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J.E. Varner

See the attached summary of our work on in vitro xylogenesis and the preprint on "Hydrogen Peroxide and Lignification." One paper is in press: Cooper, J.B., J.E. Heuser and J.E. Varner, "3,4-Dehydroproline Inhibits Cell Wall Assembly and Cell Division in Tobacco Protoplasts". Plant Physiol., In Press, 1993.

During the period 1 June '93 through 31 May '94 we plan to continue the work on in vitro xylogenesis (this is Zheng-hua Ye's Ph.D. Thesis research) and the simple approaches described in our 1990 research proposal for the determination of the mechanical properties of the walls of cells of different tissue types (this is J.E.V's principal research effort).

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Project Description
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Plant Cell Wall Architecture

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Our previous work showed that cell wall proline-rich proteins (PRPs) and glycine-rich proteins (GRPs) accumulate in the cells which are lignified in several dicot species. Since xylem tissue includes the main cell types which are lignified, we are interested in gene expression of GRPs and PRPs, and other proteins which are involved in secondary cell wall thickening during xylogenesis. Xylogenesis is a dynamic process which involves hormonal induction, microtubule reorganization, microtubule-orientated secondary cell wall thickening, lignin deposition and finally, breakdown of cytoplasm. Since the main feature of xylogenesis is the deposition of additional wall components, study of the mechanism of xylogenesis will greatly advance our knowledge of the synthesis and assembly of wall macromolecules.

We are using the *in vitro* xylogenesis system from isolated *Zinnia* mesophyll cells. Our first goal was to isolate genes which are specifically expressed during xylogenesis. We have used subtractive hybridization methods to isolate a number of cDNA clones for differentially regulated genes from the cells after hormonal induction. So far, we have partially characterized 18 different cDNA clones from 239 positive clones. These differentially regulated genes can be divided into three sets according to the characteristics of gene expression in the induction medium and the control medium. The first set is induced in both the induction medium and the control medium without hormones. The second set is induced mainly in the induction medium and in the control medium with the addition of NAA alone. Two of these genes are exclusively induced by auxin. The third set of genes is induced mainly in the induction medium. Since these genes are not induced by either auxin or cytokinin alone, they may be directly involved in the process of xylogenesis.

Our experiments on the localization of H_2O_2 production reinforce the earlier ideas of others that H_2O_2 is involved in normal lignification.

Plant Cell Wall Architecture

Zheng-Hua Ye and Joseph E. Varner

Our previous work showed that cell wall proline-rich proteins (PRPs) and glycine-rich proteins (GRPs) accumulate in the cells which are lignified in several dicot species. Since xylem tissue includes the main cell types which are lignified, we are interested in gene expression of GRPs and PRPs, and other proteins which are involved in secondary cell wall thickening during xylogenesis. Xylogenesis is a dynamic process which involves hormonal induction, microtubule reorganization, microtubule-orientated secondary cell wall thickening, lignin deposition and finally, breakdown of cytoplasm. Xylogenesis has been considered to be a model for the study of cell differentiation because of easy manipulation of xylogenesis *in vivo* and *in vitro*, and the simple visualization of xylem cells by staining of lignin. Since the main feature of xylogenesis is the deposition of additional wall components, study of the mechanism of xylogenesis will greatly advance our knowledge of the synthesis and assembly of wall macromolecules, and cell wall architecture in general.

We have established *in vitro* xylogenesis system from isolated *Zinnia* mesophyll cells. The mesophyll cells isolated from 2-week-old *Zinnia* leaves are incubated in the induction medium containing 0.5 μM of 1-naphthaleneacetic acid (NAA) and 0.5 μM benzyladenine (BA). After 48h culture at 27°C, weak secondary wall thickening is visualized in 10% of cells. After 60h culture, about 60% of cells show strong secondary wall thickening, and lignin deposition in a few of cells is detectable. After 72h culture, 60% of cells become tracheary elements, and are stained an intense red color by phloroglucinol. The cells cultured in control medium without hormone or with either hormone alone do not show any differentiation.

Our first goal was to isolate genes which are specifically expressed during xylogenesis. The *in vitro* xylogenesis system from isolated *Zinnia* mesophyll cells provides an ideal tool for this goal since the starting materials are homogeneous cells composed of palisade cells and spongy mesophyll cells without tracheary elements, and the differentiation to tracheary elements is semi-

synchronous with high frequency. We have successfully used subtractive hybridization methods to isolate a number of cDNA clones for differentially regulated genes from the cells after hormonal induction. So far, we have identified 18 different cDNA clones from 239 positive clones. RNA gel blot results indicated that these differentially regulated genes can be divided into three different types according to the characteristics of gene expression in the induction medium and the control medium. The first type of genes are induced in both the induction medium and the control medium without hormones. Presumably the expression of these genes is regulated by either mechanical wounding, stress conditions, or culture medium components. The significance of the induction of these genes for tracheary element formation is unknown. It is known that many chemical and physical factors other than plant hormones, such as carbohydrates, inorganic nitrogen sources, amino acids, and osmotic pressure affect xylogenesis. One of the genes, P48h-1 is highly expressed in both young and old *Zinnia* stems, but only slightly in leaves and root. Partial sequences of these cDNAs show that two of them, P48h-114 and P48h-229 are wounding-induced genes, i.e., a pathogenesis-related protein (PR protein) and a proteinase inhibitor. The mRNAs of these two proteins are heavily accumulated in the cells cultured in the control medium without any hormone, and in the control medium with the addition of BA alone, while addition of NAA alone inhibits the mRNA accumulation of these two proteins. Hence, the mRNAs of the PR protein and the proteinase inhibitor in the cells cultured in the induction medium appear after 12h culture, then quickly diminish after 24h culture. This indicates that wounding-induced genes are turned off during xylogenesis, and this down-regulation is accomplished by auxin. Proteinase inhibitor mRNA is not detected in *Zinnia* leaves, stem, root and flower bud, while PR protein mRNA is detected in the root only probably due to the induction by soil microorganism. The second type of genes are induced mainly in the induction medium and in the control medium with the addition of NAA alone. Two of these, p48h-10 and p48h-3 are exclusively induced by auxin. The mRNAs of these two genes appear after 24h culture and continue to be present after 60h culture. The mRNAs can also be detected in *Zinnia* stem, root and flower bud, but are barely detectable in the leaves. It will be of interest to characterize further these two genes to see whether their expression is associated with

xylogenesis. The other two genes, p48h-42 and p48h-102 are expressed at low level in the control medium, and show elevated expression in the control medium with the addition of auxin alone. p48h-42 shows high level expression in the young and old *Zinnia* stems, but only slight expression in the leaves, root and flower bud. Tissue print hybridization will be used to localize p48h-42 mRNA in *Zinnia* stem. Partial sequence results indicate that p48h-102 shows high homology to sunflower anther-specific proline-rich protein. The encoded protein has a hydrophobic N-terminal signal peptide indicating that it enters the secretion pathway, a cysteine-rich N-terminal domain and a proline-rich C-terminal domain. It has been shown that the gene is exclusively expressed in the anther epidermal cells in sunflower. Our results indicate that p48h-102, an anther-specific proline-rich protein homologue, is stress- and auxin-induced. The significance of p48h-102 related to xylogenesis remains to be investigated. The third type of genes are induced mainly in the induction medium. Since these genes are not induced by either auxin or cytokinin alone, they may be directly involved in the process of xylogenesis.

According to the characteristics of xylogenesis, we can expect that four main classes of genes are expressed during this process. The first is the genes which are involved in microtubule reorganization, such as microtubule-binding proteins. The second is the genes which are involved in secondary cell wall thickening, such as xylan synthase whose activity is specifically associated with xylem differentiation. The third is the genes which are involved in lignification. The fourth is the genes which are involved in the breakdown of the cytoplasm, such as DNase, RNase and proteinase. At present, we have cloned 6 different cDNAs whose expression is induced mainly in the induction medium. Partial sequence results indicate that two of these, p48h-107 and p48h-17 are adenylate kinase and cysteine proteinase, respectively. RNA gel results show that adenylate kinase mRNA is low in *Zinnia* organs, while it is highly accumulated in the cells cultured in the induction medium after 48h culture. This coincides with the onset of secondary cell wall thickening, and the accumulation is maintained at the same level through 60h of culture. Adenylate kinase mRNA is low at 36h although some cells are actively dividing. In the pathway to the synthesis of phenylalanine, the substrate for the biogenesis of lignin, ATP is consumed for the production of

phosphoenolpyruvate and as a result AMP is produced. Since 60% of the cells are converted into tracheary elements, and lignin production must be predominant after 48h culture, we expect that AMP must be generated rapidly. Therefore, the high level of expression of adenylate kinase may be required to convert AMP to ADP. Cysteine proteinase gene is highly expressed from 48h to 60h culture. The quick appearance of its mRNA at 48h indicates that cellular contents are being degraded at 48h. Indeed, the cytoplasm is being cleared visually after 48h culture, especially chloroplasts are disappearing.

The cDNA clones described above are only a small portion of the subtracted library, but they have already provided an insight into this dynamic process. Further work will continue the identification of differentially regulated genes, analysis of some of the more interesting genes by tissue and cellular localization of mRNAs and their proteins, and assignment of function to these genes. The results of these experiments will advance our knowledge of the synthesis and assembly of wall macromolecules during xylogenesis.

Hydrogen Peroxide and Lignification

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Abstract

The production of hydrogen peroxide in plant tissue is demonstrated in minutes with a simple histochemical test. The test solution, 50 mM potassium iodide in a 4% (W/V) potato starch suspension, is applied to the cut surface of the tissue to be tested. Hydrogen peroxide oxidizes iodide ions to iodine; the iodine is complexed by the starch to form a blue-purple color. This test detects hydrogen peroxide production in cells undergoing lignification, i.e. tracheary elements and phloem fibers, and in epidermal cells. In addition there is a rapid production of hydrogen peroxide in crushed cells. The test is negative under 1) anaerobic conditions and 2) in the presence of catalase.

Abstract

The production of hydrogen peroxide in plant tissue is demonstrated in minutes with a simple histochemical test. The test solution, 50 mM potassium iodide in a 4% (W/V) potato starch suspension, is applied to the cut surface of the tissue to be tested. Hydrogen peroxide oxidizes iodide ions to iodine; the iodine is complexed by the starch to form a blue-purple color. This test detects hydrogen peroxide production in cells undergoing lignification, i.e. tracheary elements and phloem fibers, and in epidermal cells. In addition there is a rapid production of hydrogen peroxide in crushed cells. The test is negative under 1) anaerobic conditions and 2) in the presence of catalase.

Hydrogen peroxide is an actor in a variety of phytochemical roles (8). In this paper we focus on the tissue-specific localization of hydrogen peroxide production, its apparent involvement in lignification and its rapid production in response to wounding. Lignification occurs through a series of enzymatic steps, starting with a phenylalanine ammonia-lyase catalyzed reaction to produce lignin precursors, and terminating with a step that most probably requires H_2O_2 and a cell-wall-bound peroxidase to bring about polymerization of the C_6-C_3 units into lignin (15). It is possible that any of the steps in the lignin pathway would be useful for detection and localization of the process in living tissue. Indeed, simple assays for localization of a peroxidase and beta-glucosidase are available to detect possible zones of lignification in fresh-cut tissue, but a histochemical test for H_2O_2 could serve as one of the best markers of the lignification process since it is thought to be involved in the final step of polymerization.

In addition to being necessary for lignin formation, H_2O_2 may serve as an immediate mechanism for disease resistance in response to elicitors (4) and may play an important role in wounding response (6). In elicitor signal transduction studies on cultured cells of soybean, exogenously added marker compounds were destroyed by rapidly stimulated H_2O_2 evolution in conjunction with endogenous cell wall peroxidases. H_2O_2 was also postulated to be a messenger employed in signaling other pathogen resistance mechanisms such as phytoalexin production (4). In studies of wound response, certain proteins disappeared more rapidly from the electrophoretic profiles of the extracts of wounded *Phaseolus* stems receiving an exogenous application of H_2O_2 than in profiles of the extracts of untreated wounded stems (6). In contrast, addition of catalase to stem wounds delayed the disappearance of the electrophoretically detected proteins (6). This disappearance was presumed to be due to insolubilization due to oxidative cross-linking.

Despite diverse and important roles proposed for H_2O_2 in plants, histochemical applications of the peroxide spot test (8) have been rare other than those that include polyamine substrates for the detection of H_2O_2 produced by polyamine oxidase catalyzed reactions (17,21). Furthermore, only two other studies (3,18) examined fresh, undigested tissue. In this report we apply starch-KI reagent to fresh cut sections of stem and root tissue in order to examine the process of lignification and the production of H_2O_2 induced by wounding.

Materials and methods

Hydrogen peroxide localization and lignin staining

Presence of H_2O_2 in living tissue was assayed with an aqueous solution of potato starch (4%, w/v) and potassiums iodide (0.10M). Lower concentrations (50mM) of KI yielded qualitatively similar results; however, higher concentrations permitted greater contrasts for photography while appearing to do no damage to tissue. Reagents were dissolved in water and heated to boiling; this colorless solution was allowed to cool to room temperature before application to fresh hand-cut sections (500-1000 μ thick). Localization of H_2O_2 is indicated by the development of dark starch-I_2 complexes on the cut surface. This same reaction has been utilized as an organic analysis test for peroxides (9) and in the localization of H_2O_2 produced by polyamine oxidases in chick-pea, jack bean, maize, and oat tissue (3,17,18,21).

Theoretically I^- can be oxidized by other endogenous electron acceptors, for example the Fe^{+++} of phytoferritin (20). Therefore, several controls were used to evaluate this H_2O_2 assay. 3 μL aliquots of catalase (from Bovine liver, 105 mg protein/ml, 58,000 units/mg, 2X crystalline, suspension in water containing 0.1% thymol, (Sigma Chemical Co.) and 3 μL aliquots of 0.1% (w/v) thymol were mixed in separate 200 μL aliquots of

starch-KI and applied to different tissue sections. Additionally, fresh sections coated with only starch-KI reagent were placed in anaerobic conditions (a N_2 glove bag purchased from I^2R in Cheltenham, Penn., U.S.A.) and observed for indications of H_2O_2 production. Control sections in water were also prepared for comparison.

Lignin was detected with phloroglucinol in 20% (v/v) HCl (16). Staining for lignin can be done on the same section on which H_2O_2 has been assayed. Before applying the acidified phloroglucinol, the starch I_2KI is removed and/or reduced to colorless starch-KI with 0.1M ascorbic acid.

Enzyme localization

Peroxidases were localized in fresh-cut tissue sections by adding a few drops of paraphenylenediamine solution (0.02M in citrate buffer pH 4.5 containing 0.012% (v/v) H_2O_2) to the section. Indoxyl beta-d-glucoside (0.0816M indican dye in acetate buffer pH 6 was used to detect beta-glucosidase (10).

Developmental study

A series of sections was made from the apical, mid, and basal internodes of seven-week old *Zinnia* plants. In each internode, a cross-sectional cut was made, and then a cross section of tissue was taken from both sides of that cut. This yielded a pair of mirror-image sections to represent each internode. For each of the three pairs of sections, one face was stained with para-phenylenediamine and the mirror-image face was stained first with starch-KI. After the later section was photographed, it was washed with ascorbic acid (to reduce the I_2 to the colorless KI) and placed in a drop of phloroglucinol reagent to be photographed again. Two more adjacent sections were also cut at each internode and were stained either with indican dye or placed in pure H_2O as a control.

Wounding

In some sections several layers of cells were crushed under the unsharpened end of a razor blade in order to study the association of H_2O_2 with the wounding response. The region of crushed tissue extended from the epidermis into the pith parenchyma. Comparative observations were made between crushed and unwounded cells in the rate of H_2O_2 production. Effects of anaerobic conditions and the presence of catalase were tested on wounded tissue using the protocols described above.

Plant materials

Plant materials were collected from the campus and greenhouse at Washington University, St. Louis, Missouri. *Zinnia elegans* L. var. Peter Pan was used in all of the protocols described above.

Results

Starch-KI is a useful histochemical assay for H_2O_2

Zinnia sections treated with starch-KI only and sections treated with 0.1% thymol and starch-KI showed a sharply-localized darkening in the xylem and phloem fibers within five to thirty minutes after start of treatment. Sections soaked in water before application of starch-KI showed a similar pattern although time required for color appearance was increased. In contrast, other control sections (those soaked in water without starch-KI reagent, those treated with catalase and starch-KI, and those treated with starch-KI but held under anaerobic conditions) exhibited little or no oxidation of the starch-KI reagent (see Fig. 1A and 1C). When sections covered with reagent were placed in the N_2 glove bag, the oxidation of the starch-KI proceeded at the same rate as oxidation under aerobic conditions for about two minutes. After two minutes oxidation of the substrates stopped, whereas under aerobic conditions it continued. Also, sections

held in the anaerobic glove bag for five minutes prior to application of the starch-KI reagent developed no color. Because catalase facilitates degradation of H_2O_2 and in our experiment catalase inhibited the fresh tissue from oxidizing the reagents, we concluded that the starch-KI solution serves as a useful assay for H_2O_2. Similar effects of catalase and anaerobic conditions were observed using wounded tissue sections.

The developmental story

At young stages localization of $H_2O_2$and lignin staining showed a closely correlated distribution in the vascular bundles; however, lignin occurred in the cell walls whereas H_2O_2 was detected in the lumen and appeared more highly concentrated adjacent to the cell wall (see Fig. 1A through ID). Additionally, within the same section, difference in the amount of H_2O_2 produced by neighboring vascular bundles is evident (see Fig. 1C). In later stages H_2O_2 detection occurred only in the younger portions of vascular bundles and in phloem fibers (see also Fig. 1D), whereas lignin was detected throughout vascular bundles and phloem fibers.

In the developmental stages and from those sections (stained with paraphenylenediamine and indoxyl beta-D-glucoside but not pictured) essentially a one to one correspondence in staining for peroxidase and H_2O_2 occurred in the xylem and phloem fibers. At the youngest state the H_2O_2, peroxidase, and beta-glucosidase distributions also correlated with that of the lignified xylem. However, in older stages (especially in the most mature stage) staining for peroxidase, beta-glucosidase, and H_2O_2 seemed most intense in the youngest metaxylem and phloem fibers, indicating that the primary xylem had reached full maturity and was no longer producing H_2O_2, while younger xylem and phloem fibers were still maturing and lignifying (see Fig. 1D). Some differences in the distribution of the stains appeared when examined at a finer resolution. Beta-glucosidase and

peroxidase stained in the cell walls; in the xylem beta-glucosidase was prominent in walls of the bands of large vessel elements while peroxidase appears equally whatever the xylem cell size.

The rates of accumulation of H_2O_2 in living tissue were estimated by comparisons of color intensity with known concentrations of H_2O_2 spotted on filter paper. Two μl aliquots of H_2O_2, ranging in concentration from 10 to 100 μm were spotted onto Whatman #3 (qualitative) filter paper. A thin layer of starch-KI was spread over these. The range of responses was evaluated; intensities were compared to those seen in fresh sections. By such comparisons the rate of production of H_2O_2 can be estimated.

Wounding response

As shown in Fig. 1E and 1F crushed cells showed a highly localized oxidative burst in the zone of damaged cells from the epidermis into the cortex. With catalase present or when wounding was done under anaerobic conditions the dramatic oxidation of the starch-KI regent on wounded tissue did not occur.

Discussion

H_2O_2 detection and lignification

The results presented here agree with the proposal of Gross *et al.* (14 and Goldberg *et al.* (12) that H_2O_2 is involved in the lignification process; indeed, we believe the histochemical test for H_2O_2 using starch-KI can serve as a simple method for localizing zones of ongoing lignification. Our results also confirm (in an additional taxon, i.e. Taxus ____,) the association of beta-glucosidase and peroxidase with lignification as demonstrated by Freundenberg *et al.* (10). Moreover, this test adds further support to the proposed role of H_2O_2 in wounding and disease responses (1,4,6). Applications of this histochemical test for H_2O_2 may extend

beyond localization of lignification, induc ed oxidative bursts and detection of polyamine oxidases, since other biological processes also involve H_2O_2 formation. H_2O_2 has been implicated in lignin degradation (1,19). Peroxidase activity has been suspected in auxin catabolism (13) and in *Medicago* root nodule cytosol (5). Oxidative coupling reactions of ferulate groups have been proposed as a mechanism to cross-link polysaccharides in cell walls (11). The proposed histochemical test for H_2O_2 has yet to be tested in association with these processes.

A different histochemical assay for H_2O_2 using guaiacol (12) has been utilized for assessing cell wall activities related to the final stages of lignin biosynthesis. But guaiacol also requires presence of peroxidase and conditions conducive for activity of that enzyme. The starch-KI assay on the other hand is specific when confirmed with added catalase and does not require peroxidase to detect H_2O_2. It has further advantages of low toxicity and low cost. However, as with the other possible H_2O_2 assays, a negative test result is difficult to interpret. Absence of substrate oxidation could result from several different scenarios. First, H_2O_2 production might be in fact absent in tissue. Second, endogenous catalase may be decomposing the H_2O_2 in tissue as quickly as it is evolved. Indeed, catalase inhibitors such as aminotriazole and assays to localize catalase can partially test this alternative; however, not all forms of catalase are inhibited by aminotriazole and negative results from histochemical tests for catalase are also difficult to interpret. Third, a negative test could result from the presence of some endogenous reductant(s) such as ascorbic acid which could reduce H_2O_2 and/or I_2 back to colorless KI before enough of the complex had accumulated to be detected. A combination of the last two scenarios is also a possibility. Therefore, the starch-KI histochemical reagent is not likely to give false positive tests but could give false negative tests.

Wounding

The detection of increased H_2O_2 production in response to wounding in intact tissue finds possible consonance with a recent paper describing a H_2O_2-mediated oxidative crosslinking of proline-rich cell wall proteins in soybean cell cultures (6). In that study a rapid increase in the oxidative cross-linking occurred in response to stress, fungal elicitors, and glutathione (1mM). Perhaps a similar response occurs in the crushed regions of the *Zinnia* thick sections.

Furthermore, in or tests if malate (10mM) in glutamate buffer (pH 5, 50mM) accompanies the starch-KI reagents on wounded tissues, an accelerated oxidative burst occurs in association with the wounded tissue (data not shown). This response is roughly three times greater than the the oxidative burst seen on wounded sections in buffer (pH 5) and starch-KI reagents *without* malate. This finding will need further investigation, but it is especially interesting in light of the theory outlined by Gross *et al*. (13) that suggests a cell-wall-bound malate dehydrogenase provides NADH for the reduction of O_2 to produce the H_2O_2 involved in the coupling of extensin and lignin subunits. Interestingly, our tests fail to detect any effect of malate on H_2O_2 production in zones of lignification of fresh unwounded tissue. And at pH 4 and lower, H_2O_2 appears in large quantities throughout the section. More experiments will be needed to determine if malate and/or highly acidic conditions actually increase H_2O_2 production or somehow impede normal cell processes that continuously eliminate H_2O_2. In this connection we note that <u>in vitro</u> ascorbate is a reversible inhibitor of catalase (7).

Other applications of the starch-KI reagent

The relative ease of iodine oxidation and reduction makes other applications of the starch-KI reagent potentially useful. For example, the

interference by reductants of this H_2O_2 assay has potential applications in providing another histochemical assay for reductants. As an assay for reductants like ascorbic acid, if starch-KI substrate is spread thinly on a slide and oxidized (by blotting with filter paper moistened with very dilute H_2O_2), then endogenous reductants in tissue sections will be indicated by the appearance of clear zones in the thin matrix of reagent. In *Taxus*, preliminary trials of the proposed test showed a distribution of reductants correlating with a distribution indicated by an assay for reductants involving the reduction of $AgNO_3$ (22).

Other preliminary experiments indicate the starch-KI reagent can also be utilized for detection of catalase. Tissue sections were covered with a 0.3% (v/v) H_2O_2 solution for 45 seconds and then blotted with tissue to remove most of the excess. Then 30 seconds were allowed to pass before the starch-KI reagent was applied. The characteristic blue color developed on the surface of the sections in reproducible patterns. The major drawback of this assay is that strong reductants can give a false positive result.

To conclude, application of this simple histochemical test for H_2O_2 has already provided further evidence of the role of H_2O_2 in lignification and wound response. In the future we hope use of the assay and variants of it may illuminate other areas of plant chemistry in which H_2O_2 is believed to play a role. It also has great potential as a teaching tool to illustrate basic biochemistry involving activated oxygen species.

Acknowledgements

We thank Zheng-Hua Ye and Anjana Raina for helpful discussion and Mike Dyer for care of the plants.

Figure 1. Cross-sections of Forsythia petioles stained with

(A) Starch-KI (H_2O_2)

(B) Phloroglucinol (lignin)

(C) Toluidine blue (anatomy)

Figure 2. Cross-sections of Zinnia stems with various treatments (phloem fibers, P; and xylem, X).

(A) Control section in water (20X)

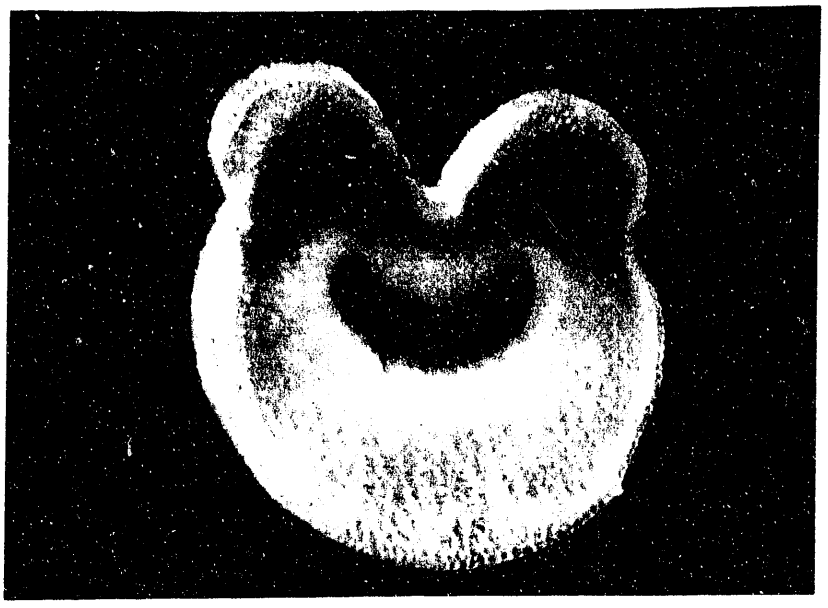
(B) Section stained with phloroglucinol to show lignin (20X)

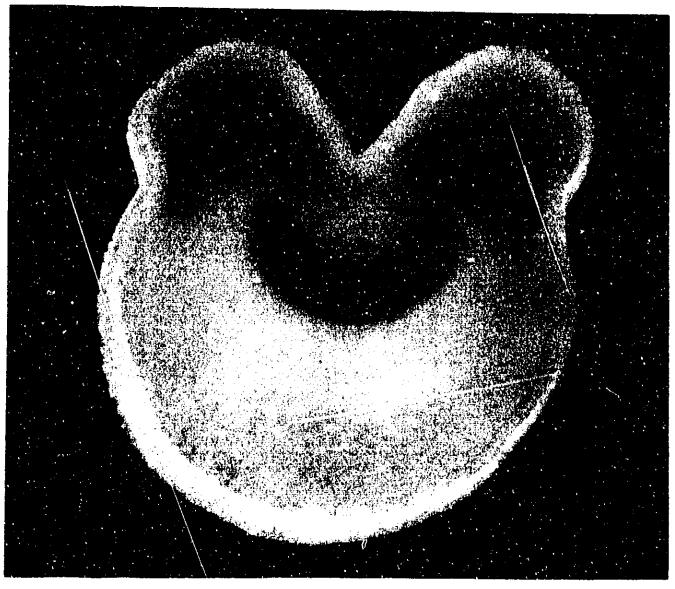
(C) Section stained with starch-KI reagent showing H_2O_2 production in phloem fibers and xylem--especially the outermost (and youngest) metaxylem (20X).

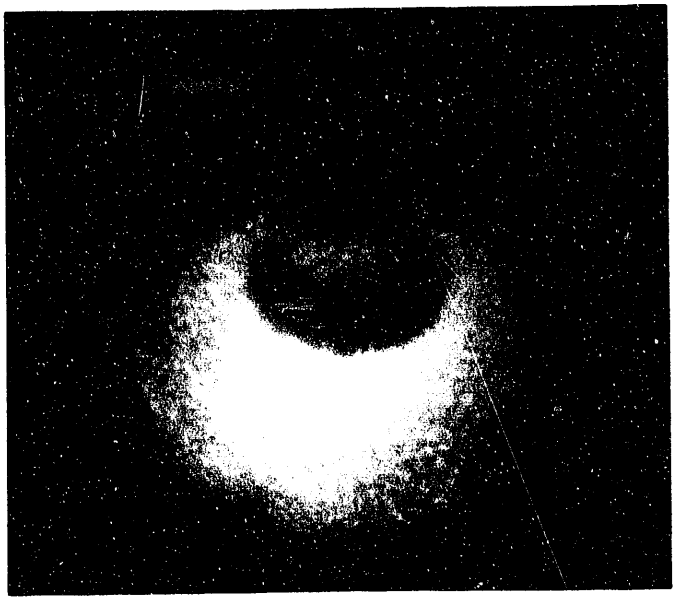
(D) Higher magnification showing starch-KI detected H_2O_2 production in individual cells (70X).

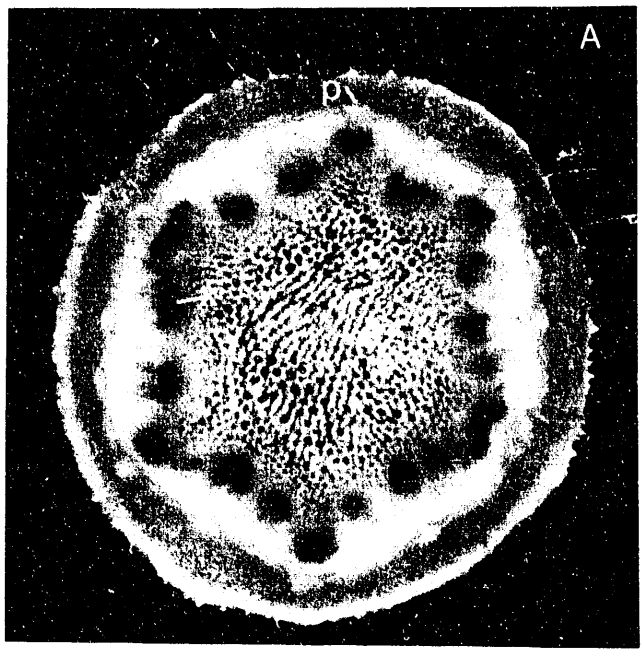
(E) Section from a relatively younger node, wounded with the blunt end of a razor blade, and stained with starch-KI reagent (20X)

(F) Same section and treatment but at higher magnification (60X)





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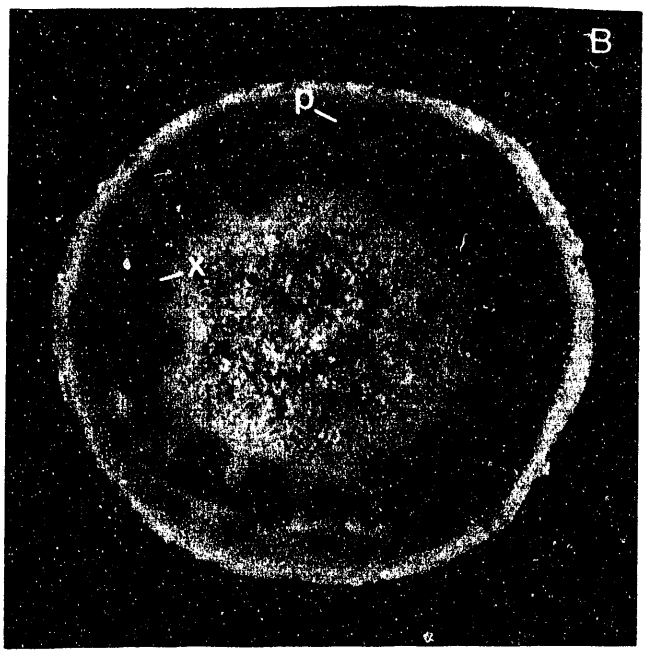
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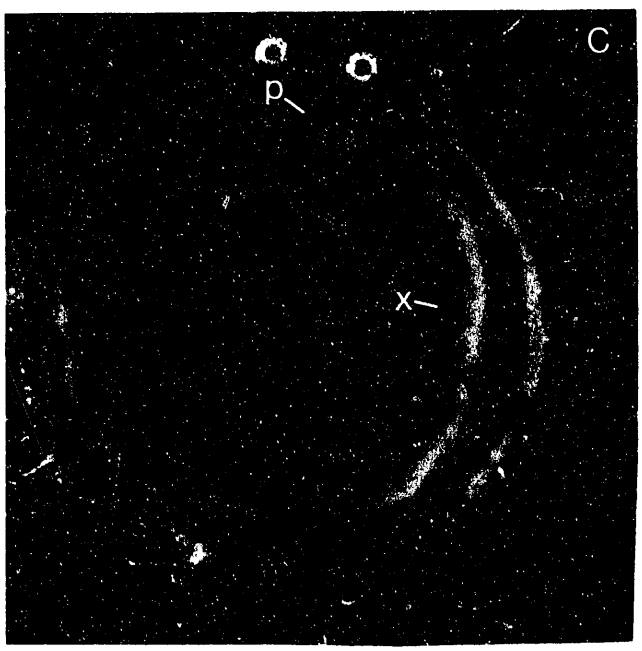
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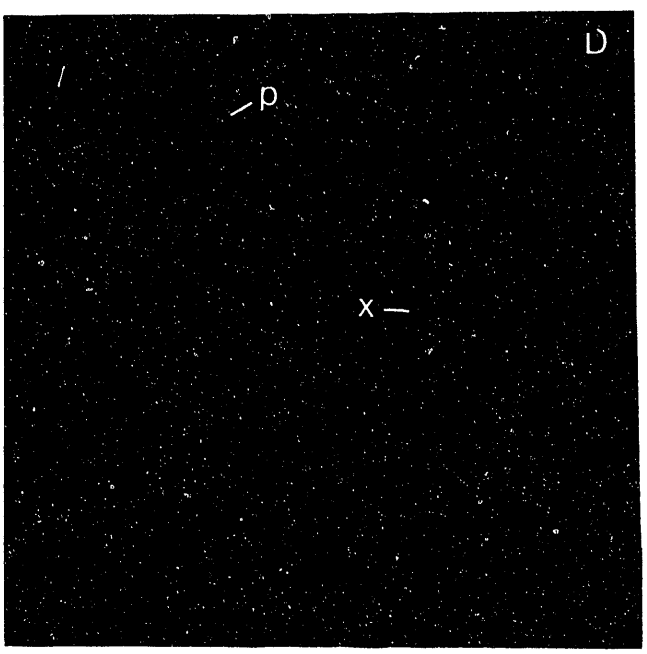
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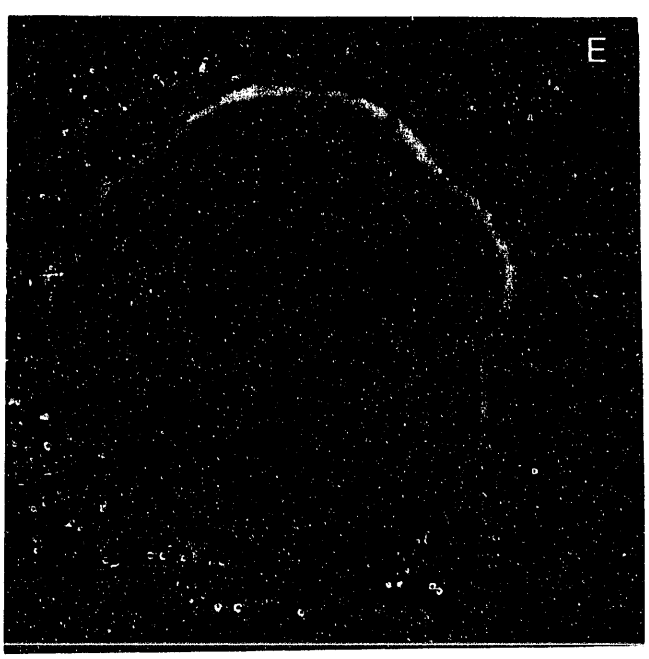
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