKININS IN FRENCH BEAN: IDENTIFICATION, ORGAN DISTRIBUTION, AND REGULATION OF LEVELS BY LIGHT

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The composition of cytokinins in *Phaseolus vulgaris*, one of the best studied plants with respect to cytokinins, was re-investigated. The plant material was extracted by a procedure that ensures the inactivation of enzymes known to be able to degrade cytokinin nucleotides. A cytokinin fraction was obtained by immunoaffinity chromatography with antibodies that are able to bind isopentenyladenine, zeatin, cis-zeatin, dihydrozeatin and benzyladenine, as well as their ribosides, ribotides and 9-glucosides. The cytokinin nucleotides are present at levels much higher than nuclosides and free bases, a feature often reported for other plants. No cis-zeatin or dihydrozeatin compounds were detected, but high levels of isopentenyladenosine monophosphate, never before reported in this species, were found. Cytokinin levels were markedly higher in the stems and petioles than in the roots and leaves. Overall cytokinin levels increased with increasing light intensity, especially in the stems.

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EFFECT OF MANIPULATING THE DOMINANCE INTERRELATIONSHIP WITHIN TOMATO FRUIT AND FRUIT PEDUNCLES

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Primigenic dominance - a phenomenon appearing in many parts of the plant - describes one way of correlative inhibition. In this type of dominance not the position of the organ, as in apical dominance, but the time of origin is important. Fruit developing first within an inflorescence dominate during the entire period of development over those initialized later. A number of reports create evidence that auxin is not the only hormonal signal involved in the regulation of dominance phenomena. In the experiments described below the importance of auxin, cytokinins and abscisic acid was examined. Tomato inflorescences were manually thinned to an artificial "4 fruit system" consisting of 4 flowers per inflorescence only. Within this experimental unit the distally developing organs were pollinated 5 days after the proximal ones. 4 days later the proximal fruitlets in half the fruit clusters were excised releasing the distal fruit from dominance. At different times of development after this treatment inhibited and uninhibited fruitlets were harvested. After extraction and purification the following hormones were analyzed quantitatively: zeatin/zeatin riboside (Z/ZR), isopentenyl adenine (iP)/ isopentenyl adenosine (IPA), abscisic acid (ABA) as well as free and conjugated indole acetic acid (IAA). The diameter of the uninhibited fruit was significantly larger than the inhibited variant. In fruit and fruit peduncles of the uninhibited variant an increase in Z/ZR concentrations as well as higher amounts of IAA were found. The concentrations of inactive, conjugated IAA were inverse to this. In inhibited variants the conjugated IAA was higher at comparable times. There was no definite tendency in IPA/iP concentration in correlation to the treatments. The increase in growth in correlation to the increased Z/ZR content shortly after the treatment allows a cytokinin - auxin - interaction as explanation as proposed for apical dominance.

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POLYAMINES AND WINTER DORMANCY OF TEA (CAMELLIA SINENSIS L. (O) KUNTZE)

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The phenomenon of dormancy is viewed as a mechanism for species to survive adverse climatic conditions. In northern India, tea is harvested throughout the growing season and the yield in winter (mid November-mid March) is negligible due to bud dormancy. In addition to environmental factors like temperature, endogenous plant growth substances play an important role in winter dormancy and subsequent to bud break. In recent years, increasing evidences have appeared on the role of polyamines (PAs) in various plant growth and developmental processes. Changes in PAs levels including free, conjugated and bound have been observed during onset and release of dormancy in tea shoot cvs. Upasi-9 (high yielding) and T-78 (low yielding). Conjugated and bound PAs (spermidine and spermine) were comparatively higher in cv. Upasi-9 as compared to cv- T-78 only at the time of dormancy setting in the first week of November. However, PAs (in any form) were almost absent till March in both the cultivars. Spermine was detected as free and conjugated form in cv. T-78, and only in free form in cv. Upasi-9 during dormancy break in the first week of April. It appears that spermidine and spermine but not putrescine do play some role during onset and break of dormancy in tea.

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ENDOGENOUS ABA, IAA, AND CYTOKININS IN MAIZE AND PEA DAMAGED BY APHIDS.

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Our knowledge of the role of phytohormones in the relations between plants and biological factors as such insect pests is still insufficient. The purpose of the present investigations was to study endogenous content of phytohormones (ABA, IAA, and cytokinins) in plant leaves damaged by feeding aphids (*Shizaphis graminium* Rond., *Aphis fabae* L.). Resistant and susceptible forms of plants (*Zea mays* L., *Pisum sativum* L.) were utilised. The endogenous phytohormones (abscisic acid (ABA), indole-3-acetic acid (IAA), zeatin (Z), zeatin-riboside (ZR), dihydro- zeatin (DZ), dihydro-zeatin-riboside (DZR) and iso-pentenyl-adenine (iPA) were assayed by means of HPLC- methods. It was found that the damaging effect of insects on leaves caused changes in the levels of phytohormones in all studied plants. The increasing of damage by insects on plants caused increase of ABA and decrease of the IAA in leaves of plants. Besides, the damage by insects on the maize apical leaves of resistant forms caused considerable changes in the cytokinins level. It was also established that all studied forms of cytokinins in the uninfested plants much differing from infested them. It is assumed that the interaction of plants and insects is closely associated with the functioning of the plant hormonal system. It has also been suggested that the resistance of plants against insect pests may be determined by different abilities to form and accumulate cytokinins (ZR, Z) in plant tissues. It is also possible to claim that the change in the ABA content in the damaged leaves of maize and pea occurs due to the disturbance in the water exchange (water stress).

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ENDOGENOUS ABA AND CYTOKININS DURING SEED DORMANCY BREAK OF COCKLEBUR.

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Cocklebur (*Xanthium strumarium* L.) is a major weed in soybeans and cotton in the former Soviet Union. In recent years, much attention has been given to the problem of different ability of cocklebur seeds to germination. It is known that the existing two seeds within each fruit usually differ both in size and germination ability. The larger seed normally germinates in the spring, whereas the smaller seed does not germinate until the following year. This raises the question of whether the endogenous phytohormones such as ABA and cytokinins, are involved in the process of dormancy and germination of seeds. Investigations were performed on the freshly-harvested seeds of cocklebur. The degree of dormancy was evaluated by ratio of germinated and non-germinated seeds both in darkness and light at alternating temperatures of 25/20°. The level of phytohormones was estimated by HPLC both in the seeds and their coat. The connection was established between the ABA level of seeds's coat and the seed ability to germinate. The ABA content in larger seeds (dormant) was greater than that in smaller seeds. The increase in the cytokinin content during the break dormancy and germination of both seeds was observed. It was assumed that the dormancy of both cocklebur seeds might be caused by the ABA accumulation in both seed's coat and inside the seeds. It may be possible also that cytokinins are included in the process of break of dormancy.

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CYTOKININ AND ABSCISIC ACID CONTENTS IN GENERATIVE ORGANS OF TOMATO MUTANTS

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Cytokinins zeatin (Z) and zeatin riboside (ZR), free and bound abscisic acid (ABA) was identified and their contents was measured in generative organs of tomato mutants possessing various genes, responsible for the reproductive system - multiflora (mult) and falsiflora (fa/+), using high pressure liquid chromatography. Maximum quantity of cytokinins, especially that of ZR, as well as both ABA forms was found at the flower bud stage, at the phase of marker gene display in mutants. The ABA content is higher and that of cytokinins, especially Z, is lower in mult-mutants, in contrast to fa/+. During generative organ differentiation the content and the ratio of the phytohormones changed. In inflorescences the content of Z and ZR decreases by 3 and 10 times, respectively, whereas ABA variation are insignificant. Fruit formation is accompanied by the increase in the Z level by more than twice, ZR by 6 times and free ABA by 1.5 times; and by the decrease in conjugated ABA by more than 4 times. The blocking of the gene controlling flower formation leads to the decrease of the both ABA forms and the increase of cytokinins. A specific pattern of the distribution of the phytohormones studied at appropriate stages of generative development (flower bud stage, flowering, fruit formation and maturation) has been shown which creats a needed hormonal situation for the development of these stages.

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CYTOKININS IN TENDRIL TYPE OF PEA PISUM SATIVUM L.

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The leafletless forms of pea were obtained by means of mutational selection methods. A leafletless form of pea leaf is determined by the mutant gene af. In this case pleiotropic influence of the recessive af gene is expressed in the formation of highly developed and multibranched tendrils. Thus leaves of the af af genotype consists of stipules and small axis which converts into the tendrils in its upper part. The tendril lenght and the dry weight daily gain in the leafletless mutant were extremely sensitive to the light intensity. In is Known that cytokinis play the essential Role in the photosynthetical apparatus formation and productivity of plants. The cytokonins (CYT) were determined in the different parts of three upper leaves and three upper internodes by ELISE-method. The CYT content was higher in all tissues of the leafletless form. The tendrils of both leaflet and leafletless forms are characterized by the extremely high level of CYT. It can be evidence of the essential role of CYT in the photosynthetical apparatus formation and productivity in the tendril form. It is based on the fact of the tendril guota of the tendrils in the dry weight daily gain in the mutant is extremely higher than it is in the normal form.

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THE CYTOKININ CONTENT IN CLOVER PLANTS GROWN AT VARIOUS NITROGEN SUPPLY

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The effects of various nitrogen concentrations on cytokinins and relative compounds in red clover (Trifolium pratense L. cv. VIK-7) as well as on their activity have been investigated. Clover plants were grown for 4 weeks in Knop mixture (12 mM NO_3^-). After that, a part of plants was transferred to the same mixture free of nitrate nitrogen or to a mixture containing 60 mM NO_3^-. Cytokinins were separated by thin layer chromatography and determined spectrophotometrically at 260-270 nm. It has been discovered that the content of cytokinin-like substances changed sharply both at the absence of nitrate and the highest nitrate concentrations. Growth substances extracted from the plants were divided into three groups. The first group containing cytokinin compounds showed the absorption maximum at 264 nm and no absorption shift was observed at alkaline pH values. It has been suggested that these hormones are cytokinin--sugar conjugates. The content was low in plants grown continuously at the highest nitrogen concentration and increased with a decrease of nitrogen supply. However, the cytokinin activity changed reciprocally, i.e. it was high at 60 mM NO_3^- and low without any nitrates. The second group contained unknown substances which demonstrated blue fluorescence under UV-light at 254 nm and there was an absorption shift into the red spectrum at 10. Their concentration increased 2 to 3-fold in plants grown in nitrate-free solutions. The third group consisted of alkaline indole derivates. These compounds having weak auxin activity were discovered only in root tissues.

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POLYAMINES IN GERMINATING SEEDS WITH RESPECT TO CELL DIVISION AND CELL ELONGATION

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In germinating *Vicia faba minor* seeds the growth initiation in axial organs starts by cell elongation of hypocotyl followed by elongation of basal root cells. The proliferation in root meristems begins 20 h after root protrusion, that favors the temporal discrimination between the two growth processes. The polyamines (putrescine, spermidine and spermine) were analyzed by ion-exchange chromatography and TNBS color reaction, measured at 420 nm. Growth initiation was accompanied by Put and Spd synthesis. Put accumulation occured only in the course of cell elongation, whereas Spd was synthesized more slowly only upon cell division. If cell elongation was prevented by polyethylene glycol (6000) treatment, no Put accumulation proceeded. In γ-irradiated seeds (100 kR) no mitoses took place and no increase in Spd amount was observed; the elongation in irradiated seeds occurred slowly and was accompanied by slow Put production. Therefore, the polyamine synthesis does not precede the cell growth, but starts after its initiation. Put formation appears to be related to the enhancement of protein synthesis during elongation, whereas Spd synthesis upon cell division provides de novo formed DNA stabilization.

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CHANGES IN HORMONAL LEVELS IN SHOOTS OF BROMELIADS IN RESPONSE TO UREA AND AMMONIUM NUTRITION.

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Levels of endogenous indole-3-acetic acid (IAA) and cytokinins (zeatin, zeatin riboside, isopentenyladenine and isopentenyladenosine) were evaluated in the shoots of in vitro cultivated plantlets of two epiphyte bromeliads. The effect of urea and ammonium on the level of endogenous hormones was quantified by the HPLC-ELISA procedure. The plantlets of both species did not present roots when cultivated with $(NH_4)_2SO_4$ neither with urea as sole nitrogen source. Urea induced a higher level of IAA in shoot tissues of both species. In relation to cytokinins, there was a predominance of the isopentenyladenosine (2iPA) form. For *Vriesea philippocoburgii* 2iPA was accumulated in plantlets cultivated with urea and *Tillandsia pohliana* presented this same result in the presence of ammonium. The first mentioned species is adapted to organic nutrition in nature while *T. pohliana* is an atmospheric plant. The results suggest that urea, in spite of also being a reduced form of nitrogen, affected phytohormone production in a different way in comparison to ammonium. Besides this, the genome itself had a great importance in determining these hormonal levels.

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ENDOGENOUS PHYTOHORMONES OF ALGAE WITH THE PARENCHYMATOUS ORGANIZATION

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The endogenous phytohormone complex (auxins, gibberellins, cytokinins and ABA) of marine macroalgae of the parenchymatous (tissue) organization with a different complexity of cell morphofunctional differentiation was studied. Using bioassay and HPLC techniques IAA, GLS, zeatin, riboside zeatin, dihydrozeatin, izopentenyladenine,izopentenyladenosine and ABA were identified and quantified at various ratios. An endogenous level of IAA in algae with ordinary nondifferentiated tube thallus *(Enteromorpha)* was positively correlated with a relatively high gibberellin-like activity. Maximum levels of cytokinins were found in algae with an apical type of growth. Dynamics of phytohormones in algae with a complexly differentiated thallus *(Cystoseira)* shows a year rythm of branch formation. Continually high content of ABA was observed in algae with a simple plate like thallus having a diffusion growth *(Ulva)*. The involvement of phytohormones in metabolic processes in algae with an integrated thallus is discussed.

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A LOCALISED DECREASE OF GA_1 IN THE ELONGATION ZONE OF SALIX PENTANDRA PRECEEDES GROWTH CESSATION INDUCED BY SHORT PHOTOPERIOD

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Short day induced cessation of apical growth of seedlings of the woody species *Salix pentandra* is thought to be mediated by GA_1. By applying a novel metod for GC-MS analysis of GAs in mg amounts of plant tissue, the effect of photoperiod on the levels of GAs in various parts of shoot tips was investigated. Stem elongation in long days was correlated with a gradient of GA_1 within the elongation zone of the seedlings. The highest levels were found in stem segments from 5-20 mm below the apical tip. The gradient had flattened out after 5 short days, but morphological and anatomical changes were not detectable. Growth cessation was complete after 15 SD, but no further derease in level of GA_1 was observed relative to the situation at 5 SD. Thus, there was a temporal separation of decrease of GA_1 and decrease of growth rate in SD. This provides further support for involvement of GA_1 in photoperiodic control of shoot elongation of this woody species.

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DO SUSPENSOR'S HORMONES FUNCTIONS AS CORRELATIVE SIGNALS IN THE ESTABLISHEMENT OF DOMINANCE AMONG SEEDS OF *PHASEOLUS COCCINEUS*?

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Some studies have shown that the position of the ovule, within the ovary of *P.coccineus*, had a strong effect on the probability of maturation. The ovules in the top of the ovary (stylar) in *P.coccineus* seem to have an advantage over the ovules at the basal end (peduncolar). They are more likely to produce heavier seeds, or, in the case of ovule position two, are both more likely to mature and produce heavier seeds than ovule at the bottom of the ovary. It has been reported that is not the morphological position of fruits/sinks that determines their degree of dominance or inhibition but rather their sequence of development relative to each other. Plant hormones have suggested has the dominance signal among seeds. In this report we have quantified GA_1, GA_5 and GA_8 in each ovule position within the ovary in order to examine if suspensor's GAs may function as correlative signals in the establishment of dominance among seeds of *P.coccineus*. Accurate quantification of endogenous GA levels has been accomplished by GC-MS with the aid of deuterated GAs as internal standards. Results show that stylar ovules (position 1-2-3) contain significantly higher levels of the major biologically active GAs (GA_1 and GA_5). GA_8, inactive GAs, produced by metabolism of GA_1 shows also a higher level in stylar ovules. Therefore this adds to the difference in the total level of active Gas produced by the two types of ovules during development.

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PHYTOHORMONE CHANGES DURING CELL CYCLE IN SYNCHRONIZED TOBACCO CELLS

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The pattern of cell division in plants is influenced by multiple signals of developmental and environmental nature.

Plant hormones, in particular auxins and cytokinins, have a pronounced effect on cell division. Most plant cells or tissue culture systems are dependent on exogenous hormone supply. However exogenous application of phytohormones results in incomplete information due to problems related to uptake and metabolism.

To study the hormonal regulation of cell division we followed endogenous balances of three major groups of phytohormones during cell cycle of synchronized tobacco cells.

Out of a minimal amount of cells cytokinins (free bases, nucleosides and nucleotides),indole acetic acid (IAA) and abscisic acid (ABA) were extracted by means of solid phase anion exchange chromatography in combination with immune affinity purification. The different cytokinins were quantified by electrospray LC-MS/MS. IAA and ABA were analyzed by GC-MS after derivatization.

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ROOT GROWTH AND ENDOGENOUS IAA LEVELS IN TALL AND DWARF LINES OF PEA

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Primary root elongation, the short term response of roots to exogenous IAA application, and endogenous IAA levels in tips of primary roots were compared in tall (*Le*), dwarf (*le*) and ultradwarf (*na*) lines of pea. Roots of tall and *le* dwarf lines elongate at the same rate over a 10 day period, while roots of the ultradwarf elongate at about one half the rate of the tall and *le* dwarf. IAA, applied exogenously to roots, rapidly inhibited primary root elongation in all lines tested; inhibition was rapid, occurring within 15 minutes after application, and lasting several hours. Measurements of endogenous IAA levels in 3 cm root tips of the different lines show comparable IAA levels in tips of both tall and *le* dwarf lines, while the *na* ultra-dwarf contains about one half the amount of the tall, when measured on a ng/g fresh weight basis. Endogenous IAA level has previously been shown to correlate with internode elongation in pea (Law and Davies, Plant Physiol. 93:1539-1543, 1988). From these results, it appears that endogenous IAA level also correlates to primary root elongation rate of pea.

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ENDOGENOUS CYTOKININS AND POTATO TUBER DORMANCY

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Previous research from this laboratory has demonstrated that the initiation and maintenance of potato (*Solanum tuberosum* L.) tuber dormancy requires the continuous synthesis and presence of ABA. However, the termination of dormancy was not associated with any particular threshold level of endogenous ABA. This indicated that other internal factors must be involved with the breaking of dormancy. Therefore, a study examining the involvement of endogenous cytokinins in the termination of tuber dormancy was initiated. Following fractionation by HPLC, endogenous cytokinins were characterized by a combination of bioassay and ELISA using group-specific monoclonal antibodies. Endogenous cytokinins identified to date include: Z, ZR, IP, IPA and IP9G. Injection of ZR or IPA, but not IP9G, into fully dormant tubers resulted in the premature termination of dormancy. The effects of postharvest storage on endogenous levels of each of these cytokinins is currently being determined in order to identify possible precursor-product relationships and possible points of biosynthetic control.

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FLUCTUATION IN LEVELS OF AUXIN AND CYTOKININS AFTER DECAPITATION AND 6– BENZYL AMINO PURINE TREATMENT OF AZALEA, AND THEIR RELATIONSHIP TO APICAL DOMINANCE

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Recently, Prof. F. Bangerth (1994) showed that cytokinin concentration in xylem exudate of bean increased rapidly when the plants were decapitated. We obtained the similar result in 'Siji' azalea(*Rhododendron obtusum spp.*). The amount of ZRs and DHZRs in 0.5cm shoot below the terminal bud(shoot segment,where lateral bud will or may emerge) increased 2.5–fold and 3– fold 8h after decapitation (determined by ELISA ,detection range:0. 064– 200pmol / L),respectively.That of IAA (ELISA detection range: 0. 78–800pmol / L) decreased by 96 % 12h after decapitation. Besides, the fluctuation in levels of IAA and cytokinins in both terminal bud and shoot segment were observed when intact plants had been sprayed with 1000ppm 6–benzyl amino purine (6–BA).The amount of IAA in shoot segment decreased by 95% after 24h,whereas in terminal bud almost increased to 3200% after 12h compared to control.Moreover, the amount of iPAs in shoot segment increased 7–fold,24–fold and 35–fold after 4h,8h and 24h, respectively.That of ZRs increased 5–fold and 50–fold after 4h and 8h. That of DHZRs only increased 1. 5–fold after 4h. Interestingly, the amount of iPAs,ZRs and DHZRs also increased from 2–fold to 5–fold in terminal bud 4–48h after 6–BA treatment. These results indicate that decapitation increases the levels of root cyctokinins, which are thought to be under the control of the polar IAA transport system, by removing the source of IAA.6–BA treatment, which can also overcome the inhibition to lateral bud,not only increases the levels of endogenous cytokinins but also alters the distribution of IAA between terminal bud and lateral buds.Therefore,auxin / cytokinin interaction seems to be a two–sided regulation in the system of apical dominance.

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SHOOT GROWTH AND ENDOGENOUS GAs, IAA AND ABA IN *Cryptomeria japonica*

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Sugi (*Cryptomeria japonica*), in the *Taxodiaceae* family is an important forest species in Japan. As an indigenous conifer, it has been planted in Japan for several centuries. The shoots of three *Cryptomeria japonica* varieties, "elongated", "normal" and "dwarf" types, were collected and analyzed by gas chromatography-mass spectrometry-selected ion monitoring [GC-MS-SIM] for gibberellins [GAs], indole-3-acetic acid [IAA] and abscisic acid [ABA] after purification and separation on reversed phase C_{18} HPLC and normal phase nucleosil $N(CH_3)_2$HPLC. Gibberellins $A_9$and A_4were found in shoot tips at 1.0 and 31.4 ng/g dry wt, respectively, in the "elongated type". Both GA_9 and GA_4, however, were undetectable levels in parallel tissue samples of normal and dwarf types. New tissue of all three cultivars has been obtained, and analyses are now being repeated for endogenous GAs. Shoot tips of the "elongated" type also contained the lowest levels of ABA [2953ng/g dry wt] and IAA [19.7ng/g dry wt], whereas shoot tips of dwarf cultivar contained the highest levels of ABA [3668ng/g dry wt] and IAA [82.0ng/g dry wt]. High IAA levels and reduced shoot elongation in conifers has been reported previously. High GAs and low ABA and IAA in shoot tips of the elongated type may thus be causally associated with the rapidly growing "elongated" genotype of *C. Japonica.*

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SYSTEM ANALYSIS OF REGULATION OF ENDOGENOUS HORMONE IN SENESCENCE OF WHEAT LEAF

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Many experiments have showed that various endogenous hormones do not play roles in senescence of plant leaf isolatedly,but there are interaction amony them. These interacted hormones constructed a system of hormone. Based on the principles of system theory ,hormone regulation on senescence of the first euphylla of wheat(triticum asetivum L.) were analyzed systematically by means of statistics in this paper. Artificial population of the asychronous senesced wheat were made with various treatments. The chlorophyll content was used as the quantative index of senescence.The contents of Z, IAA, GA_3 and ABA were determined through HPLC,the contents of DHZR and iPA were determined through ELISA,and the amounts of expelled Eth were determined through gas chromatagraphy. First, through regression and correlation analysis, it was proved that endogenous hormone integratedly play roles in regulation of wheat leaf senescence. Secondly , the structure of endogenous hormone system was analyzed by stepwise regression. the following equal was obtained: $[Chl] = 1.0935 - 1.4702[ABA] + 0.7386[Z] + 0.4852[GA3]$ $R^2 = 0.9452^{**}$. It showed that of all seven endogenous hormones studied , Z, GA_3 and ABA play direct roles in regulation of senescence , the other four play indrect roles through affecting the contents of those three hormones. Thirdly, path analysis were conducted on the endogenous hormone system ,the results as following, $P_{ABA \to Chl} = -0.474$, $P_{ABA \to Z \to Chl} = -0.150$. $P_{GA_3 \to Chl} = 0.450$, $P_{GA_3 \to Z \to Chl} = 0.221$, $P_{Z \to Chl} = 0.289$, $P_{Z \to GA_3 \to Chl} = 0.344$, $P_{Z \to ABA \to Chl} = 0.245$. These showed that on one hand ABA could prompt senescence,on the other hand, it also could resist the slow-down effect of Z on senescence. GA_3 not only could retard senescence directly ,but also could increase endogenous Z level. Z could retard senescence independently ,in the meantime,it also could play slow-down roles in regulating senescence through raising level of GA_3 and slowing the level of ABA.

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AUXINS AND CYTOKININS AS COORDINATIVE SIGNALS IN CORRELATIVE EVENTS

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Following decapitation of pea (*Pisum sativum*) or bean (*Phaseolus vulgaris*) plants, lateral buds start to grow 7 to 8 hours later. However, already 5 to 6 hours after decapitation the cytokinin concentration in bean xylem exudate and in pea stem tissue increased considerably. In the xylem exudate this increase lasted for more than 80 hours, whereas in pea stem tissue a rapid decline to control levels was observed. Replacing the apex of the plants by NAA in lanoline prevented the increase in cytokinins, suggesting that in intact plants the auxin produced in the apex maintaines a low cytokinin level. On the other hand, applying cytokinins to the apex of these plants or raising the endogenous concentration of this hormone greatly stimulates the basipolar transport of endogenous IAA. A hyposesis is presented suggesting that a self regulated IAA/cytokinin homoeostasis controls lateral bud growth as well as a number of other correlative events.

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DEVIATIONS FROM THE STANDARD GROWTH RESPONSE TO EXOGENOUS AUXIN IN SEGMENTS OF LIGHT-GROWN PEA ARE DEPENDENT ON THE DEVELOPMENTAL STAGE AND GENOTYPE

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Responses to exogenous auxin of excised stem segments of three genotypes (tall, dwarf, and severe dwarf) of light-grown pea (*Pisum sativum* L.) were characterized at two developmental stages. Segments taken from internodes at 20% full expansion are termed "old", and segments from internodes at 7% full expansion are termed "young". Using angular position transducers and a computerized data collection system, high resolution measurement of growth kinetics was performed on stacks of segments incubated in aerated buffer over 20 hours. For long-term growth studies, segments were incubated on Petri plates of buffer for 48 hours and their final lengths measured. The magnitude and pattern of growth rate changes in response to exogenous auxin varied with developmental stage and genotype. The "standard" auxin response, recorded in the literature to consist of an initial growth response (IGR) and prolonged growth response (PGR), was demonstrated by old segments of all genotypes. Young segments of tall and dwarf plants gave auxin responses that differ both from the "standard" and from each other. Young segments of dwarf pants showed a late growth response we have termed the delayed growth response (DGR). Young segments of tall plants were unique in showing auxin-induced inhibition of growth. Use of the ethylene action inhibitor norbornodiene in growth studies, and measurement by GC of ethylene evolved from segments, demonstrated the inhibition to be mediated by heightened ethylene sensitivity. Young segments of tall plants had 25% more endogenous auxin, as determined by GC/MS, than did old segments.

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IAA PARTIALLY COUNTERS THE EFFECTS OF A WIDE RANGE OF ROOT FORMATION INHIBITORS IN LEAFY SPURGE

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In aseptic, isolated hypocotyl segments of the perennial weed leafy spurge (*Euphorbia esula* L.), root formation is inhibited, whereas shoot formation is stimulated by cool white fluorescent light. Exogenous IAA stimulates root formation in light or darkness. In most cases, shoot formation is inhibited by IAA, irrespective of the presence or absence of light. Canaline and canavanine, inhibitors of polyamine biosynthesis, inhibit root formation. The inhibition by canaline is partially overcome by IAA, but the results of canavanine treatment varied. Arcaine and pentamidine, two presumed competitors of polyamine interactions with N-methyl-D-aspartate in animals, also inhibit root formation in leafy spurge. This inhibition and the inhibition of root formation caused by calcium or zinc deprivation are also partially reversed by IAA. However, IAA did not reverse the inhibition of root formation by gabaculine, which inhibits photosynthesis as well as phytochrome biosynthesis or action. These results support the hypothesis that IAA (or a major metabolite of IAA) is involved in a regulatory mechanism that controls root formation in leafy spurge hypocotyl segments, but is not the sole regulator of root formation.

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EFFECT OF SIMULTANEOUS TREATMENT OF 4-CL-IAA AND GIBBERELLIN ON PEA PERICARP.

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Pea fruit require the presence of seeds for continued growth. Deseeded pericarp continue to grow when treated with the plant growth regulators gibberellin (GA_1, GA_3) or auxin (4-chloro-indole-3-acetic acid, 4-Cl-IAA) which naturally occur in pea fruit (Plant Physiol 105:S-762). The interaction between gibberellin and auxin was examined by treating deseeded pea fruit with GA_1 + 4-Cl-IAA, or GA_3 + 4-Cl-IAA (half log concentrations from 10^{-4} M to 10^{-7} M). GA_1 + 4-Cl-IAA and the GA_3 + 4-Cl-IAA treatments gave similar log linear growth curves. Both the GA_1 + 4-Cl-IAA and the GA_3 + 4-Cl-IAA treatments were more effective in stimulating growth than GA_1, GA_3, or 4-Cl-IAA applied alone. The gibberellin + auxin treatments were not completely additive for increase in length. For example, $5X10^{-5}$ M GA_1, GA_3, or 4-Cl-IAA treatments gave 23±1.0, 24.3±2.1, or 25.9±1.7 mm increase in pericarp growth, respectively, while GA_1 + 4-Cl-IAA and GA_3 + 4-Cl-IAA gave 43.3±1.4 and 44.8±0.5 mm increase in growth, respectively. However, final fresh weight (7 days after initial treatment) was additive or more than additive for GA_1 + 4-Cl-IAA and GA_3 + 4-Cl-IAA treatments. This data suggests that pea pericarp have the ability to respond to both GAs (GA_1 & GA_3) and auxins (4-Cl-IAA) and that GA's and auxin can interact to stimulate pericarp growth.
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IAA AND 4-CL-IAA EFFECT ON PEA PERICARP GROWTH.

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The pea (*Pisum sativum*) pericarp growth assay is the first system to test IAA and 4-Cl-IAA *in planta* on the growth of a tissue known to contain IAA and 4-Cl-IAA. We have previously shown that 4-Cl-IAA is active in promoting pea pericarp elongation, while IAA is inactive (Plant Physiol S105:139). We have begun studies to determine what function IAA and 4-Cl-IAA may play in pea fruit development, and why only 4-Cl-IAA application stimulates pericarp elongation.

Pea pericarp are split down the dorsal suture, and solutions of IAA or 4-Cl-IAA are applied. Only 4-Cl-IAA actively promotes sustained pericarp elongation at 10^{-4} to 10^{-6} M while IAA inhibits growth or has no activity when applied at 10^{-3} to 10^{-7} M. When IAA is applied simultaneously with 4-Cl-IAA, the pericarp growth response is the same as 4-Cl-IAA alone. Gibberellin (another class of plant hormone) is also active in promoting pericarp growth, but IAA inhibits the activity of GA_3. Ethylene may be induced by the application of auxin; however, silver thiosulfate an ethylene antagonist does not cause IAA to stimulate growth. IAA is labile to peroxidase catalyzed decarboxylation. However, ferrulic acid, a peroxidase inhibitor does not reverse IAA's lack of activity in the pericarp assay. We are currently examining how IAA is metabolized by pea pericarp tissue to test whether IAA's lack of activity is related to how IAA is metabolized.

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THE INFLUENCE OF TRIIODOBENZOIC ACID AND DECAPITATION ON CIRCUMNUTATION IN PEA SEEDLINGS

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Circumnutation is a growth phenomenon the mechanism of which is not fully understood. We investigated the involvement of indole-3-acetic acid (IAA) in the circumnutation of green pea seedlings by applying triiodobenzoic acid (TIBA) to the uppermost node of the plant. Movement before and after the treatment was recorded using time-lapsed video photography and analysed using computer-based digitizer. After a time lag of not more than 60 minutes, TIBA severely inhibited circumnutation. The TIBA treatment did not completely stop elongation growth of the stem axis below the point of application, but enhanced elongation above the point of application. Decapitation of the shoot tip also stopped circumnutational movement without markedly affecting the elongation growth of the stem axis basipetal to the point of excision. These results indicated that IAA transported from the shoot tip is involved in controlling the circumnutational movement of pea seedlings.

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GROWTH AND MORPHOGENETIC RESPONSES INDUCED BY AUXINS AND CYTOKININS IN TWO RED ALGAE, *GRATELOUPIA DICHOTOMA* AND *GRACILARIOPSIS TENUIFRONS*.

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Effects of growth regulators and their role in the control of the morphogenetic processes in algae are poorly known. Few experimental systems have been described with absolute control of environmental conditions. We tried to study morphogenetic processes using small thallus segments and callus-like structures (CLS) in a synthetic medium, liquid or semi-solid. Apical and intercallary thallus segments, and CLS were cultured in ASP 12-NTA medium, 30% salinity, 40-50 $\mu mol.m^{-2}s^{-1}$ irradiance, 16:8 h light dark cycle. Two auxins (IAA and 2,4-D) and one cytokinin (BAP) were tested at 0.5 and 5.0 $mg.L^{-1}$, alone or combined. The main results were: *Grateloupia dichotoma*: a) auxins inhibited adventitious lateral branching while BAP promoted; b) elongation of lateral branches was promoted by auxins; c) plant regeneration in CLS was promoted by BAP or BAP + IAA. *Gracillariopsis tenuifrons*: a) high concentration of 2,4-D promoted the growth of apical or intercallary segments; b) high auxin promoted the formation of lateral branches, while apical branches were induced by low IAA plus high BAP; c) growth of lateral branches were promoted by high auxin; d) all treatments, except 2,4-D, increase the formation of callus at the apical end e) auxins increased growth of the calli. Our results show that natural morphogenetic processes occurring in algae are strongly affected by growth regulators, and others can be induced, such as callus formation with indirect plant regeneration.

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USING IAA AND GA_3 TO MANIPULATE WOOD FORMATION IN 1-YEAR-OLD SHOOTS AND 20-YEAR-OLD STEMS OF WHITE ASH *FRAXINUS AMERICANA* (L.)

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Cambial growth rate and xylem differentiation determine wood quantity and quality, respectively. To investigate hormonal regulation of these variables, different concentrations of IAA and GA_3 were applied, individually or in combination, in nutrient media to 1-year-old debudded cuttings and 20-year-old stem segments of white ash. For both ages, IAA alone induced wood with the highest vessel percentage (by volume), and with only vessel elements and surrounding parenchyma cells lignified. A combination of high IAA/low GA_3 (i.e., 5:1 by mass) resulted in a relatively high ratio of vessels to fibres, and partially lignified fibres as well as lignified vessel elements and parenchyma cells. A low IAA/high GA_3 combination (i.e. 1:10 by mass) promoted massive cambial growth and primarily fibres that were mostly (in 1-year-old cuttings) or partially (in 20-year-old stem segments) lignified. GA_3 alone did not stimulate wood production in 1-year-old cuttings, but in 20-year-old segments resulted in wood similar to that induced by IAA alone, except vessel elements being fewer and smaller. In the presence of IAA, GA_3 promoted more wood formation, elevated the percentage of fibres, and reduced number of vessels and axial parenchyma. Ray percentage was unaffected in all treatments. Significant differences in response to IAA and GA_3 occurred between 1 cm and 3 cm below the site of hormone application in 1-year-old cuttings, as well as between the two ages. This research was supported by Repap Technologies Inc. and NSERC.

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GROWTH INHIBITION OF EXPLANTS OF *HANCORNIA SPECIOSA* (A TROPICAL FRUIT TREE) INDUCED BY DIFFERENT GIBBERELLIC ACIDS.

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Attempts to induce internode elongation of *in vitro*-grown shoots of *Hancornia speciosa* through the use of gibberellic acid A_3 in the culture medium resulted in 89% decrease on internode elongation and lateral meristem development (growth), and increased mortality of the explants. To verify whether or not the apparent inhibition of growth was a specific response to GA_3, shoot tips and nodal segments of *in vitro*-grown *H. speciosa* were cultured in media containing five different concentrations of the gibberellic acids A_4 (GA_4) and A_7 (GA_7) (0 to 1 μM). GA_4 and GA_7 were more effective than GA_3 at inhibiting growth. The addition of a mixture of gibberellic acids A_{17} and A_{20} ($GA_{17} + GA_{20}$) to the culture medium led to results similar to what was found for the other GA(s) tested. Hydroxi-isoesteviol, an inhibitor of gibberellic acid biosynthesis, was able to slightly increase growth. Another inhibitor of gibberellic acid biosynthesis, ancymidol (0 to 2 μM), induced an exponential decrease in elongation without any noticeable change on the mortality rate. Higher ancymidol concentrations (2.5 to 10μM) resulted in complete growth inhibition. Shoots grown in high ancymidol concentrations were able to recover their ability to grow after transference to an ancymidol-free medium; the degree of recovery was inversely dependent on the ancymidol concentration. In conclusion, low concentrations of different GAs result in growth inhibition of *H. speciosa* explants growing *in vitro*; the assumed reduction of the endogenous levels of GA(s) due to ancymidol and hydroxi-isoesteviol treatment did not significantly increase growth.

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HORMONAL CONTROL OF ROOT HAIR GROWTH IN CLOVER

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Root hairs elongate by tip growth, but little is known about endogenous factors regulating their growth. We examined effects of known plant hormones (2,4-D, GA_3, kinetin, ABA, brassinolide) on the growth of clover (*Trifolium repens* L.) root hairs. Germinated seeds were transferred onto Fåhråus media containing hormones of various concentrations, and grown under 16hr light (23°C) - 8hr dark (20°C). The length of root hairs was measured after three days under a light microscope. Matured root hairs located at given intervals along the root axis were chosen for the measurement. All the hormones examined except GA_3 promoted elongation of root hairs. 2,4-D and kinetin were most effective with greatest stimulation 180% at 10^{-6}M and 147% at 10^{-6}M, respectively. The nucleus in the root hair cell remained positioned at a constant distance (approx. 25μm) from the tip during its growth. The hormones examined had no effect on the nucleus position. Some hormones induced morphological effects such as branching, swelling, curling, twisting, etc. Although GA_3 had no effect, uniconazole-P, an inhibitor of GA biosynthesis, markedly inhibited root hair elongation, and exogenous GA_3 recovered this inhibition. This result indicates that GA is required for normal root hair growth in clover. Since uniconazole-P treatment induced a distinct change in the cytoplasm of the root hair tip, we are now investigating the cytological aspects of the change in connection with a possible role of GA.

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THERMOPERIODIC CONTROL OF STEM ELONGATION AND ENDOGENOUS GIBBERELLINS IN *CAMPANULA ISOPHYLLA*

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The effect of day/night temperature regimes on elongation growth and on the content of endogenous gibberellins in vegetatively propagated plants of *Campanula isophylla* cv Hvit have been studied. Growth experiments were carried out under controlled conditions in a phytotron. Endogenous gibberellins were identified and quantified by combined GC-MS. Compared with a constant temperature regime at 18°C (18/18°C), stem and internode elongation was significantly enhanced by a combination of high day/low night temperature (21/15°C) and inhibited by an opposite temperature regime (15/21°C). No effect of temperature regimes on the number of nodes or on the length of time to anthesis could be observed. Gibberellin application stimulated elongation growth at all temperature regimes, GA_1 being more active than GA_{20}, and GA_{20} was more active than GA_{19}. Gibberellins A_1, A_{19}, A_{44}, A_{53} and 2-β-OH A_{53} were identified as endogenous constituents in *Campanula*. Quantitative analysis of the endogenous gibberellins indicated that temperature regimes that stimulated elongation growth increased the levels of GA_1, GA_{19} and GA_{44}. On the other hand, in plants grown at conditions with reduced elongation growth, there was an increased level of 2-β-OH A_{53}.

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ROLE OF GIBBERELLINS IN DELAYING LEAF SENESCENCE: A GA UPTAKE STUDY

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Leaves of the cut flower Alstroemeria pelegrina L. exhibit rapid senescence in the dark (i.e. loss of photosynthetic activity, decrease in chlorophyll- and protein content). In addition, some specific proteins are synhesized during senescence. The senescence process is strongly delayed by gibberellins (GAs). We have determined the characteristics of uptake of 4 radio-labelled gibberellins (GA_3, GA_4, GA_9, GA_{20}) in alstroemeria leaf protoplasts to investigate whether GA uptake can be described as diffusion of protonated GAs or in addition has a carrier-mediated component.

In order to get additional insight into the uptake mechanism, we have investigated the interaction of gibberelllins with modelmembranes. The characteristics of GA-uptake and the spontaneous penetration of GAs into lipid modelmembranes are consistent with a mechanism of membrane transport by permeation of neutral (protonated) GAs and subsequent accumulation of GAs inside the protoplasts (anion trapping).

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EFFECT OF DIURNAL TEMPERATURE ALTERNATIONS AND GIBBERELLINS ON STEM ELONGATION IN *BEGONIA X HIEMALIS*

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The aim of the experiments was to study the role of gibberellin in the thermoperiodic regulation of stem elongation in *Begonia x hiemalis* Fotsch. Growth experiments were carried out under controlled conditions in a phytotron. Endogenous gibberellins were identified and quantified by combined GC-MS. Compared with constant temperature, stem elongation was significantly inhibited by a combination of low day temperature and high night temperature and enhanced by an opposite regime, high day temperature and low night temperature. Gibberellins A_1, A_{19}, A_{20}, A_{44}, A_{53}, A_4 and A_9 were identified as endogeous compounds in stem and leaf tissues of *Begonia x hiemalis*. When applied exogenously to the plants, both GA_1, GA_4 and GA_9 stimulated stem elongation while similar amounts (20 µg/plant) of GA_{19}, GA_{20}, GA_{12}, kaurenoic acid and *ent*-kaurene had no significant effect. The stimulatory effect of GA_1 was enhanced by simultaneous application of BX-112 (gift from Kumiai Chemical Industry Co., Ltd., Tokyo). The relative effect of GA_1, GA_4 and GA_9 was greater at the temperature regime of low day/high night temperature than at constant temperature. No apparent relationships could be found between levels of endogenous GAs and stem elongation.

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EFFECTS OF BAP, NAA AND TRIACONTANOL ON NITROGEN REDISTRIBUTION IN BUCKWHEAT

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The aim of studies was to determine the effects of phytohormones on nitrogen accumulation dynamics and extent of this nutrient translocation during buckwheat flowering and seed maturation in the conditions of different level of nitrogen nutrition. Buckwheat (*Fagopyrum esculentum* Moench.) plant were grown in cold greenhouse in Kick-Brauckmann pots filled with 10 kg of soil. Nitrogen fertilizer was applied in two rates – 1.25 or 2.50 g N per pot and 50% of total rate was used as soil or foliar nutriton during plant growth. On the background of this nitrogen fertilization scheme three phytohormones were used: benyladenine (BA), α-naphtyloacetic acid (NAA) and triacontanol (TRIA) as foliar spraying seven times during growing season in the period from plant budding to flowering. Samples of plant material were taken at 46, 63, 80 and 102 day of growing. It was shown that accumulation and redistribution of nitrogen was closely related to the rate and method of nitrogen applying as well as on the phytohormone and phase of plant development. Translocation of nitrogen from stem and leaves to inflorescens and seeds took place. More intense transport from vegetative to generative plant organs was found in the case of soil than foliar applied nitrogen. Foliar nutrition affected uniform distribution of studied nutrient in plant organs. When N was introduced to soil the more intense nitrogen translocation from vegetative to generative organs was found as consequence of triacontanol using. For foliar N nutrition triacontanol also enhanced N translocation from vegetaive organs to seeds, whereas limitation of this process was observed when BAP was applied.

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EFFECT OF BENZYLADENINE ON RESPIRATORY RATE AND ENERGY CHARGE IN LEAF OF PEANUT SEEDLINGS

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Decapitated-derooted peanut (Arachis hypogaea L.) were smeared with 10^{-5}mol/L benzyladenine (BA) solution on the surface of upper leaves (sink leaves) .0-24 hours after BA treatment, the rate of respiration in mitochondria isolated treated leaves was promoted 60.5%. Inhibiting effects of carbonyl cyanide chlorophenydrazone (CCCP) and dicyclohexylcar bodiimide (DCCD) on the respiratory rate showed 49.1% and 52.7% increase respectively. We found that contents of ATP and total adenine nucleotide increased at 12 hours after BA treatment, following declined , and increased rapidly again at 48 hours. Energy Charge valus significant stimulated by BA. Sink leaves by BA-treated had a higher ability using for resynthesing ATP. The activity of ATPase located in mitochondria increased by 23.9% in comparison with control at 24 hours. These results indicate that role of BA may act on stimulating the oxidative phosphorylation and energy transport process.

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STRUCTURAL OPTIMIZATION OF CARBON-SUBSTITUTED CYTOKININ ANALOGS OF PYRIMIDINE

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Carbon-substituted purine derivatives involving 6-styrylpurines are important for comprehensive understanding of structure-activity relationships (SAR) of cytokinins as well as their active conformation. Our designed synthesis on the basis of the analyses of SAR of cytokinins led to the finding of 4-styrlpyrimidines of potent activity. Thus, structural optimization of 2-, 5-, 6- and β-substituents was carried out in combination with evaluation of biological activity against betacyanin biosynthesis of *Amaranthus* seedlings, growth of tobacco callus and chlorophyll retention of rice leaves. Substituted 4-styrylpyrimidines were synthesized conveniently from dichloropyrimidines. Palladium-catalyzed coupling of terminal acetylenes with 2,4-dichloropyrimidine, 4,6-dichloropyrimidine and 2,4-dichloro-5-substituted pyrimidines afforded the corresponding 4-monoalkynylated products. Nucelophilic replacement of the alkynylated products and subsequent electrophilic and nucleophilic addition of hydrogen halide, alcohol and mercaptan produced (*Z*) and (*E*)-4-styrylpyrimidines. Photoisomerization of the (*Z*)-isomers under sunlight gave their (*E*)-isomers. An *Amaranthus* betacyanin assay revealed that i) amino and ethoxy group were the most favorable for 2- and β-substituent, respectively, ii) for the side chain at the 4-position, phenyl group was preferable to alkyl groups, iii) any substituent at the 5-position disfavored the activity, iv) 2,4-substitution prevailed over 4,6-substituion. The most active was the (*Z*)-ethoxy derivative which was 5 times as potent as N^6-benzyladenine (BA). Interestingly, both compounds equaled in activity in a tobacco callus bioassay. The decreased activity of the (*Z*)-ethoxy derivative suggested the involvement of metabolism during the bioassay. In a chlorophyll retention bioassay, it was more active than BA. Our findings in this study stress the significance of the development of carbon-substituted cytokinin analogs.

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KINETIN RETARDS AGING IN HUMAN CELLS

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Cytokinin plant growth hormones are well known to have certain senescence-retarding effects in plants. Their effects on animal cells have been, until now, best considered as growth inhibitory and anti-tumorigenic. Using the well established experimental system of cellular aging of normal cells in culture, we have observed that an addition of 40–200 μM kinetin (Kn) in the culture medium of human skin fibroblasts both delays the onset and decreases the extent of many of the aging characteristics that appear during serial passaging. Age-related changes that are affected by Kn include morphological alterations, growth rates, cell size, cytoskeletal organisation, macromolecular synthesis and the intensity of autofluorescence due to the oxidative damage product lipofuscin. These anti-aging effects of Kn are achieved without any increase in the cell culture lifespan in terms of maximum proliferative capacity of normal human cells in culture. Studies on the kinetics of uptake, intracellular accumulation and release of [^{14}C]-labelled Kn suggest a rapid internalization of the hormone by human cells reaching stable concentrations within 3 days. However, the rate of uptake of Kn was slower in senescent cells than young cells. Although the exact mode of action of Kn in human cells is not known at present, our preliminary studies show that Kn may become incorporated in tRNAs and thereby affect the efficiency and accuracy of protein synthesis during aging. (Acknowledgement: This work was supported by a grant from Senetek PLC).

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IMAZALIL ENHANCES THE SHOOT INDUCING EFFECT OF BENZYLADENINE IN *SPATHIPHYLLUM FLORIBUNDUM* SCHOTT

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In *Spathiphyllum floribundum* 'Petite' Schott in *vitro* shoot induction by benzyladenine was enhanced dramatically by adding the imidazole fungicide imazalil to the medium. As the concentration of imazalil increased, the number of shoots increased and finally their size was reduced to a small meristematic dome. An average of 127 shoots per explant developed when 2.5 mg/l BA was combined with 16 mg/l imazalil. Doubling the BA concentration had no significant effect on shoot induction. Imazalil did not affect the root inhibiting effect of BA. When imazalil was applied without BA, no new shoots developped, increasing its concentrations only reduced number of roots and total root length per plant. Problably imazalil has more than one mode of action and can offer a tool for studying the metabolism of cytokinins as well as auxins.
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SOLANUM TUBEROSUM L. MORPHOGENESIS: EFFECT OF GASEOUS ENVIRONMENT OF *IN VITRO* FLASKS

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An ability to accumulate ethylene and other hydrocarbon gases in vessels used for *in vitro* culture of *Solanum tuberosum* L. explants in respect to flask volume, type of clossure, composition of nutrient medium, as well as its effect on the morphogenesis was investigated. Nodal stem explants and excised leaves of *Solanum tuberosum* L. cv. Zile and cv. Agrie dzeltenie were cultivated in a hormone-free MS medium. By varying the tightness of clossure of *in vitro* flasks a different degree of accumulation of ethylene, ethane and propylene in the internal atmosphere was achieved that correlated with an effect on early shoot and root development and with long-term effects such as stimulation of rooting on stem, initiation of stolon growth and tuberization. In experiments where IBA, IAA or BAP was applied before multiplication, the role of hormone balance in the control of rooting, stolon initiation and tuberization of *Solanum tuberosum* L. explants in different cultivation conditions was established.

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EFFECTS OF ETHYLENE AND GIBBERELLIN ON THE ELONGATION OF RICE SEEDLINGS (*Oryza sativa* L.)

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Ethylene induces shoot elongation of normal rice like deep water rice. To study the effects of ethylene on gibberellin (GA) biosynthesis in normal rice, we investigated the levels of endogenous GA and the turnover of [^{14}C] labeled GA_1 in rice seedlings (cv. Fujiminori) in the presence of ethylene. The results of measured endogenous GA levels show that although ethylene induced elongation of rice seedlings, the endogenous GA_1 level was reduced. Ethylene also stimulated the conversion of GA_1 to GA_8 and an unknown GA-conjugate. When the levels of endogenous GA in rice seedlings were reduced by uniconazole, the application of GA_1 and ethylene together stimulated elongation of the 3rd leaf more than application of only GA_1. These results show that ethylene increases the response of rice seedlings to endogenous GA_1 levels. These results suggest that when ethylene stimulates the elongation of rice seedlings, ethylene increases both the response to GA_1 and GA_1 turnover. We also plan to investigate these results with other cultivars of rice to find differences between normal rice and deep water rice.

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RESPONSIVENESS OF *AMARANTHUS RETROFLEXUS* L. SEEDS TO ETHEPHON, ACC AND GIBBERELLIC ACID IN RELATION TO TEMPERATURE AND DORMANCY INTENSITY

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Seed germination is regulated by various external factors among which temperature, oxygen and light play an essential role. In some species ethylene and gibberellins may also affect germination. All these factors can be involved on both breaking of seed dormancy and on germination of non-dormant seeds. The aim of the present work was (1) to determine the effect of temperature and oxygen on germination of redroot pigweed (*Amaranthus retroflexus* L.) seeds with different depth of dormancy and (2) to investigate the effect of ethephon, 1-aminocyclopropane-1-carboxylic acid (ACC) and gibberellic acid (GA_3) on seed germination in relation to temperature.

Dormancy of redroot pigweed seeds corresponds to the inability to germinate in the dark at relatively low temperatures (below 30°C).Dormant seeds only germinated in a very narrow range of temperatures (35 °- 40°C) whereas non-dormant ones germinated in a wider range of temperatures When they are non-dormant germinated in atmospheres containing at least 10% 0_2 at 25° and 35°C. However, sensitivity of seeds to oxygen deprivation increased with increasing depth of dormancy. Ethephon, GA_3 and ACC strongly improved germination of dormant seeds. The results obtained are discussed in relation to dormancy of seeds and temperature. GA_3 action was less dependent on temperature than that of ethephon, while ACC stimulated redroot pigweed seed germination only at high temperatures (25°-30°C).Involvement of ethylene biosynthesis in regulation of primary dormancy releasing of redroot pigweed seeds is suggested.

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INDUCTION OF SECONDARY DORMANCY IN *AMARANTHUS RETROFLEXUS* L. SEEDS BY HIGH TEMPERATURE AND ITS REMOVAL BY ETHYLENE

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Seeds of many species become secondarily dormant and its fail to germinate under the condition which previously allowed germination. Secondary dormancy can be induced by natural or artificial means following harvest. This dormancy may be broken by some plant growth regulators. The purpose of the present study was to analyse development of secondary dormancy in redroot pigweed (*Amaranthus retroflexus* L.) seeds caused by high temperature and to examine the effect of 2-chloroethylphosphonic acid (ethephon) and 1-aminocyclopropane-1-carboxylic acid (ACC) on germination of these seeds.

Non-dormant redroot pigweed seeds germinate rapidly in a wide range of temperatures (15 °- 40°C), however germination remains impossible at 45°C. Incubation of seeds at 45°C for 1 to 3 days induces a secondary dormancy which is associated with a progressive decrease of germination at suboptimal temperatures (15° - 25°C) and reduces germination rate at the thermal optimum (40°C). Ethephon, a ethylene releasing compound, and ACC, the precursor of ethylene biosynthesis, strongly improve germination of thermodormant seeds. The possible involvement of ethylene biosynthesis and seed sensitivity to this hormon are discussed in relation with induction of secondary dormancy.

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INVOLVEMENT OF ETHYLENE IN DEVELOPMENT OF *BOTRYTIS CINEREA* AND *ALTERNARIA ALTERNATA*

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Exogenous ethylene has been shown to affect the germination of spores, hyphal and mycelial growth of many fungi species, including *Botrytis cinerea* Pers.ex.Fr. and *Alternaria alternata* (Fr.) Keissl., but little is known about the role of endogenous ethylene in germination and development of plant fungal pathogens. The aim of the study was to examine the role of endogenous ethylene in development of these two important plant pathogens.

A competitive inhibitor of ethylene binding, 2,5-norbornadiene (NBD), inhibited spore germination, hyphal and mycelial growth of *Botrytis cinerea* and *Alternaria alternata*. The transfer of spores or mycelium from an atmosphere containing NBD to air relieved the inhibition, indicating that its effects are non-toxic and reversible. The addition of ethylene to an atmosphere enriched with NBD effectively reduced the inhibition of spore germination and mycelial growth. Inhibition due to NBD was also antagonized by ethephon, an ethylene releasing factor. An inhibitor of ethylene synthesis, aminoethoxyvinylglycine (AVG), inhibited spore germination and this inhibition was reduced by a precursor of ethylene biosynthesis, 1-aminocyclopropane-1-carboxylic acid (ACC). ACC was also very effective in antagonizing the inhibitory influence of NBD. The stimulatory effect of ACC on mycelial growth, inhibited by NBD, was associated with increasing of ethylene production. The level of ACC, MACC and activity of ACC oxydase in mycelia was determined. The presented results suggest (1) that ethylene produced by *Botrytis cinerea* and *Alternaria alternata* is indispensable factor for its growth and development (2) that endogenous ACC serves as a precursor of ethylene biosynthesis and the enzymic system converting ACC to ethylene is active in those fungi.

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NORBORNADIENE AND SILVER ION OVERCOME ETHYLENE INHIBITION OF *POTENTILLA NORVEGICA* L. SEED GERMINATION

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It is reported that ethylene inhibits the phytochrome dependent germination of *Potentilla norvegica* L. seeds at a low concentration (<1 ppm). In this study the effects of ethylene antagonists, norbornadiene(NBD) and silver ion(Ag+) upon the germination, respiration and amino acid metabolism of the seeds were investigated. A lot of a hundred seeds were imbibed on a filter paper moistened with 0.4 ml DW in a 22 ml flask. The flasks were sealed with a vaccine rubber stopper through which ethylene and /or NBD was injected using a syringe. The seeds routinely were imbibed at 25 C in dark for 3 days, exposed to white fluorescent light for 24 hr and kept in the dark for 5 days to allow germination. Respiration of the seeds was investigated by measuring CO_2 concentration in the head space by TCD gas chromatography. Amino acid was extracted with 70 % ethanol and analyzed with HPLC. Whereas NBD and Ag+ themselves did not promote the germination, they specifically restored ethylene inhibited germination. Light exposure to the seeds triggered an increase of respiratory activity and a mobilization of amino acid metabolism but exogenously applied ethylene completely canceled these effects of light. Furthermore, NBD and Ag+ also restored the inhibitory effects of ethylene on respiratory activity and amino acid metabolism. These results indicate that some of the enzymes relating amino acid metabolism may be a possible site of action of phytochrome or ethylene.

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THE ROLE OF METHYL JASMONATE IN GERMINATION OF *AMARANTHUS CAUDATUS* SEEDS

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Jasmonic acid (JA) and/or methyl jasmonate (JA-Me) are widely distributed in many plant organs including seeds. JA-Me and abscisic acid (ABA) frequently show similar biological effects. ABA has been shown to be a strong inhibitor of germination of many seeds e.g. *Amaranthus caudatus* L. Inhibition caused by ABA can be reversed by ethephon, an ethylene releasing compound, or by 1-aminocyclopropane-1-carboxylic acid (ACC), a precursor of ethylene biosynthesis. The aim of studies was to determine the effect of methyl jasmonate on *Amaranthus caudatus* L. seed germination at a range of temperatures from 20^0 to 35^0C and the interaction between jasmonate and ethephon, ACC, gibberellins (GA_3,GA_{4+7}) or benzyladenine.

JA-Me inhibited seed germination of *Amaranthus caudatus* L. at a range of temperatures between 20^0 and 35^0C. It was found that inhibition of seed germination at 24^0C by JA-Me was associated with inhibition of ethylene production. Ethylene, GA_3 and benzyladenine partially or completely reversed the inhibition at 20^0-35^0C. ACC was effective in reversing the inhibition of seed germination at 24^0C although to a lesser extent than ethephon or gibberellins. Both GA_3 and GA_{4+7} enhanced the stimulatory effect of ethephon or ACC on germination of seeds inhibited by JA-Me.

The results indicate that methyl jasmonate increased requirement for ethephon, ACC, gibberellins and benzyladenine. The participation of ethylene in germination of JA-Me inhibited seeds will be discussed.

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THE EFFECT OF METHYL JASMONATE ON DEVELOPMENT OF *ALTERNARIA ALTERNATA* AND ITS REVERSAL BY ETHEPHON OR ACC

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Jasmonates are considered as a new class regulators of plant growth and development. They are widely distributed in higher plants and affect multiciplicity of plant physiological processes by inhibition, promotion or induction when exogenously applied to plants. Although jasmonic acid was isolated from pathogenic fungus *Lasiodiplodia theobromae* physiological action in the regulation of growth and development of fungus is unknown. The present study was carried out to determine the effect of methyl jasmonate (JA-Me) on germination of spores and the growth of hyphae and mycelium of *Alternaria alternata* (Fr.) Keissl. and to investigate the interaction between JA-Me and ethephon, releasing ethylene, and 1- aminocyclopropane-1-carboxylic acid (ACC), a precursor of ethylene biosynthesis.

JA-Me inhibited germination of spores of *Alternaria alternata*. The inhibitory effect of JA-Me was also manifested when growth of hyphae was estimated. JA-Me only slightly affected growth of mycelium. Although ethephon had a slight stimulatory effect on spore germination, it strongly stimulated when applied in presence of JA-Me. The reversing effect of ethephon was also evident when length of hyphae was determined. Ethephon almost totaly decreased slight inhibitory effect of JA-Me on growth of mycelium. ACC completely reversed the inhibitory effect of JA-Me on spore germination and growth of hyphae. The presented data suggest that methyl jasmonate may increase sensitivity of fungus to ethylene and probably does not inhibit the conversion of exogenous ACC to ethylene.

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EFFECTS OF METHYL JASMONATE ON ETHYLENE BIOSYNTHESIS IN MUNGBEAN

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Methyl jasmonate (MJ) inhibited auxin-induced ethylene production in mungbean hypocotyls with a half inhibitory concentration of 200 μM, while it stimulated the ethylene production in detached leaves. The inhibitory effect of MJ in hypocotyls was rapid, detectable within 4 h after treatment. Auxin-induced accumulation of ACC was found to be decreased by MJ in hypocotyls, but not in leaves. MJ also exerted differential effects on in vivo ACC oxidase activity. It inhibited ACC oxidase activity in hypocotyls, while the ACC oxidase activity was greatly stimulated by MJ in leaves. We investigated the MJ effect on the expression of genes encoding ACC synthase (VR-ACS1 and VR-ACS6) and ACC oxidase (VR-ACO1). In hypocotyls, MJ decreased both the auxin-induced accumulation of ACS6 and ACO1 transcripts. MJ treatment, however, resulted in a marked increase in the level of ACS1 transcripts. These results indicate that the MJ effects are primarily on the gene expression involved in ethylene biosynthesis. MJ is suggested to play different roles in different tissues of mungbean.

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DIFFERENTIAL EFFECTS OF JASMONIC ACID IN LIGHT AND DARKNESS, IN *Solanum tuberosum* EXPLANTS

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Jasmonic acid is able to strongly induce tuberization in 'in vitro' explants cultured in the dark. However its lack of effectiveness reported when sprayed to whole plants argues against jasmonic acid itself being the tuber-inducing signal, and poses some doubts on the results from in vitro assays.
Experiments with two different explants, in vitro dark-obtained stolons and single-node sections after subculture under long photoperiod, for several cultivars of different maturity groups, were performed in order to clarify the jasmonic acid 'in vitro' effects. Explants were cultured under several photoperiods in the presence or absence of jasmonic acid; tuberization and general development were periodically recorded. Althought variations among cultivars were noteworthy, only explants which had never received light tuberized, while those cultured under any photoperiod or which had been previously micropropagated in photoperiod did not tuberized but strongly increased its vegetative development. Root formation notably increased for all jasmonic acid treated explants, and they were most abundant in no tuberizing explants. It is suggested that light, either previously received or during jasmonic acid treatments, may have altered the 'in vitro' jasmonic acid promotion of tuberization.

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PGR EFFECTS AND PRACTICAL USES OF JASMONIC ACID ANALOGUES

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Our studies is related to put jasmonate compounds to practical use on agricultural production. Jasmonic acid(JA) or methyl jasmonate(JA-Me) showed the strong effects on plant growth in the bioassay systems, however PGR effects were not stable in the field conditions. We investigated the relationship of chemical structure and PGR effects. Jasmonate compouns showed the inhibitory effects on plant growth in a high dosage, but had the promotive effects in a low dosage. We expect Jasmonate compounds has a great potential for promotion of germination, growth, fruit-maturation and cold-resistance.

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CHANGES IN THE PROTEIN SYNTHESIS AND SEED CELL STRUCTURE OF ACER *TATARICUM L.* DURING JASMONIC ACID TREATMENT

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Acer tataicum L. seeds are able to germinate after a long cold stratification in wet conditions or jasmonic acid (JA) treatment. We studied JA treatment effects on the cell structure and protein synthesis in seeds. JA treatment resulted in the restoration of cell membrane structure, protein and lipid bodies breakdown. The protein synthesis in vivo was measured by means of [^{35}S]-metionine introdution in protein. When JA was used to treat dormant seed in cotyledons the transcription activity increased up to that of seed stratified for 4 months. In embryo axes JA had the same effect but to a considerably less extent. Radioactively labelled protein was analysed by two-dimentional gel electrophoresis. JA stimulated almost similarly the synthesis of all protein groups in cotyledons while during the cold stratification a general increase in the protein sythesis involved some singling out of a number of specific protein zones. JA influence on the spectrum of synthesized polypeptides in embryo axis is considerably weaker. This differentiated effects of JA on the protein syntesis may be associated with a more significant role of cotyledons in the regulation of dormancy mechanism.

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EFFECTS OF ABSCISIC ACID, FUSICOCCIN AND POTASSIUM ON MORPHOGENESIS OF LEAVES AND INTERNODES IN DARK-GROWN RICE SEEDLINGS.

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Seedlings with mesocotyl and foliage leaves engulfed in coleoptile are here refered to as MC seedlings and are considered to be suitable for deep sowing. We investigated the effects of abscisic acid(ABA), fusicoccin(FC) and potassium on the appearance of MC or non-MC rice (*Oryza sativa* L.)seedlings. Seedlings were grown on 0.8% agar medium with or without ABA, FC and KCl under aseptic conditions at 30℃ in the dark for 14 days. In the MC dominant cultivar, Basmati 217, 2×10^{-2}(M)KCl or 10^{-6}(M)FC both promoted the emergence of foliage leaves from coleoptile. Meanwhile mesocotyl growth was inhibited about 45% by KCl and 90% by FC. In the non-MC dominant cultivar, JC 91, 1.5×10^{-6} (M)ABA inhibited emergence of foliage leaves from coleoptile and also reduced coleoptile growth by 78% and second leaf growth by 87%. Meanwhile, during this treatment mesocotyl growth was stimulated by 195%. 2-trans ABA had no activity. Effects of ABA were almost entirely overcome by FC. It has been reported that FC stimulates the development of K^+ uptake coupled to H^+ extrusion and ABA inhibits this function. We concluded that ABA and FC may regulate the morphogenesis of leaves and internodes of rice seedlings via the H^+/K^+ exchange system.

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GROWTH, PHOTOSYNTHESIS AND HISTO-ANATOMICAL MODIFICATIONS IN YOUNG OLIVE TREES TREATED WITH PACLOBUTRAZOL

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An investigation was carried out on 2-year-old self rooted potted plants of the cv.Leccino. The plants were treated with Paclobutrazol-(2RS, 3RS)-1-(4chlorophenyl)-4,4dimethyl-2-(1H-1,2,4-triazol-1-yl)pentan-3-ol - at the beginning of vegetative activity either as a single application to the foliage, (2000 ppm), as a single application to the soil (1 g a.i. plant) or to both. The rates of photosynthesis and carboydrate content were determined in both shoots and leaves 7, 14, 21 and 28 days after treatment. Plant height, shoot lenght, number and lenght of internodes , leaf area and the histo-anatomical characters of shoots, leaves and roots were recorded at the end of growth. Foliar and soil applications of Paclobutrazol reduced plant height, shoot lenght, number and lenght of internodes and leaf area. The most marked effect was achieved by soil application and by combined foliage and soil application. The photosynthetic rate and carbohydrate content was increased slightly by foliage application of Paclobutrazol and decreased by treatments applied to the foliage and root system. A histo-anatomical study of leaves, shoots and roots of trees treated with Paclobutrazol showed the reduction in size was due to a reduced cell elongation particularly in the wood, and a reduced differentiation since resulting in fewer fibres and vessels and a higher number of tracheids.

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NEW COMPOUNDS REGULATING INDUCTION AND SECRETION OF α-AMYLASE IN ALEURONE CELLS OF BARLEY SEEDS

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By the action of an active gibberellin, α-amylase is synthesized *de novo* in aleurone cells in barley seeds and secreted out of cells. The purpose of the study is to elucidate the machanism of the α-amylase induction and secretion by using specific inhibitors of this system as molecular probes. As a first stage of this study, we investigated the action of many compounds. Phloroglucinol, 2,4-pyrandione and 4-pyrone derivatives inhibited the α-amylase induction. Phloroglucinol derivatives are known as a potent uncoupler in respiratory electron transport system, but their modes of action in inhibition of α-amylase induction were not due to their uncoupling effect. This was sugested through the structure-activity relationships study, that is, they showed potent activity of uncoupling after structural modification, but they lost the inhibitory activity on α-amylase induction. While, folimycin and brefeldin A, inhibitors of intracellular translocation of virus glycoproteins, inhibited the secretion of α-amylase but did not inhibit its induction in the cells.

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EFFECTS OF BAP, NAA AND TRIACONTANOL ON PHOSPHORUS REDISTRIBUTION IN BUCKWHEAT

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The aim of studies was to determine the effects of phytohormones on phosphorus accumulation dynamics and extent of this nutrient translocation during buckwheat flowering and seed ripening in the conditions of different level of nitrogen nutrition. Buckwheat (*Fagopyrum esculentum* Moench.) plant were grown in cold greenhouse in Kick-Brauckmann pots filled with 10 kg of soil. Nitrogen fertilizers were applied in two rates – 1.25 or 2.50 g N per pot and 50% of total rate was used as soil or foliar nutriton during plant growth. On the background of this nitrogen fertilization regime three phytohormones were used: benyladenine (BA), α-naphtyloacetic acid (NAA) and triacontanol (TRIA) as foliar spraying seven times during growing season in the period from plant budding to flowering. Samples of plant material were taken at 46, 63, 80 and 102 day of growing. In the studies was found that in the conditions of soil applied nitrogen, BAP stimulated P tranport from vegetative to generative organs, whereas inhibition of phosphorus translocation under the effect of NAA applying was found. Triacontanol positively affected phosphorus accumulation in buckwheat inflorescens only at the beginning of flowering. This phytohormone interacting with foliar applied nitrogen enhanced phosphorus translocation at the phase of buckwheat maturation. It was shown that accumulation and redistribution of phosphorus at some particular development phases was remarkably modified by used phytohormones, nitrogen rates and interaction of studied phytohormones with nitrogen.

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EFFECT OF GROWTH REGULATORS AND BLOCKATOR OF Ca^{++}-CHANNELS ON INTERACTION OF AUTOTROPHIC AND HETEROTROPHIC ORGANS OF PLANTS

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The effect of new growth regulators with hormonal and antioxidant properties - ambiol and fonk (10^{-6}-10^{-12}M), blockator of Ca^{++}-channels - verapamil (10^{-4}M) and different light spectrum composition (white, red, blue light) on growth, development and change in acid-base equillibrium of autotrophic (leaves, stems) and heterotrophic (roots) organs of C3- and C4-plants has been studied. Ambiol activated growth and development of shoots and roots in pea to a higher degree than in maize and caused an increase in the size of leaves. Fonk at 10^{-6}-10^{-7}M concentrations decreased the growth of shoots but not of roots and stimulated the growth of shoots and roots at 10^{-8}-10^{-10}M concentrations. Verapamil caused a decrease in the growth of shoots and roots and a decrease in the size of leaves with white and blue light in particular. In red light the inhibiting effect of verapamil was expressed less. Ambiol reduced the growth inhibiting effect of verapamil. Fonk, depending on concentration, increased or decreased the growth inhibiting effect of verapamil. Characteristic changes in acid-base equillibrium in shoot and root tissues under action of ambiol, fonk and verapamil have been noted. Effect of the studied factors on coordination of functions of autotrophic and heterotrophic organs of plants is discussed.

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THE INFLUENCE OF PLANT HORMONES ON THE REPLICATION OF WHITE CLOVER MOSAIC VIRUS (WClMV) IN *Phaseolus vulgaris* AND TOBACCO MOSAIC VIRUS IN *Nicotiana tabacum*

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Plant hormones of varying concentration were supplied to the xylem of *P. vulgaris* and *N. tabacum* via a wick. These plants were then challenged with WClMV and TMV respectively. The replication of the virus was determined daily using ELISA, and viral dsRNA was extracted and quantified. A reduction in viral replication was recorded following the feeding of two cytokinins (dihydrozeatin and dihydrozeatin riboside), jasmonic acid, salicylic acid, 1-amino-1-cyclopropane carboxylic acid (ACC) and to a lesser extent IAA. A slight increase in viral replication occurred following feeding of ABA, while GA_3 had no effect. Qualitative and quantitative changes occurred in endogenous cytokinins following infection. Levels of jasmonic acid are currently being monitored during infection.

Viral infection causes changes in the synthesis and metabolism of plant hormones, particularly the cytokinins. The ability to control these changes may provide a novel approach to the production of virus-resistant plants.

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PRACTICAL APPLICATIONS OF BRASSINOSTEROID COMPOUND [TS303]

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Since Brassinolide(BR) was first isolated in 1979, many people have been interested in applying to growth control of crops and many field experiments have been carried out by utilizing BR, Epi-BR or Homo-BR. However, significant growth control activities have not been clearly proven yet even though these activities were well demonstrated in laboratory experiments. This might be due to instability in the field conditions. We selected a new compound [TS303] that have persistent effects. There have been a lot of studies on agricultural applications of [TS303]. Thus, highly effective growth control, now can be expected; (1)promotion of seed and fruit setting, (2)promotion of germination and rooting, and (3)prevention from low temperature and frost injury.

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THE POSSIBLE REGULATORY EFFECT OF ZINC AMMONIA ACETATE ON GERMINATION AND EARLY SEEDLING GROWTH OF THREE MAIZE (*Zea mays* L.) GENOTYPES

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Four concentrations (0, 0.85, 8.5, and 85 ppm) of zinc ammonia acetate (ZAA) were evaluated for effects on germination, early seedling growth and nutrient status of 'Dekalb', 'Pioneer', and 'Jacques' maize hybrids. At 0.85 and/or 8.5 ppm, ZAA tended to increase length of total seminal root, and shoot and root dry weight (DW) of germinated seeds of all genotypes. In hydroponic test, plant height and whole plant DW of 2-week-old seedlings either increased or tended to increase at 0.85 and/or 8.5 ppm of ZAA. The number of seminal and lateral root for all genotypes, and total seminal root length for 'Dekalb' and 'Jacques' were significantly increased by ZAA at either 0.85 or 8.5 ppm. However, the observed high root : shoot ratio of ZAA treated plants was not due to increased root growth over shoot growth, but resulted from a greater reduction in shoot growth at higher ZAA levels. The B content in shoots of all genotypes had a positive linearly response to increasing ZAA concentrations. Either 0.85 or 8.5 ppm of ZAA increased Cu and Fe contents. The Ca, K, Mg, and P contents were not significantly affected by ZAA. In greenhouse test, plant height, leaf area and shoot DW of 3-week-old seedlings of all genotypes were increased by ZAA at 0.85 and/or 8.5 ppm. Root volume increased at 0.85 and 8.5 ppm, and at all concentrations for 'Pioneer' and 'Jacques', respectively. These data show that variability existed among genotypes in response to ZAA. Considering the extremely low concentration of ZAA required to induce a plant response, ZAA may also act as a plant growth regulator rather than just as a nutrient supplement.

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SYNERGISM BETWEEN IBA AND PLANT GROWTH RETARDANTS IN THE ADVENTITIOUS ROOT FORMATION IN MUNG BEAN HYPOCOTYL

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The effect of IBA and plant growth retardants, such as triadimefon, daminozide and paclobutrazol, on the formation of adventitious root and its hormone change during rooting were studied. The action of rooting by IBA and three retardants was sequential, former followed by latter. IBA was applied during the first 24h followed by retardants at 48h induced more rooting than application in reverse order. This result proved that induction phase of root primordia is promoted by IAA accumulation and the early initiation phase of root primordia is inhibited the biosynthesis of GA. Mixture of three retardants with IBA have proven generally more effective than IBA alone in promoting adventitious root formation. The physiological basis of synergistic effect on the combination of IBA and triadimefon were increasing endogenous IAA level, decreasing GA level, enhancing the activities of peroxidase and IAA oxidase. In addition, several ornamental plants cuttings treated with the mixture of IBA plus triadimefon showed better adventitious root formation than those treated with IBA alone.

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SHOOT PROLIFERATION OF GARDENIA (GARDENIA JASMINOIDES ELLIS)

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Optimum proliferation of Gardenia jasminoides Ellis shoot tip was obtained on Murashige and Skoog salts medium supplemented with BA or kinetin (0-5 mg/L) alone or with combinations of NAA at concentrations of 0-2 mg/L. The results indicated that proliferation was maximal after 6 weeks of culture. Significantly greater proliferation of shoot tips (8.4 and 9.1) respectively, occurred on medium with BA alone at 2 and 3 mg/L, and with kinetin at 3 and 5 mg/L (4.7 and 5.7) respectively. BA proved superior to kinetin for promotion of shoot proliferation, while combinations of cytokin with auxin produced shoot proliferation lower than cytokin alone. Plantlets were transferred to growing medium and acclimated successfully.

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IDENTIFICATION OF POTATO CULTIVARS BY ISOZYME ANALYSIS

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This research evaluated the response of seven potato (Solanum tubersom L) cultivars (Diamant, Famosa, Marfona, Mirka, Desiree, Agria, and Jearla) to tissue culture using electrophoresis for genotype determination. The results revealed that shoot explants represent a suitable source for propagation of these cultivars. Diamant, Famosa, Marfona, Mirka and Desiree showed good response to tissue culture, while Agria and Jearla were less responsive. Isozyme analysis results revealed the existence of genetic variation among the cultivars under study.

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PRIMARY IDENTIFICATION OF RICE EMBRYOGENIC-CELL PROTEINS

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Up to now little is known about the molecular events occurring during processes of embryogenic competence induction. Native / SDS-PAGE was used to isolate and identify proteins specific to embryogenic competence in various embryogenic calli. Firstly, two proteins (46KDa, 35KDa) were isolated from embryogenic calli on 15th day of induction culture in explant-difference-type system through comparison between embryogenic callus from young panicles and nonembryogenic callus from mature embryos of the rice variety Guangluai No.4. These proteins also existed in other two kinds of embryogenic calli from young panicle explants of varieties Yangdao No.2 and Yanjing No.2, and were not found in their mature-embryo nonembryogenic calli. Appearance of the two proteins was closely relative to higher percentages of plantlet regeneration (PPRs) in three kinds of embryogenic calli from the three varieties in which PPRs reached 73.9%, 50.2% and 24.3% respectively, while in three kinds of nonembryogenic calli from mature embryos of the three varieties PPRs were only 1.6%-3.2%. Secondly, a protein (about 45KDa) was isolated by SDS-PAGE in embryogenic callus in osmosis-induction-type system, which resembles the 46KDa protein as above. Thirdly, the 46KDa protein disappeared and the 35KDa protein only accumulated little in nonembryogenic callus from young panicle explants of the variety Liaoyan No.2. It was known PPR in this callus as low as 1.6%. Fourthly, 2ppm of KT could enhanced the accumulation of the two proteins (46KDa, 35KDa) in callus from mature embryo of Guangluai No.4, which coincided with appearance of embryogenic competence and then improvement of PPR after treated by it. Lastly, by treatment of callus from young panicles of Guangluai No.4 with Actinomycin D and Actidione at 40μg/ml and 15μg/ml respectively from 7th to 15th day, it was observed that transcription inhibitor did not affect the two proteins while translation inhibitor blocked their expression. According to these results, it is suggested that 46KDa and 35KDa proteins may function in embryogenic competence induction.

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INFLUENCE OF FRUIT AND SEED LOCATION ON COTTON FIBER DEVELOPMENT IN VITRO

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Cotton fibers are single cells derived from the epidermis of cotton ovules. Fiber quality parameters include length and cell wall thickness. Cotton (*Gossypium hirsutum* L. cv. Deltapine 50) plants were greenhouse grown. Fruit was collected 30 to 48 h post anthesis, ovules were removed and cultured on modified Beasley and Ting medium. The gibberellic acid concentration was lowered to permit any differences in the physiological state at the time of excision not be obliterated by high GA_3 and IAA concentrations. Fiber quality was determined for plant and culture-grown fiber using the Advanced Fiber Information System. Fiber from the micropylar end was shorter and thicker than fiber from the chalazal end of the seed in both plant and culture-grown fibers. In greenhouse-grown fruit as seed weight increased fiber cell wall thickness increased. An increase in ovule weight was not accompanied by an increase in cell wall thickness in vitro. Late set fruit has poor fiber quality. Ovules cultures from different fruiting sites did not differ in quality. It was concluded that assimilate distribution was primarily responsible for differences seen in fiber quality.

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THE INFLUENCE OF JA-ME AND ABA ON ETHYLENE PRODUCTION AND GROWTH OF CALLUS OF *MEDICAGO SATIVA* L.

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Jasmonates, considered as a new class of plant growth and development regulators, are widely distributed in higher plants.They affect multiciplicity of plant physiological processes by inhibition, promotion or induction when exogenously applied to plants. The effects of methyl jasmonate (JA-Me) in comparison to abscisic acid (ABA) on ethylene production and growth of callus of *Medicago sativa* L. during induction stage of somatic embriogenesis have been investigated.
The SH medium containing 2,4-dichlorophenoxyacetic acid and kinetin was used for the callus formation from petioles of *Medicago sativa L.*. The cultures were grown in conical flasks sealed with a rubber puncture cap until the amount of ethylene was determined by gas chromatography. In order to determine the ability of callus to ethylene production, Erlenmayer flasks were sealed with rubber puncture caps for 24 hours before measurement.
A high rate of ethylene production on the third day and its gradual declining afterwards was observed. Decrease of ethylene production was correlated with rapid callus growth. Methyl jasmonate and abscisic acid did not affect ethylene accumulation determined on the 21st day of culture but they greatly inhibited callus growth. Those compounds significantly decreased ability to ethylene production on the 3rd day but not on the 21st day of culture. Both, JA-Me and ABA showed similar activity. The inhibition of callus growth caused by JA-Me or ABA can not be reversed by exogenous ethylene or ACC. The influence of JA-Me or ABA in relation to induction of somatic embryogenesis and subsequent embryo maturation will be discussed.

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HABITUATION TO HORMONES AND PLANT CANCER IN THE ABSENCE OF PATHOGENS : MORPHOLOGICAL AND BIOCHEMICAL ASPECTS

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A comparative investigation of habituation (hormone independence) in plant calli and vitrification (malformations associated to hyperhydricity) of shoots under vegetative multiplication *in vitro*, has led to draw neoplasic progressions in the absence of pathogens at the plant cellular and organismic levels. The cancer terminal phase in calli is characterized by the irreversible loss of capacity for undifferentiated cells to organize themselves into regenerative primary meristems. Vitreous shoots subcultured in vitrifying conditions become fully habituated with many characters similar to habituated calli and animal cancerous cells. Progressive reduction of cell-cell adhesion inducing shoot breakability (friability is the term used for calli) leads to apex necrosis. This is considered as whole plant death through abandon of the meristems. The still surviving cells in culture are further incapable to organize themselves in primary meristems. The irreversible loss of organogenic totipotency represents the typical plant cancer trait.

Fully habituated nonorganogenic cells are characterized by deficient/uncomplete differentiation of walls, mitochondria and chloroplasts. They show very large nuclei with deep invaginations and many nucleoli. Micronuclei can also be seen. Abnormal DNA distribution indicates polyploidy and aneuploidy. Several interdependent perturbed metabolisms, common to habituated calli and vitrified shoots, will be shown.

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ORIGIN OF POLYAMINE ACCUMULATION ALONG NEOPLASIC PROGRESSIONS OF PLANT TISSUES

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Hormone independent cell lines of sugarbeet accumulate polyamines at levels superior to those of (normal) hormone dependent ones. Shoots undergoing vitrification (hyperhydric malformations; another neoplasic process) in *in vitro* cultures also accumulate polyamines. A deviation or adaptation of nitrogen metabolism thus characterizes the acquisition of the cancer state in plant cells as in animal cancerous cells.

Polyamine accumulation in hormone independent cells is accompanied by elevated levels of NH_4^+ and NO_3^- ions, proline, glutamate and glutamate dehydrogenase. Relationships between proline catabolism, and glutamate and polyamine biosyntheses, will be established. It will also be shown how these biosyntheses are facilitated through activation of the pentose phosphate pathway and non-photosynthetic fixation of CO_2.

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CHEMICAL STUDIES ON PLANT CELL GROWTH FACTOR

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It is well known that the proliferation of plant cells in culture is highly dependent on the initial cell density and diluted suspension cell cultures have a relatively low colony formation frequency in any condition. The colony formation frequency in such diluted suspension cell cultures is increased by supplementation of conditioned medium (CM) isolated from rapidly growing cell cultures. This promoting factor in CM can not be substituted by any known plant hormone and is considered to be a plant cell growth factor. In spite of numerous researches, the molecular nature and chemical structure of this factor remain unidentified. We established a novel and highly sensitive bioassay system to detect this factor adopting the mechanically isolated mesophyll cells from *Asparagus officinalis* L.. Colony formation frequency of the cell suspension in relatively low cell density is dramatically increased by supplementation of CM prepared from 10-day cell suspension cultures in high cell density. This bioassay method is about ten times as sensitive as the previously reported methods using protoplasts or cells derived from callus and requires only 5-7 days to detect the activity of plant cell growth factor in CM. Characterization studies of this factor indicated that the factor was a relatively heat-stable, water-soluble and acidic compound with MW of ca. 1300, and possibly had peptide moiety as judged by the enzymatic digestion experiments.

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Callus was induced from different explants of *Taxus baccata* using different concentrationsof growth regulators such as 2,4-D , NAA, IBA in combination with KN, BAP etc. Callus obtained from neddles was dark in colour and grew very slowly,calhus tissue from stem / rachis dispayed the best growth in MS media with 0.5 mg / l 2,4-D and 0.01 mg / l KN. Poly vinyl pyrrolidone (PVP) was added to the media to adsorb the excessive phnolic leakage.Growth rate of the callus tissue improved upon continuos subculturing at every18 day interval and subsequent reduction of growth regulator concentrations to 1/10 th of the induction media.Callus tissue were extracted for *Taxol* after 3 months in culture and analysed both by HPLC and ELISA monoclonal antibody test.Variation in Taxol content was noticed at different growth regulator conacentrations.Effect of various concentrations of growth regulators on growth and secondary product accumulation will be disscussed.

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RELATIONSHIP OF PLANT HORMONES TO SHOOT REGENERATION ON EXPLANTS OF LESQUERELLA

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The genus Lesquerella contains several species which produce industrially important long-chain hydroxy fatty acids in the seed oil. To explore the usefulness of *in vitro* culture techniques in the improvement of these species experiments were conducted on plant regeneration from explants of *Lesquerella fendleri*. Seeds were surface sterilized with 1% sodium hypochlorite and germinated on ½ MS medium at 25°C in light of 100 $\mu E.M^{-2}Sec^{-1}$ for 16h. Leaf, stem and root explants were taken from 4 wk old seedlings and incubated on MS medium with various combinations of plant growth regulators. All cultures were maintained in light, 100 $\mu E.M^{-2}Sec^{-1}$ for 16h at 25°C. Auxins, 2,4-D, NAA and IBA at 0.5 to 5 μM promoted callus proliferation on all explants but not shoot formation. At low auxin levels root proliferation occurred on callused explants. All cytokinins, BAP, Zeatin, 2IP and Kinetin promoted shoot proliferation on all explants in the range of 1.0 to 5.0 μM. An average of 30 shoots developed on a 2 cm segment of root and 12-14 on stem and leaf explants. Addition of silver nitrate, 25 μM, influenced the time to shoot emergence and the number per explant. Auxins suppressed cytokinin-induced shoot proliferation and enhanced callusing. Shoot buds developed on auxin-induced callus in the presence of high levels of cytokinin and silver nitrate. Regeneration occurred by organogenesis and embryos were never observed. These results indicate that efficient shoot regeneration on all explants require cytokinins and is inhibited by auxins. The differential response of root explants compared to leaf and stem explants may be due to endogenous levels of plant hormones.

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EFFECT OF HORMONE FOR DIVISION AND ELONGATION OF TOBACCOS FREE CELL

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Used the free cells formed by inducing calli of the leaf blades of young tobacco(Nicotiana tobacum)plants as material. The free cells are got by puttion fresh calli into H′ s liquid medium, shaking and culturing 3 weeks, using nylon net to filter, and, getting rid of the dregs. Add different consistencies of 2, 4-dichlorophrnoxyacetic acid (2, 4-D), kinetin (KT), abscisic acid (ABA) ect. into H′ s liquid medium during the time of shaking and culturing free cells. Study how theis 3 kinds of hormone effect cell′ s division and elongation. Use phase microscope to observe the division and elongation of the livion cells. Results show that auxin such as 2, 4-D is good for cells to elongate toward one single direction and good to divide and form long cells, that cytokinin such as KT isn′ t good to cells elongation but is good for globular cells to devide and form, that ABA is similar to KT but the cells is senescent easily and medium containing ABA.

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ANALYSIS OF CYTOKININS BY COMBINED LIQUID CHROMATOGRAPHY-ELECTROSPRAY IONISATION TANDEM MASS SPECTROMETRY

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Combined liquid chromatography-mass spectrometry (LC/MS) has proved to be a valuable tool in plant hormone science, especially for the analysis of thermolabile and non-volatile hormone conjugates and hormone metabolites. N^6-substituted adenines, cytokinins, are polar compounds wich are difficult to analyse by standard GC/MS techniques. We have combined a capillary LC system with a four sector tandem mass spectrometer (B_1/E_1-B_2/E_2 geometry) via an electrospray ionisation (ESI) interface. Full scan spectra obtained by "in-source fragmentation" were compared with daughter ion spectra obtained by collision-activated dissociations (CAD) of parent ions in the third field-free region of the instrument. The ESI technique gave both high sensitivity and low background noice. In combination with tandem analysis, therefore, ESI provides a valuable tool for identification of trace amounts of cytokinins and their metabolites.

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DEVELOPMENT OF A DIRECT ELISA FOR THE MEASUREMENT OF CYTOKININ O-GLUCOSIDES IN PLANT TISSUES

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The side chain conjugation of cytokinins to sugar molecules plays an important role in maintaining cytokinin homeostasis in many plant tissues. The measurement of these compounds, however, is often neglected due to the lack of suitable methods. Using antisera raised against zeatin O-glucoside (ZOG) conjugated to BSA via the side chain glucose, a direct ELISA specific to ZOG was developed. The assay is based on competition for antibody binding between cytokinin in the sample and a tracer of ZOG conjugated to alkaline phosphatase via the side chain glucose. The assay is accurate over a range of 20 - 6000fmol. ZOG is required at 250fmol for 50% inhibition of tracer binding. Cross-reactivity of other cytokinins shows that the free bases Z, DHZ and iP can compete for antibody binding as efficiently as ZOG. Any substitution on the adenine ring, however, reduces cross-reactivity to less than 1% of the ZOG - antibody affinity. Importantly, cross-reactivity with adenine is approximately 1% illustrating that the N^6 side chain is necessary for epitope-antigen recognition. This therefore represents a much more sensitive assay for the measurement of free bases than existing assays based upon cytokinin ribosides. The assay has therefore been used, in conjunction with HPLC, to measure the concentration of ZOG and free base cytokinins in mature leaves of *Phaseolus vulgaris* L. and senescing leaves of *Ginkgo biloba* L.. These results will be discussed with reference to the use of the assay in clarifying the importance of the flux between free base and side chain conjugates in the control of the level of active cytokinins *in vivo*.

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IMMUNOHISTOCHEMICAL LOCALIZATION OF GIBBERELLINS

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Several immunohistochemical and immunocytochemical analyses of plant hormones have been reported. In these analyses, plant hormone fixation was performed in liquid media and the possibility remains that the plant hormones might have drifted from their original location during the fixation process. To elucidate the problem of drifting, we developed a new fixation method for the immunohistochemical localization of gibberellins (GAs) in rice spikelets. Spikelets before anthesis were frozen in liquid nitrogen and lyophilized at -20°C. They were fixed with diisopropylcarbodiimide gas in a desiccator, which catalyzed amide formation between 7-carboxyl groups of GAs and amino groups of proteins in the tissue. The fixed spikelets were embedded in paraffin and sectioned (10μm thick). The sections were stained with anti-GA_1-Me antibody and visualized using an immunoperoxidase system. Anthers containing mature pollen were stained well, while those containing immature pollen were not stained. This result correlates with the quantitative results of GA_4 and GA_7 in these anthers.

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RESOLUTION OF RACEMIC JASMONIC ACID AND ABSCISIC ACID BY CHIRAL HPLC

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The endogenous plant growth regulators (-)-jasmonic acid ((-)-JA) and (+)-abscisic acid ((+)-ABA) are involved in the regulation of various processes of plant development. Synthetic products of ABA and JA, however, consist of racemic mixtures, which in respect to physiological studies are of limited use only. Former attempts to resolve the enantiomers were based on the separation of derivatives (esters) by chiral HPLC followed by hydrolysis[1,2].

We found that free racemic acids of JA and ABA can efficiently be separated by HPLC on the chiral cyclodextrin phase Nucleodex ß-PM. With synthetic (3RS,7RS)-JA, the native (-)-(3R,7R)-enatiomer was obtained to elute prior to the artificial (+)-(3S,7S)-isomer. Likewise, racemic *cis, trans* -ABA could be separated into its enantiomers with (+)(S)-ABA showing a shorter retention than the (-)-(R)-enantiomer. The method can be applied to separations on preparative scale (milligram amounts). The resolved pure enantiomers were characterized by CD and ORD.

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UNDERSTANDING THE MOLECULAR STRUCTURE OF ABSCISIC ACID

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(+)- and (-)-ABA are almost equally effective growth inhibitors so it was suggested in 1969 that the (-)- fits into receptor in the same way as the (+) both with a 6+-Me in the place where the 7+-Me of the (+) should be and vice versa. This can occur only when the ring is in the pseudochair conformation with the sidechain equatorial. X-ray and NMR data show that the sidechain is normally axial.

The steric requirements deduced from a number of optically pure synthetic analogues are strict and can be satisfied only by side-chain-equatorial conformers. It is suggested that H bonding to the 4+-ketone and by the 1+-OH pulls the ring into the receptor until the axial 8+-Me is pushed outwards and thereby thrusts the sidehcain into the equatorial position.

Four epimers of 2+-dihydro ABA show that the 2+-double bond is not required for activity, both 1+-S- and 1+-R-are active provided that the 7+-Me does not project from the same face of the ring as the 1+-OH. This is considered to cause steric interference with the receptor surface. Consequently, the lack of effect of phaseic acid is attributed to a steric effect of the oxymethylene bridge and to the ring being constrained with the sidechain axial, not to the absence of a 2+-ring double bond.

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METHODS FOR QUANTIFICATION OF PLANT HORMONES IN MILLIGRAM AMOUNTS OF PLANT TISSUE

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Two methods for quantification of IAA and GAs in milligram amounts of tissue have been developed at the department and will be presented. The plant extract for quantification of IAA was purified with XAD-7 slurry only, before the final analysis by combined gas chromatography-mass spectrometry (GC/MS). Low (LR)- and high-resolution selected-ion-monitoring (HR/SIM) and selected-reaction-monitoring (SRM) mass spectrometry techniques were compared for selectivity and precision. The best selectivity was obtained with SRM analysis, and 1 mg samples containing 500 fg of IAA could be analysed accurately with this method. GAs were purified from plant extracts by a solid phase extraction. Analysis by HR-SIM, SRM and four sector MS/MS were compared. The best selectivity was found with four-sector MS/MS analysis, but the sensitivity was too low for analysis in mg amounts of plant tissue. HR-SIM and SRM had similarly low limits of detection (<pg) for most of the anlysed GAs but SRM analyses provided better selectivity. Examples will be presented of how the two methods can be used for investigation of distribution patterns in specific plant tissues.

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CYTOKININ ANALYSIS BY ELECTROSPRAY TANDEM MASS SPECTROMETRY

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In the last decade, procedures described for phytohormone analysis as well as the available hardware has improved substantially, allowing the analysis of phytohormones starting from a minimal amount of plant tissue (0.1-1g). Analysis by RIA or ELISA was favoured due to its high sensitivity and short analysis time. Different cross reactivities of the antibodies available towards the individual different cytokinin-conjugates are however a severe disadvantage. UV (diode array) analysis requires a complete absence of interfering plant-matrix components and a baseline separation of all cytokinin compounds. The analysis by combined ES LC-MS/MS, presented here is a very sensitive and accurate method to analyze all different cytokinins (free bases, ribosides and glucosides) without extensive HPLC-separation. A single step immune affinity purification procedure is sufficient. Cytokinin-phosphates are removed from the crude extract on a solid phase anion exchange column prior to immune affinity purification and analyzed, without hydrolysis, using a separate ES LC-MS/MS run.

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A MICROTECHNIQUE FOR THE ANALYSIS OF FREE AND CONJUGATED IAA BY GC-MS-SIM

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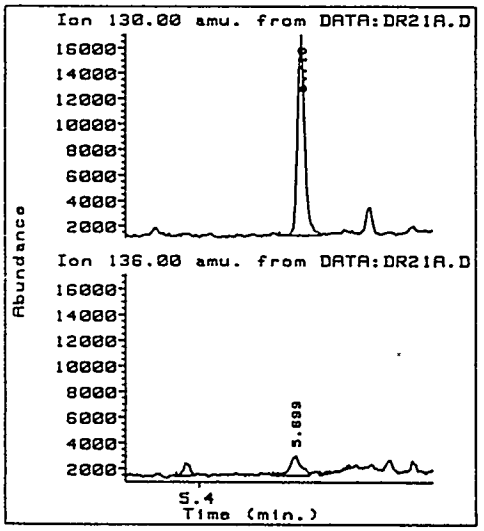
Many developmental processes such as somatic embryogenesis are thought to be controlled by the concentration of IAA. IAA content in specific tissues could not previously be readily investigated because at least 100 mg of plant tissue was routinely required. Such a large sample size is often the limiting factor for many types of investigations.

Therefore, a microtechnique was developed to measure samples as small as 1 mg. The technique relies on micro-SPE preparatory columns made in 200 µl pipet tips and microbore HPLC prior to analysis by GC-MS-SIM. The impetus for the development of this technique was to investigate IAA involvement in carrot zygotic embryogenesis and relate it to our previous work on carrot somatic embryogenesis. Such a comparison is important in order to give biological significance to the changes in IAA observed in the somatic system. This technique is widely applicable; for example, individual parts of 1 *Arabidopsis* seedling can be analyzed separately. The Figure shows the chromatograms for the total IAA analysis of a 3.2 mg seedling of the *Arabidopsis* mutant GORDO. This work is supported by *D.O.E. grant DE-AI02-94ER20153.*



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PRELIMINARY CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST INDOL-3-YLACETIC ACID (IAA) AND DEVELOPMENT OF ENZYME-LINKED IMMUNOSORBENT ASSAYS (ELISA)

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Despite the development over the last two decades of several immunological assays for quantifying endogenous IAA, a reliable and highly sensitive method has remained wanting. Using IAA linked through the C-5 position to bovine serum albumin (BSA), and 5-hydroxy-IAA linked through the C-6 position to BSA as antigens in Balb\c mice, 28 monoclonal antibodies (McAb) against IAA (non-derivatized) were obtained following screening of ~10^5 clones. Following cross reactivity studies, one McAb raised against each antigen was selected; both showed very high specificity toward the combined presence of the acetic acid side chain, the indolic nitrogen and the benzene moiety of IAA. An indirect ELISA (plate coated with IAA-BSA, incubated with IAA + anti-IAA McAb, and following washing detected using alkaline phosphatase-labelled rabbit anti-mouse IgG). This indirect ELISA was determined to be useful over the range of 3.5 - 350 ng of IAA. To develop a direct ELISA, the anti-IAA McAbs were purified using IAA-N-Sephadex G-75 chromatography. For competitive binding, IAA was linked to alkaline phosphatase through the C-5 position. In direct ELISA (plate coated with rabbit anti-mouse IgG followed by anti-IAA McAb, then incubated with IAA + IAA-alkaline phosphatase), less than 0.1 pg of IAA was readily detected and the working range extended from 35 fg to 350 pg per assay. Data are provided on the levels of extractable IAA in cambium and developing xylem of jackpine (*Pinus banksiana* Lamb.) as determined by direct ELISA and combined gas chromatography - selected ion monitoring mass spectrometry. This research was supported by a grant from the Natural Sciences and Engineering Research Council of Canada to R.A.S.

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ANALYSIS OF GIBBERELLIN GLYCOSYL ESTERS BY LC / ESI - MS

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Among sugar conjugates of plant hormones the sugar esters are most difficult to analyze because of their lability. Nowadays, modern LC-MS techniques based on fast atom bombardment (FAB) ionization[1] or atmospheric pressure ionization (API)[2] have been shown to provide useful conditions to identify intact gibberellin glucosyl esters. Electrospray ionization (ESI)-MS represents another more recent ionization technique, which is expected to work efficiently with gibberellin glycosyl esters, too. We investigated a series of synthesized gibberellin glycosyl esters and their labelled derivatives with LC / ESI-MS negative and positive mode. From the positive ESI spectra an abundant ion of $[M+Na]^+$ is useful to recognize the molecular weight of a sugar ester. In the negative ESI spectra the $[M\text{-sugar}]^-$ ion represents the base peak, which allows to search for a group of different sugar esters of distinct gibberellins by SIM. For the final identification, MS/MS techniques were included, e.g. „daughter" ion or „parent" ion spectra, which also *on line* provide convincing data. The application of LC/ESI-MS to the quantification of the endogenous pools of gibberellin glucosyl esters was investigated by measuring differently labelled standards.

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LOCALIZATION OF ABA IN MUSKMELON SEED TISSUES DURING DEVELOPMENT

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Previous studies have shown that muskmelon seeds attain maximum dry weight 35 d after anthesis (DAA) but fail to reach full germinability until 45 DAA. Seed germination rate increases dramatically after dry storage, and sensitivity to exogenous ABA dramatically decreases after 50 DAA. To further investigate the role of ABA in muskmelon seed development, ABA contents were measured in the embryonic axis, cotyledons, liquid endosperm, perisperm envelope, testae, endocarp, and pericarp tissue from 20 to 60 DAA using indirect ELISA. Intra-cellular ABA was visualized using transmission electron microscopy to detect gold particles conjugated to a polyclonal antibody raised against conjugated BSA-ABA. ABA concentrations were highest (250 ng (g dwt)$^{-1}$) in embryonic axis tissue at 25 DAA during the time of rapid dry weight accumulation and the development of desiccation tolerance and then dropped rapidly to a low of 25 ng (g dwt)$^{-1}$ at 50 DAA. Concentrations in the perisperm envelope tissue were 150 ng (g dwt)$^{-1}$ at the same stage of development. ABA concentrations were also high in the endocarp but less than 75 ng (g dwt)$^{-1}$ in other tissues. Rapid drying decreased ABA content in embryonic axis tissues and increased germination percentages in seeds < 40 DAA. After-ripening caused isolated embryos to lose sensitivity to exogenous ABA, while intact seeds were not affected. All cell layers of the perisperm envelope were gold-labeled at 25, 45, and 55 DAA. In the embryonic axis of 25 DAA seeds, ABA was detected in the cytoplasm, nuclear membrane, protein bodies, and endoplasmic reticulum. Cell walls in the embryonic axis were only labeled at 45 DAA. At 55 DAA, the amount of labeling was reduced, and most labeling was in microbodies and endoplasmic reticulum.

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