JOINT FAO/IAEA DIVISION

OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE

REPORT OF SECOND FAO/IAEA

RESEARCH COORDINATION MEETING ON

IN VITRO TECHNIQUES FOR SELECTION OF RADIATION-INDUCED MUTANTS ADAPTED TO ADVERSE ENVIRONMENTAL CONDITIONS

15-19 April 1996

Cairo, Egypt

IAEA 312.D2.RC.545
Working Material Reproduced
by the IAEA
Vienna, Austria
(July 1997)

JOINT FAO/IAEA DIVISION

OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE

REPORT OF SECOND FAO/IAEA

RESEARCH COORDINATION MEETING ON

IN VITRO TECHNIQUES FOR SELECTION OF RADIATION-INDUCED MUTANTS ADAPTED TO ADVERSE ENVIRONMENTAL CONDITIONS

15-19 April 1996

Cairo, Egypt

IAEA 312.D2.RC.545
Working Material Reproduced by the IAEA
Vienna, Austria
(July 1997)

JOINT FAO/IAEA DIVISION
OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE

WAGRAMERSTRASSE 5, PO BOX 100, A-1400 VIENNA, AUSTRIA
FAX: (+43) 1-20607 TELEPHONE: (+43) 1-2060

EDITORIAL NOTE

In preparing this document for press, staff of the IAEA have made up the pages from the original manuscripts as submitted by the authors. The views expressed do not necessarily reflect those of the governments of the nominating Member States or of the nominating organizations.
Throughout the text names of Member States are retained as they were when the text was compiled.
The use of particular designations of countries or territories does not imply any judgement by the publisher, the IAEA, as to the legal status of such countries or territories, of their authorities and institutions or of the delimitation of their boundaries.
The mention of names of specific companies or products (whether or not indicated as registered) does not imply any intention to infringe proprietary rights, nor should it be construed as an endorsement or recommendation on the part of the IAEA.
The material in this document has been supplied by the authors. The authors are responsible for having obtained the necessary permission for the IAEA to reproduce, translate or use material from sources already protected by copyrights. In preparing this material for the press, staff of the International Atomic Energy Agency have retyped the majority of the manuscripts and made corrections in English.

CONTENTS

FOREWORD

This working material is a collection of papers presented at The Second Research Coordination Meeting of the FAO/IAEA Coordinated Research Programme on "*In vitro* techniques for selection of radiation-induced mutations adapted to adverse environmental conditions", 15-19 April, 1996, Cairo, Egypt.

This meeting was attended by scientists from nine countries: Bangladesh, China P.R., Colombia, Egypt, Ghana, India, Pakistan, Peru and United States of America. The participants in this Research Coordination Meeting are working on the improvement of potato, sweet potato, garlic, sugarcane, pineapple and alfalfa by combining *in vitro* techniques with induced mutagenesis to select for resistance to salinity, freezing, heat, drought, and water-logging depending upon adverse condition prevailing in their region. The participants reported results of their experiments on the radio-sensitivity tests on *in vitro* cultured plant material, such as micropropagated plants and organogenic or embryogenic callus cultures. In addition, reports on the modifications of culture media required to regenerate and multiply local varieties and to carry out *in vitro* selection for specific stress conditions were presented. The research progress reports included the following significant achievements:

- In Ghana, the objective is to obtain variation in pineapple for tolerance to drought and high temperature. *In vitro* irradiation of shoot cultures of pineapple with gamma rays ranging from 15 to 120 Gy showed that doses above 80 Gy were lethal. Multiple shoot buds were produced on Murashige and Skoog (MS) medium supplemented with 3.5% sucrose, 3μM/l thiamine HCl, 3μM/l naphthalene acetic acid (NAA) and varying concentrations of 6-benzylaminopurine (BAP). Shoot proliferation was best on MS medium supplemented with 20 μM/l BA.

- Irradiation of *in-vitro* grown plantlets of local potato cultivars in India, Pakistan, Egypt, and Colombia with different doses showed that 20 Gy gamma rays was optimal.

- In India, variation for tolerance to heat and resistance to late blight in potato is being investigated. *In vitro* cultured shoots and calli were irradiated with 20 and 40 Gy gamma rays and micro-tubers were obtained from irradiated shoots and soil planted to observe variation in plant morphology and tuber characters. To study variation for late blight resistance, irradiated calli were cultured on medium with filtrate of *Phytophthora infestans*. To induce variation for heat tolerance, *in vitro* shoots were mass-propagated and allowed to form micro-tubers at high temperature.

- In Egypt, induced variation is being sought for resistance to high temperature and soil salinity in potato. *In vitro* grown potato plants were irradiated with 20 to 40 Gy gamma rays, cut into single nodes and cultured on media supplemented with 2 g NaCl. Salt resistant plantlets were transferred to liquid medium supplemented with 2g NaCl/l, and micro-tubers were collected after 6 weeks from which mini-tubers were produced under the same level of salinity.

- In Pakistan, radiation induced variation is being investigated for resistance to salinity in sugarcane and potato. Sugarcane embryogenic calli were irradiated with 0, 5, 20, 40 and 60 Gy gamma rays. Irradiated and non-irradiated calli were subjected to various

levels of salt stress. Plant regeneration was inhibited on the medium containing 50 mM NaCl. Variation was detected by RAPD markers in the irradiated and salt stressed calli. The regenerants are under field evaluation. In potato, *in-vitro* grown plants were irradiated with 20 and 40 Gy gamma rays. With 20 Gy, only from 50% of the axillary buds formed shoots. The cultures were subjected to 50 to 200 mM NaCl stress, which resulted in shoot-tip necrosis and reduction in plant height.

- In China P.R., heat and drought tolerance is being studied in garlic and sweet potato. In garlic, callus proliferation and plant regeneration were inhibited with irradiation doses of 8 and 10 Gy; doses of 3 and 5 Gy were suitable to induce mutations. Somatic embryos were induced on MS medium supplemented with 2.0 mg/l 2,4-D + 500 mg/l casein, 1000 mg/l yeast extract and 5% sucrose.

- In Bangladesh, induction of variation for delayed flowering, tolerance to red-rot disease and water-logging is being studied by irradiating cuttings and callus cultures. Cuttings of local sugarcane cultivars were irradiated with 20-40 Gy gamma-rays and advanced to M_1V_4 generation, and inoculated with red-rot pathogen. Four clones with resistance and 64 with moderate resistance to red-rot disease were selected which have been grown as M_1V_5 at two locations. In addition, somaclones regenerated from callus cultures have been screened for resistance to red-rot disease and tolerance to waterlogged condition. Callus cultured were irradiated with 2 to 10 Gy gamma-rays. Shoots were regenerated from all calli except those treated with 8 and 10 Gy. The highest regeneration was from calli irradiated with 3 Gy.

- In Colombia, induction of genetic variation for resistance to frost and late blight in the local potato is being investigated. *In vitro* grown plantlets were irradiated with 25 to 100 Gy gamma rays; the optimal radiation dose was 20 Gy. The derived clones were subjected to simulated frost conditions. Fungal extracts were added to the culture medium for *in vitro* selection for resistance to late blight. The selected clones are being field tested.

- In Peru, the effect of gamma irradiation on different explants of sweet potato has been investigated to induce tolerance to salinity. Clonal lines tolerant to NaCl were obtained by combining *in vitro* culture and gamma radiation in two local varieties. The most suitable explants were pedicel sections and leaf blades and embryogenic callus was induced on basal MS basal medium containing 0.5 ppm 2,4-D. The embryogenic calli were irradiated with 5 Gy and several putative mutants were selected.

- In USA, *in vitro* selection of mutants to salt-tolerance and inducible gene regulation for salt tolerance is being studied. In alfalfa, cell lines were selected and salt-tolerant plants were regenerated. The regenerated plants were tested and were considerably more salt tolerant than the parent which is salt-sensitive. The selected plants passed on the salt-tolerance trait through seed in a semi-dominant fashion. These studies have been extended to rice using a single step *in vitro* protocol to select salt-tolerant calli, followed by regeneration and progeny-test.

The reports presented and the conclusions and recommendations drawn from the deliberations at the meeting have been compiled as the working material as a landmark of progress and source for future reference. The working material was provided by the participants in the RCM, and has been edited by Dr. B.S. Ahloowalia, Scientific Secretary, Joint FAO/IAEA, Division, International Atomic Energy Agency, Vienna and the manuscripts were retyped by Ms. K. Entekhabi.

INTRODUCTION

Stress conditions during growth and development cause major loss of crop yield and quality. Abiotic stress caused by heat, salinity, frost, water-logging and drought limit nutrient uptake and reduce metabolism which is reflected in reduced yield and quality. During the course of evolution, many plant species and land races have adapted to such adverse environments by developing mechanisms which regulate rapid osmotic changes under stress situation. Molecular techniques have shown that such regulatory mechanisms are under genetic control and in some cases can be induced to respond to the adverse change in the environment.

A number of plants such as pineapple, potato, sweet potato, sugarcane, garlic are vegetatively propagated and can be propagated through *in vitro* techniques of micropropagation and regeneration. The cell and tissue culture techniques allow production of large plant populations for mutagenesis by physical and chemical mutagens. Following mutagenic treatments, such as irradiation with gamma rays, *in vitro* cultured tissues and plants can be propagated rapidly to generate solid mutants which can be subjected to selection pressure for obtaining the desired genotypes. In some cases, the selection pressures may be applied under *in vitro* conditions to select for tolerance to stress. There is increasing evidence that pre-selection for tolerance to freezing injury, drought and salinity might be possible by exposing cell and tissue populations grown *in vitro*. Plants selected *in vitro* can later be tested under field condition. The selected clones can then be rapidly multiplied as disease-free material for release to the growers.

Considering the negative effects of various stress conditions on crop production, an FAO/IAEA Coordinated Research Programme (CRP) was established on *"In vitro* techniques for selection of radiation-induced mutations adapted to adverse environmental conditions", in 1993. The First FAO/IAEA Research Coordination Meeting of this CRP was held 11-15 April 1994, in Vienna. The Second FAO/IAEA Research Coordination Meeting was held 15-19 April 1996, in Cairo, Egypt. This meeting was attended by scientists from nine countries, Bangladesh, China P.R., Colombia, Egypt, Ghana, India, Pakistan, Peru and United States of America. This publication is a record of the meeting and has been reproduced by editing the research reports presented and discussed by the participants at the meeting.

OPENING ADDRESS

H. ALY
Chairman
Atomic Energy Authority
Cairo, Egypt

Distinguished Guests, Ladies and Gentlemen,

It is a great pleasure for me to welcome the participants in Cairo to the Second Research Coordination Meeting on "*In vitro* techniques for selection of radiation-induced mutations adapted to adverse environmental conditions". Stress caused by heat, salinity, frost, water-logging and drought reduces crop yields and impairs quality. Under extreme stress conditions crops fail completely which in turn may lead to food shortage, high food prices, migration of populations from villages and in turn to rural instability.

We in Egypt have problems of high temperature and salinity as leading stress factors, which often get accentuated with drought. The farmers know only too well the damage to their crops and their suffering when the rainfall is either not enough or not on time. One of the solutions to have a sustainable and secure food production is to breed varieties which are tolerant of stress conditions during their growth and development. Modern day technologies of plant tissue culture allow the production of large populations of plants in a short duration and on a year round basis in the laboratory. Such populations can be irradiated *in vitro* to induce mutations, multiplied and grown in the field for selection of desired genotypes. Using a combination of mutagenesis and *in vitro* techniques, new genotypes can be created in crops which are propagated from vegetative parts. Because seed of crops such as potato, sweet potato and garlic is highly variable, induction of mutations offers the possibility to produce only a limited number of desired genetic changes in genotypes and varieties which are adapted to the local eco-climatic conditions and have been grown for decades.

Using such a strategy, the Joint Division of Food and Agriculture Organization of the United Nations and International Atomic Energy Agency, created a network under the Coordinated Research Programme to stimulate research in the use of *in vitro* and mutation techniques to improve vegetatively propagated crops such as potato, sweet potato, sugarcane, pineapple, garlic which are most amenable to upgrading through such techniques. Although, the crops and stress conditions these crops suffer in various parts of the world are different, the technology of *in vitro* culture and mutation induction is common to their improvement.

During the course of this meeting, scientific reports shall highlight the progress made during the past two years when this net-work was established with the first meeting held in April 1994 in Vienna.

I, on behalf of the Atomic Energy Authority and Biotechnology Research Center welcome the participants and wish them a most successful time in their deliberations during the week. I also wish to express our gratitude to the Director General of IAEA for holding this meeting in Egypt. Thank you for your attention.

ADDRESS OF WELCOME

B.S. AHLOOWALIA
Scientific Secretary
Joint FAO/IAEA Division
International Atomic Energy Agency
Vienna

Prof. Aly, Distinguished guests, Colleagues, Ladies and Gentlemen,

On behalf of the Director General of the International Atomic Energy Agency and the Director General of Food and Agriculture Organization of the United Nations, it is my privilege to welcome you to the Second Research Coordination Meeting on "*In vitro* techniques for selection of radiation-induced mutations adapted to adverse environmental conditions". This RCM is part of the several Coordinated Research Programmes (CRP's) which we undertake to promote research and technology among the developing Member States of FAO and IAEA. Presently, we have ten such CRP's in progress.

This morning, I wish to share with you some of my thoughts not only as the Scientific Secretary of this meeting but as a plant breeder and biotechnologist.

Many civilizations around the world, among them the Mesopotamian, Egyptian, Indian and Latin American pioneered the early development of agriculture. Some, 10,000 years ago, in Mesopotamia (which is the present day Iraq, Palestine and Israel) along the rivers of Euphrates and Tigris, man domesticated cereals and perhaps lentil. This took place soon after the domestication of animals for meat and milk production. The Indus Valley civilization started cultivation of wheat, sugarcane and cotton as early as 7000 years ago, before the Aryans migrated into India. The Incas and Mayans in South America domesticated maize and potato as back as 8000 years ago. In your own country, cotton and barley were widely cultivated during ancient Egyptian civilization, dating some 7000 ago. However, as population increased, and man discovered his dependence on soil, plant and animal interaction, and human migration and trade began, not only more plants were added to cultivation, but the local ones were improved in their yield and quality. The einkorn, *T. monococcum* gave birth to the modern durum and then to the *vulgare* wheats; the two-row barley to six row types, the short-linted cotton to long-staple types, the old pod and pop type maize to flints and dents, and in sugarcane, the wild non-sugar producing species of *Saccharum spontaneum* to the modern day *S. officinarum*. However, this progress in plant improvement albeit slow was by very significant in increasing food production.

With the re-discovery of laws of heredity, the breeding and selection of plants was placed on a firm scientific footing, and plant breeding took a respectable place among the sciences. Modern day plant breeding, became based on creating genetic variation (mostly through sexual recombination), selection, evaluation, and multiplication of new varieties. In the search and creation of new varieties, only a few genes, such as those for short height and photo-insensitivity in wheat and rice, male sterility and monogerm in sugar beet, male sterility and restorer genes in maize and other crops for hybrid seed production, and disease resistance in potato have played a major role in increasing food production. The green revolution in Asia

would not have been possible but for the introduction of these genes in the local wheat and rice cultivars.

The technology of plant *in vitro* culture, which began in mid-fifties has already become a multi-million dollar business to multiply disease-free plants, particularly ornamentals such as saintpaulia, roses, orchids, gerbera, carnation. The technology is being now adopted for mass-scale propagation of potato, sweet potato, sugarcane, cassava, yams, banana, plantain, strawberry, apple, pineapple and several forest trees. This is one technology which the developing nations can utilize and benefit from in a very short duration. However, this technology is much more powerful than simply to be used for the propagation of high quality planting material. It allows us to byepass sexual process in plants for changing their genetic make-up. The para-sexual techniques of regeneration of plants from cells and calli, somatic embryos, anther and ovary culture, and from protoplast fusion allow the creation of new genotypes without going through the sexual recombination. It also allows us to irradiate thousands of date palm trees, banana, papaya, cassava, and potato in a small space under *in vitro* condition. Such plants can be then taken through the conventional process of selection and field evaluation.

The key discoveries of this Century on the structure of DNA, and to cut, rejoin and put it back into any organism, have revolutionized our concept of genes and their inheritance. The last fifty years have brought in a synthesis of old and new technologies. Biotechnology has emerged as a synthesis of genetics, microbiology and biochemistry. The technology has just started to yield the first series of new varieties based on gene insertion, what we now call as transgenesis. These include the 'Flavr savr' tomato, maize with *Bt* resistance to corn borer and resistance to Basta, cotton with resistance to boll worm beetle, squash resistant to wilt disease, potatoes resistant to Colorado beetle and with changed starch quality.

Through combination of several events, among these over-use and abuse of land and water resources, the same *Homo sapiens* who has been able to sustain its reproductive capacity, has not been able to sustain the eco-system on which he is totally dependent. Among the lands he has cultivated, he has created marginal lands, often through mis-management. These include flood basins and desertification caused by de-forestation, soil salinity by irrigation, disappearance of organic matter and soil erosion through lack of proper crop rotation.

Increasing world population cannot be sustained if the equation of food production and human reproduction cannot be placed in equilibrium and harmony. It is going to require the use of marginal land systems and increase productivity from the existing resources. Whereas other components such as land tenure system, rural credit, availability of agronomic inputs such as fertilizer, agro-chemicals, irrigation, crop rotation all are essential to sustain adequate food production, a key component is the seed and the planting material for which all the inputs are applied. An improved seed variety, suited to a particular environment and management is the key to increased production and quality of the crop.

We in the Joint FAO/IAEA Division have the mandate to use nuclear techniques in the improvement of agriculture. To this end, we promote the research and development of genotypes which can produce better and assure production under adverse conditions. To achieve this goal, we have chosen the use of irradiation to create new genotypes by producing mutants of the established cultivars. The technology of mutation induction coupled with *in vitro* tissue

culture techniques and biotechnology is a rapid and fast method to obtain the desired types.

During the next five days, scientists from 9 countries shall present their findings on the research they have carried out under the initiative started two years ago. Some of the developments in creating novel genotypes look promising while others are still at an exploratory stage.

I extend a warm welcome to all of you to attend and participate in this meeting.

DEVELOPMENT OF SALT TOLERANT POTATO AND SUGARCANE THROUGH *IN VITRO* TECHNIQUES

S. ASAD, M.J. IQBAL, M. SAIF-UR-RASHEED,
Y. ZAFAR, K.A. MALIK
Plant Biotechnology Division
National Institute of Biotechnology and Genetic Engineering
Faisalabad, Pakistan

Abstract

Improvement of sugarcane and potato in Pakistan is hampered by their intricate flowering behaviour under natural day-length conditions. The improvement of these crops for their salt tolerance can be carried out by tissue culture mediated techniques. To induce variation in sugarcane, five-week old white to yellow nodular embryogenic calli were irradiated with 5, 20, 40 and 60 Gy gamma rays. After one month, the calli were cultured on regeneration media, and plant hardening procedures were optimized. Irradiated and non-irradiated calli were subjected to various levels of salt stress and plant regeneration was investigated. Although growth of sugarcane calli was observed at 200 mM NaCl, regeneration was inhibited even at 50 mM NaCl in the medium. The regenerants from gamma irradiated material are under field evaluation. Variation was detected in both irradiated and salt treated calli by DNA fingerprinting using random amplified polymorphic DNA (RAPD) markers.

In potato, 6-7 weeks old *in vitro* grown plants with single shoots having 8-10 buds were irradiated with 20 Gy gamma rays. Shoot formation was successful only from 50% of the axillary buds. The cultures were subjected to four levels of salinity (50, 100, 150 and 200 mM NaCl). Shoot-tip necrosis was observed along with significant reduction in shoot height.

1. INTRODUCTION

Sugarcane and potato are the most important cash crops in Pakistan. Pakistan produces more than 45.7 million tonnes of sugarcane from about 1,009,000 hectares of land while potato production has crossed the figure of 1.14 million tonnes from an area of 85.4 thousand hectares [3]. Improvement in both of these crops through breeding is handicapped because of the complex flowering behaviour under natural day-length conditions in Pakistan. Tissue and cell culture techniques provide new methods for deriving genetic variation in relatively shorter duration. The use of plant cell culture techniques has expanded greatly particularly in the improvement of vegetatively propagated crops like sugarcane and potato. In these crops genetic variation can be introduced through somaclonal variation [1,4,5,8,11]. It has been reported that *in vitro* irradiation and other mutagenic agents increase variation and the level of stress tolerance among regenerants [2]. Polymorphisms in various agronomically important crops by random amplified polymorphic DNA (RAPD) markers have been extensively studied [6,10,13]. Potato and sugarcane are extremely salt sensitive crops, and soil salinity is a major constraint in the production of these crops in Pakistan. Keeping in view this situation, radiation induced mutations coupled with tissue culture based selection protocols were adopted for the development of salt tolerance in these crops. The present study was carried out to determine the effect of gamma radiation and salinity on callus growth and regeneration in sugarcane and

growth of micropropagated plants in potato. Studies were also undertaken to determine genetic changes in the callus caused by gamma irradiation and salt treatment by using RAPD markers.

2. MATERIALS AND METHODS

Plant material

Sugarcane plants, cv. 'CP-43/33' (a hybrid of 'Col-54'), were obtained from Sugarcane Research Institute, AARI, Faisalabad. Young leaves taken from the inner most whorl were used for callus induction.

Micro-shoot of a potato cv. 'Cardinal' initiated from meristem tip culture, were obtained from Virology Section, Ayub Agriculture Research Institute (AARI), Faisalabad. Micropropogated plants were obtained by *in vitro* multiplication of these microshoots.

Culture initiation

Callus cultures were initiated from the explants excised under aseptic condition from the innermost apical stem of the field grown sugarcane cv. 'CP-43/33'. Explants were sliced into 3-4 mm pieces with sterilized tools. The explants were transferred to 100 ml plastic jars (Sigma) containing 20 ml of callus induction medium (Table I). The induced calli were maintained and proliferated on the same medium by serial transfer after 2-3 weeks. Cultures were incubated in dark at 25±2°C.

TABLE I. COMPOSITION OF MEDIUM USED FOR INDUCTION SUGARCANE CALLUS

Ingredients		Quantity mg/l
1.	MS-Macro and Micronutrients	-
2.	Vitamins	
	■ Myo-inositol	100
	■ Casein hydrolysate	500
	■ Cysteine- HCl	30
	■ Nicotinic Acid	1
	■ Thiamine HCl	10
	■ Pyridoxin HCl	1
3.	Other supplements	
	■ Coconut milk	10% (v/v)
4.	Amino Acid	
	■ Arginine	50
5.	Sucrose	30 (g/l)
6.	Phytagel	2.5 (g/l)
	pH before autoclaving	5.8

In vitro micropropagation of potato was performed by nodal fragmentation [1] on modified MS media [9] and incubated at 25±1°C under light intensity of 2500 lux and photoperiod of 16 hours.

Gamma irradiation

Five weeks old yellow to white nodular embryogenic calli were irradiated with five doses of gamma rays from a ^{60}Co-source at NIAB. About 60-70 calli were exposed to each radiation dose (0, 5, 20, 40 and 60 Gy). Irradiated and non-irradiated materials were maintained and proliferated by sub-culturing after every 3-4 weeks.

Six weeks old microshoots of potato were irradiated with gamma rays from a ^{60}Co gamma source with a dose of 20 Gy.

Salt treatment

For salt treatments, 4-5 weeks old embryogenic calli of sugarcane were subjected to sodium chloride (NaCl) stress. Seven equal pieces (128±10 mg) of calli were placed on callus induction medium supplemented with various concentration of NaCl (0, 50, 100, 150 and 200 mM) on petri dishes (180 mm). Five replicates were used in each treatment. Sub-culturing was performed after every 2 weeks.

To study the effect of salt (NaCl) on bud culture of potato, axillary bud segments were placed in 250 ml conical flasks containing 25 ml modified MS liquid medium [9] supplemented with different levels of salt (0, 50, 100, 200 mM NaCl). Eight flasks for each salt level were used with ten axillary buds/flask. Flasks were maintained for one month on an orbital shaker at 100 rpm at 25±1°C under 16 hours photoperiod.

Regeneration studies

Four week old post-irradiated and non-irradiated calli were transferred to four regeneration media with following composition:

a) MS0 (Hormone free) + 500 mg l^{-1} casein hydrolysate + 30 mg l^{-1} cysteine HCL (MS0)
b) MS0 + 2 mg l^{-1} Kinetin + 5 mg l^{-1} IAA.
c) MS0 containing 6% sucrose (MS06S).
d) MS06S + 2 mg l^{-1} kinetin + 5 mg l^{-1} IAA.

Four callus pieces (0.5-1.0 g) were placed in each glass bottle containing 50 ml of regeneration medium. Jars were kept at 25±2°C with 16 hr photoperiod in a controlled growth room. For root induction, MS0 + 500 mg l^{-1} casein hydrolysate + 50 mg l^{-1} Arginine + 5 mg l^{-1} NAA was used.

DNA isolation and PCR amplification

DNA was isolated from sugarcane calli as described in a previous report [6]. The purified DNA after RNase treatment was diluted in sterilized distilled water at a conc. of 12.5 mg/ml and 50 mg of it was used in each PCR amplification. Random primers (10 nucleotide

long) obtained from Operon Technologies Inc. Alameda, CA, USA were used for amplification. The amplification conditions and PCR product analysis was done as mentioned in a previous report [6].

3. RESULTS AND DISCUSSION

In vitro culture studies

Callus initiation and proliferation in dark produced yellow to white nodular tissue at the cut edges after 2-3 weeks. Callus kept in dark when transferred to light, turned pink brown in most of the cases. However, in some calli, green pinheads developed, which differentiated into shoots [7,8]. The change in colour on transfer from dark to light even in the presence of cysteine-HCl might be due to physical effect of light or some other chemical changes [8]. Cysteine-HCl is known to inhibit the release of phenolic compounds from tissue into medium. To minimize the browning of calli, activated charcoal and polyvinylpyrrolidone (PVP) was used; however, no significant effect was observed.

Regeneration studies

It was observed that different media affected the regeneration frequency of sugarcane calli (Table II). Of the media tested, MSO6S was the best and used for routine regeneration. The calli developed green pin dot like regions which later on differentiated into shoots. The regeneration was also affected by increase of radiation dose. Although, a few regenerants were obtained from 60 Gy irradiated calli, they failed to establish in soil. A limited number of regenerants were established in soil from 20 and 40 Gy treatments. There was no significant difference between control and 5 Gy treatments in regeneration and survival frequency. Hence, the experiment were repeated to regenerate plants from 20 and 40 Gy treatments, and regenerated 150 plants from 20 Gy treated and 50 plants from 40 Gy treated calli.

Shoots regenerated from calli were transferred to rooting medium. After root formation, the regenerants were transferred to vermiculite and kept under high humidity by covering the plants with plastic envelops. Regenerants from irradiated and control calli exhibited variation in their height and colour (Fig. 2). After 3-4 weeks, regenerants were transferred into pots containing a mixture of silt and farm yard manure (FYM) in a ratio of 3:1, and kept in a greenhouse. After 4 months, the regenerants were transferred to a field. The regenerants are being studied at two locations for tolerance to salinity under field conditions.

Effect of gamma irradiation

Effect of different radiation doses on callus growth and differentiation of sugarcane calli are shown in Table II. Significant effect of increase in radiation dose was observed on the survival and differentiation of callus (Fig. 1). The colour and proliferation efficiency of callus was effected by radiation [12].

TABLE II. EFFECT OF MEDIUM COMPOSITION AND RADIATION DOSE ON

REGENERATION OF CALLUS IN SUGARCANE

Treatment Radiation (Gy)	No. of Calli	MS0 regeneration %	MS0+KI+IAA regeneration %	MS06S regeneration %	MS06S+KI+IAA regeneration %
CONTROL	100	81.20	75.00	85.00	87.00
5	100	72.50	66.00	80.00	78.00
20	100	50.00	50.00	52.30	48.20
40	100	13.00	12.00	12.50	9.50
60	100	2.50	0.00	5.60	3.50

Figure 1: Effect of radiation dose on regeneration of sugarcane calli
a) 40 Gy b) 20 Gy c) 5 Gy d) Control

Figure 2: Plants obtained from irradiated calli growing in pots
a) Control b) 5 Gy c) 20 Gy d) 40 Gy

Radiation dose of 20 Gy to potato seemed to be LD_{50} as 50% of the axillary buds failed to form shoots on the modified MS media, while in the surviving M_1V_2 material leaf size and internodes were markedly influenced by radiation dose of 20 Gy; stunted growth and bunches of small leaves formed on the lower nodes of microplants. One batch of irradiated (20 Gy) microplants of M_1V_4 generation are under salt stress evaluation. A second batch of irradiated and control micropropagated plants were planted under a plastic tunnel at NIBGE to produce minitubers. 20 Gy microtubers were also planted in the tunnel to produce minitubers. These minitubers will be then subjected to different levels of salinity.

Effect of salt stress

After one month, the fresh weight of calli of sugarcane was recorded. A slight decrease in fresh weight was observed in salt treated calli. The treated calli cultured on regeneration medium without any salt became necrotic and lost their ability to differentiate into shoots. It was interesting that the growth of calli was observed even at 200 mM salt level (Table III). However, even low level of salinity 50 mM NaCl affected the regeneration process in all the tissues. To understand this process, this experiment was repeated by culturing calli for more than one month on the salt medium and to regenerate shoots from the stressed calli. No regeneration was observed in salt stressed calli. To explore the salinity effect at cellular level, irradiated and non-irradiated calli were also cultured on regeneration medium containing different levels of salts (0, 100, 200, 300, 400 mM NaCl). Not a single regenerant was obtained from these calli. This indicates that sugarcane is very sensitive to salinity at cell level.

TABLE III. EFFECT OF NaCl ON GROWTH OF SUGARCANE CALLUS

NaCl (mM)	0	50	100	150	200
Final wt.: (mg)	250(192.3)	160(123.1)	200(153.8)	170(130.7)	210(161.5)

Initial wt of calli in each treatment: (mg) 130±10
Replicates : 5
Incubation time: 4 weeks
* Figure in the parenthesis show percent increase in weight over the initial weight

In potato various concentrations of salt had drastic effect on shoot height. This effect was significant even at a lower level of salt concentration (50 mM NaCl). At this concentration burning and decay of shoot tip was observed. It was also noted that with the increase in salt concentration (150 mM and 200 mM) growth was reduced and the number of nodes, branches and roots decreased.

Detection of stress induced variability by PCR based DNA Fingerprinting

Stress caused by tissue culture, irradiation and high levels of salt may cause change in the genetic material which may result in somaclonal variation. However, it is hard to detect any genetic variation or somaclones at the callus stage from morphology. Random amplified polymorphic DNA (RAPD) markers were used to detect changes in DNA at callus stage. DNA was isolated from sugarcane control callus, and the calli subjected to 5, 20 and 40 Gy doses

of radiation and 100, 150 and 200 mM NaCl concentration, and used for PCR amplification.

Polymorphisms in amplification profiles was detected in 5 Gy with OPJ 13 (Fig. 3) and OPJ17 primers and in 200 mM NaCl treatment with OPJ18 primers. The polymorphic bands represents the genetic changes that occurred due to salt stress and irradiation. At callus stage, these polymorphism cannot be linked to any of the morphological characters, but in plants regenerated from calli, these markers may be linked to the morphological/physiological changes.

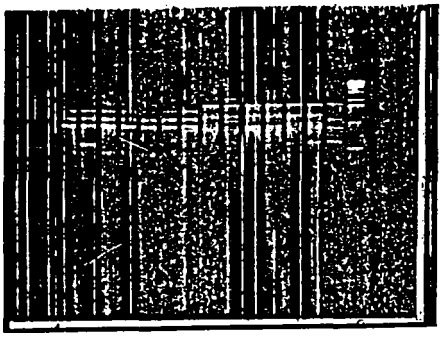


Figure 3: Amplification profiles of DNA isolated from sugarcane calli with OPJ13 (lanes 1-2) and OPJ 17 (lanes 8-14) primers. Lanes 1,8=control callus, 2,9=5 Gy treatment,3,10=20 Gy treatments, 4,11=40 Gy treatment, 5,12=100 mM salt stress, 6,13=150 mM salt stress, 7,14=200 mM salt stress, M=Size Marker (1-kb DNA ladder)

In vegetatively propagated crops, genetic variation can be increased for useful agronomic characters by various kinds of stress. Tissue cultures parameters have been optimized for the two crops and effective levels of gamma irradiation and salt stress are being studied. The selected clones of the two crops have been multiplied in the field.

TABLE IV. EFFECT OF NaCl ON SHOOT LENGTH, NUMBER OF NODES, BRANCHES AND ROOTS OF POTATO AXILLARY BUD CULTURE

NaCl Conc.(mM)	Plant Height	No. of nodes	No. of branches	No. of roots
00	5.61 a	7.40 a	1.75 a	2.93 a
50	2.70 b	5.51 b	0.55 b	2.32 a
100	1.04 bc	5.99 b	0.31 b	0.65 b
150	0.49 c	3.93 c	0.25 b	0.42 b
200	0.40 c	2.34 d	0.49 b	0.02 b

* a, b, c and d; results with same letter in each column are not significantly different at 0.05 probability.

Acknowledgements: The authors acknowledge the kind cooperation of Sugarcane Research

Institute and Virology Section, AARI, Faisalabad for providing plant materials. They also thank NIAB, Faisalabad for irradiating the material. The research was funded in part by a grant (Research Contract No. 7858/RB) from IAEA/FAO, Vienna, Austria. The help of Mr. Khalid Mehmood for typing the manuscript is appreciated.

REFERENCES

[1] BRYAN, J.E., JACKSON, M.T., QUEVEDO, M., MELENDEZ, N. Single-node cuttings a rapid multiplication technique for potatoes.Series I: Rapid Multiplication Techniques for Potatoes. Guide-Book I & II Intl. Potato Center, Lima, Peru (1981) 1-8.

[2] CHAUDHRI, M.T., et al., The regeneration of salt tolerant plants of *Medicago* media from salt selected suspension cultures. Rice Biotech. Quat. 17 (1994) 31-34.

[3] ANONYMOUS, Economic Survey 1994-95, Government of Pakistan, Finance Division, Economic Advisor's Wings. Islamabad, Pakistan.

[4] HEINZ, D.J., MEE, G.W.P. Plant differentiation from callus tissue of *Saccharum* species. Crop Sci. 9 (1960) 346.

[5] HUSSEY G., STACEY, N.J. *In-vitro* propagation of potato (*Solanum tuberosum* L.). Ann.Bot. 48 (1981) 787-796.

[6] IQBAL, M.J., ASAD, S., ZAFAR, Y. DNA Polymorphisms in banana and sugar cane varieties revealed by RAPD analysis. Proc. Intl. Symp. Induced mutations and molecular techniques for crop improvement, IAEA, Vienna (1995) 309-317.

[7] LARKIN P.J. Sugarcane tissue and protoplast culture. Plant cell, Tissue and Organ Culture 3 (1982) 149-164.

[8] LIU, M.C., HUANG, Y.J., SHIH, S.C. The *in-vitro* production of plants from several tissues of *Saccharum* species. J. Agri. Ass. of China. 77 (1972) 52-58.

[9] MURASHIGE, T., SKOOG, F. A revised medium for rapid growth and bioassay with tobacco tissue. Physiologia Planta. 15 (1962) 473-497.

[10] POWELL, W., et al. The use of DNA markers to estimate the extent and nature of genetic variability in *Solanum tuberosum* cultivars. Ann. Appl. Biol. 188 (1991) 423-432.

[11] ROCA, W.M., ESPINOZA, N.O. ROCA, M.R., BRYAN, J.E.A. Tissue culture method for the rapid propagation of potato. Amer. Potato J. 55 (1978) 691-701.

[12] SIDDIQUI, S.H., JAVED, M. Mutation breeding in sugarcane (*Saccharum* sp.) hybrid by gamma irradiation of cuttings and tissue cultures. Proc. Intl. Symp. Induced

mutations in vegetatively propagated plants II, IAEA, Vienna (1982) 155-166.

[13] YU, L.X., NGUYEN, H.T. Genetic variation with RAPD markers among upland and lowland rice cultivars (*Oryza sativa* L.). Theor. Appl. Genet. 87 (1993) 668-672.

IMPROVEMENT OF PINEAPPLE (*Ananas comosus* (L.) Merr.) USING BIOTECHNOLOGY AND MUTATION BREEDING TECHNIQUES

F. OSEI-KOFI, H. M. AMOATEY, Y. LOKKO
Biotechnology and Nuclear Agriculture Research Institute
Ghana Atomic Energy Commission
Legon-Accra, Ghana

Abstract

Micropropagation and *in vitro* mutagenesis are reported in two local pineapple (*Ananas comosus* (L.) Merr.) varieties, 'Smooth Cayenne' and 'Sugar Loaf'. Multiple shoots developed on Murashige and Skoog medium containing 3.5% sucrose, 3μM/l thiamine HCl, 3μM/l naphthalene acetic acid (NAA) and varying concentrations of 6-benzylaminopurine (BAP). Shoot proliferation was best with 20 μM/l BAP. Shoots were rooted on MS medium supplemented with 1.5 μM/l indole-3-butyric acid (IBA) and 0.75 μM/l indole-3-acetic acid (IAA). Radiosensitivity was determined by irradiating *in vitro* shoot tips with 15 to 120 Gy gamma rays. The LD_{50} was found to be 45 Gy, and doses above 80 Gy were lethal to explants. Projected methods are discussed to carry out mutation breeding for tolerance to drought and heat .

1. INTRODUCTION

Pineapple (*Ananas comosus* (L.) Merr.) can be propagated from various vegetative parts such as hapes bitts, suckers, slips and crowns. Usually, it is propagated from suckers [1]. It is highly heterozygous, and its genetics is not well understood. It is one of the few crops in which all cultivars are derived from spontaneous somatic mutations and natural evolution without controlled breeding.

In Ghana, pineapple has become a very important horticultural export commodity. Current government agricultural policies emphasize the need to promote production of non-traditional cash crops such as pineapple to reduce the over-reliance on traditional crops. Pineapple is cultivated in regions near the sea with high humidity and wide range in rainfall (635-2,500 mm per annum). The optimal rainfall for commercial production is between 1,000 to 1,500 mm [2]. Pineapple plant has xerophytic features and can tolerate drought. However, severe drought causes early withering of the peduncle resulting in reduced flow of nutrients to the fruit. The fruit pericarp produced under such adverse conditions lacks lustre and have corky micro-fissures. The sugar contents is also reduced as a result of severe drought and thus the taste changes. This in effect reduces the market value of the fruit [3].

In Ghana, pineapple cultivation is predominantly along the periphery of the forest zones and regions of coastal savannah where temperatures range between 27°C to 35°C with annual rainfall of 1,042 to 1,488 mm [4]. In Ghana, when the dry season exceeds five months, especially if accompanied by high temperature, farmers experience poor crop production [5]. Breeding of pineapples varieties capable of withstanding these adverse environmental conditions is essential. This will increase production of the crop, increase sustainable use of farm land, and

restore farmer's income to levels above subsistence.

Mutation breeding is a suitable technique to improve vegetatively propagated crops. The impact of mutation techniques for crop improvement has been described in many publications [6]. In many vegetatively propagated crops, mutation induction in combination with *in vitro* culture and other methods of plant biotechnology maybe the only effective method for their improvement [7,8]. These advances made in plant biotechnology, including the use of mutagens and tissue culture techniques, should provide a new impetus to the solution of breeding pineapple.

The objectives of this project are to modify the existing protocol for *in vitro* micropropagation of pineapple (to generate large numbers of shoots for mutation induction and for rapid propagation of planting material), and to induce genetic variation via *in vitro* culture and gamma radiation to select for tolerance to prolonged periods of drought and heat.

MATERIALS AND METHODS

In vitro culture

Two popular Ghanaian pineapple cultivars, 'Smooth Cayenne' and 'Sugar Loaf' were used in these experiments. Explants were obtained from shoot apices of young suckers. They were washed under tap water, followed by immersion in 7X detergent for about 15 minutes. The explants were then rinsed in distilled water and surface-sterilized in 20% Clorox, with 2 to 3 drops of surfactant, Tween 80, for 20 minutes. This was followed by four rinses in sterile distilled water. They were further trimmed down to the shoot tips.

Shoot tips of both cultivars were cultured on the following media: Murashige and Skoog Salts [9], 3.5% sucrose, 3μM/l thiamine HCl, 3μM/l naphthalene acetic acid (NAA) and varying concentrations (0, 10, 15, 20 and 25μM/l) of 6-benzylaminopurine (BAP) in completely randomized design with three replicates. Prior to sterilization, the pH was adjusted to 5.8. Multiple shoot buds were produced in liquid media agitated at 70 rpm on a rotary shaker, and records taken after 8 weeks of culture. Rooting was induced on media supplemented with 1.50μM/l indole-3-butyric acid (IBA), 0.75μM/l indole-3-acetic acid (IAA) and 0.8% agar. All cultures were maintained at 27°C under 16 hr cool-white fluorescent light at an intensity of 3000 lux.

Radio-sensitivity tests

Shoot tips were acutely irradiated with gamma radiation with doses 0, 15, 25, 45, 60, 80, 100 and 120 Gy from a ^{60}Co gamma source at a dose rate of 215 Gy/hr. There were 50 explants per dose, and each dose was replicated three times in completely randomized design. Irradiated shoot tips were transferred to shoot-proliferation medium with 20μM/l BAP (which gave the highest number of shoots; see results and discussions below). Radiation responses were evaluated in terms of explant survival and shoot proliferation after 8 weeks of culture. Surviving shoot tips were transferred to fresh medium, and subcultured to M_1V_1 and sequentially subcultured up to M_1V_4. Roots were induced in the shoots as described above. The data were analyzed using the statistical software Statgraphics (STSC Inc. and Statistical Graphic Corporation, USA).

RESULTS AND DISCUSSION

Shoot proliferation varied with the concentration of BAP in the medium (Fig. 1). Generally, shoot bud number increased with increasing concentration of BAP. This is in line with studies by Skoog and Millar [10] that up to a certain limit, a high cytokinin-auxin ratio favours bud and shoot formation. In both cultivars, optimal proliferation of shoot buds occurred at concentration of 20μM/l BAP. However, as reported in previous studies [11], the cultivar 'Smooth Cayenne' proliferated better than the cv. 'Sugar Loaf'. The mean shoot proliferation for 'Smooth Cayenne' at this optima was 100 compared with 85 shoots for 'Sugar Loaf'. BAP concentrations over 20μM/l reduced shoot proliferation. Multiple Range Test ($p<0.05$) showed that the concentrations of 10, 15, 20 and 25 μM/l significantly affected shoot proliferation. Shoot tips cultured on cytokinin-free medium produced few shoot-buds. For both cultivars, rooting was achieved in medium supplemented with 1.5μM/l IBA and 0.75μM/l IAA.

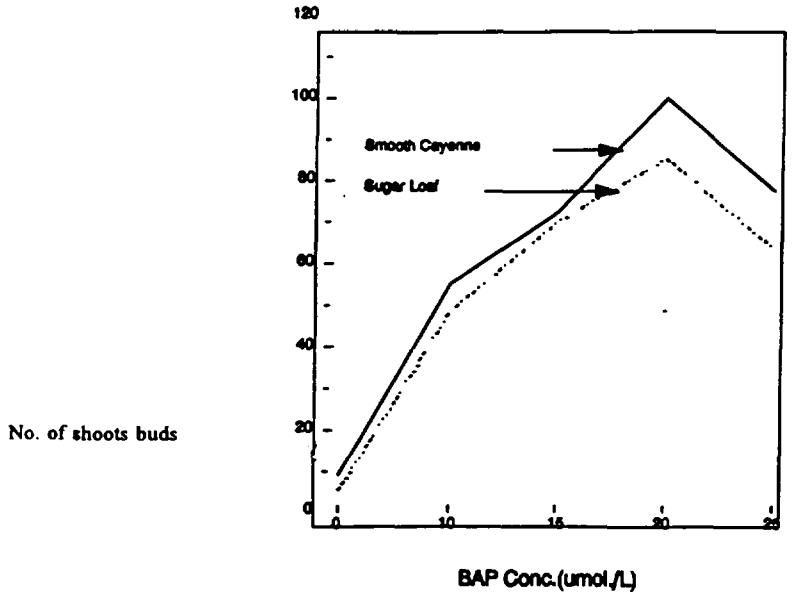


Fig. 1. Effect of BAP concentration on shoot proliferation

Radiosensitivity tests showed that dose of 80 Gy gamma rays was lethal to explants in both 'Smooth Cayenne' and 'Sugar Loaf'. The experiment established the LD_{50} was 45 Gy (Fig. 2) based on the survival of shoot tips. These results are consistent with the studies carried out by other workers on mutation breeding in pineapple [12].

FUTURE WORK

Plantlets of M_1V_1, M_1V_2, M_1V_3 and M_1V_4 propagation from radio-sensitivity studies were transferred to greenhouse for weaning and selection for heat tolerance. The plantlets of both irradiated and control experiments were exposed to high temperatures (35-40°C) in a heat chamber. As control, plantlets were weaned at 25-28°C. Records are being taken on percentage survival. This would be followed by selection in the field under prolonged drought conditions. Selection under field condition would be based on fruit size and quality.

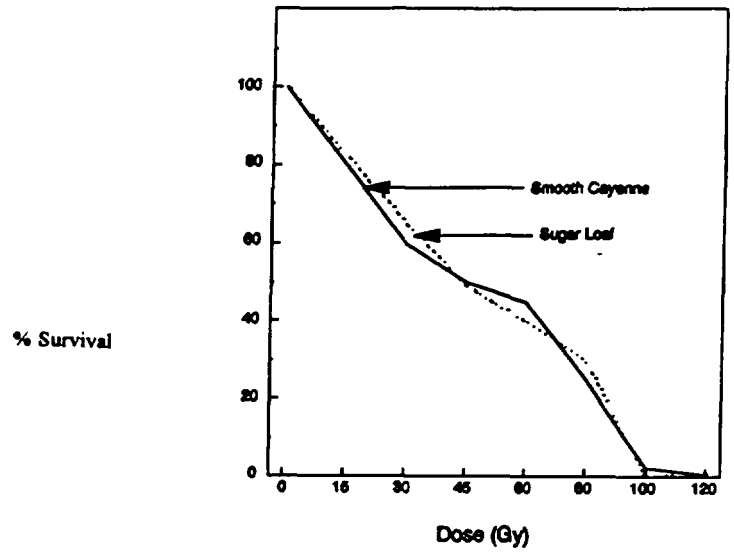


Fig. 2. Effect of gamma radiation on shoot tips.

Mutagenic treatment of shoot tips to generate variants that can tolerate severe drought and heat will be initiated this year. A total of 1000 explants of each cultivar will be treated with 30 Gy and 40 Gy gamma rays. These would be cultured through M_1V_1 and subsequently through M_1V_2, M_1V_3 and M_1V_4 by micropropagation. Plantlets would then be transferred to the greenhouse and subsequently to the field for selection, as described above. Selected types will be multiplied *in vitro* for multi-location trials. Trials will be done in the pineapple growing regions during the long dry season and also in the Northern parts of Ghana that have prolonged drought periods and high temperature.

REFERENCES

[1] SRIVIVAS, R., DORE, S., CHACKO, E.K. Differentiation of plantlets in hybrid embryo callus of pineapple. Scientia Hort. 15 (1981) 235-238.

[2] PURSEGLOVE, J. Bromeliaceae. In: Tropical Crops, Monocotyledons, Longman Scientific & Technical. ISBN 0-582-46606-7. (1988) pp. 75-91.

[3] PY, C., LACOEUILHE, J., TEISSON, C. The Pineapple Cultivation and Uses. Maisonneuve and Larose. (1987) pp.127-137.

[4] GHANA METEOROLOGICAL SERVICES DEPARTMENT, Accra Ghana. 1996. Average annual rainfall in pineapple growing areas, 1961-1995.

[5] NYAMEKYE-BOAMAH, K. 1995. (Personal communication).

[6] MALUSZYNSKI, M., AHLOOWALIA, B. S., SIGURBJÖRNSSON, B. Application of *in vivo* and *in vitro* mutation techniques for crop improvement. Euphytica 85 (1995) 303-315.

[7] NOVAK, F.J. *In vitro* mutation system for crop improvement. Plant mutation Breeding for Crop Improvement, IAEA, Vienna Vol. 2. (1991) 327-342.

[8] AHLOOWALIA, B.S. *In vitro* mutagenesis for the improvement of vegetatively propagated plants. Induced Mutations and Molecular Techniques for Crop Improvement, Proc. IAEA/FAO International Symposium on the Use of Induced Mutations and Molecular Techniques for Crop Improvement. IAEA, Vienna (1995) 531-541.

[9] MURASHIGE, T., SKOOG, F. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiology Plant. 15 (1992) 473-497.

[10] SKOOG, F., MILLAR, C.O. Chemical regulation of growth and organ formation i n plant tissues cultured *in vitro*. In: Biological Action of Growth Substances. Symp. Soc. Exper. Biol. 11 (1957) 118-131.

[11] OSEI-KOFI, F., ADACHI, T. Effect of cytokinins on the proliferation of multiple shoots of pineapple *in vitro*. Sabrao Jour. 25 (1993) 59-69.

[12] LAPADE, A.G., VELUZ, A.M.S., SANTOS, I.S. Genetic improvement of the Queen variety of pineapple through induced mutation and *in vitro* culture techniques. Induced Mutations and Molecular Techniques for Crop Improvement, Proc. IAEA/FAO International Symposium on the use of induced mutations and molecular techniques for crop improvement. IAEA, Vienna (1995) 684-687.

IN VITRO INDUCTION OF VARIATION THROUGH RADIATION FOR LATE BLIGHT RESISTANCE AND HEAT TOLERANCE IN POTATO

J.L. MINOCHA, A. DAS, J.GOPAL, S.S. GOSAL
Biotechnology Centre
Punjab Agricultural University
Ludhiana, Punjab, India

Abstract

In vitro plants were obtained from nodal sections of sprouts of cvs. 'Kufri Jyoti' and 'Kufri Chandramukhi' of potato cultured on MS medium with 3% sucrose. Callus from leaves of *in vitro* cultured plantlets was induced on modified Linsmaier and Skoog medium supplemented with 5 mg/l NAA. The obtained shoots and calli were irradiated with 20 and 40 Gy gamma rays. Irradiated shoots were transferred to MS medium with 8% sucrose for multiplication, and then to MS medium with 8% sucrose and 10 mg/l BAP to induce microtuber formation, which gave on average 1.3 microtubers per plant. The microtubers were planted in pots and variation was observed in plant morphology and tuber characters. To study variation for late blight resistance, irradiated calli were kept on Gamborg B-5 medium with culture filtrate of *Phytophthora infestans*. To induce variation for heat tolerance, *in vitro* shoots from irradiated material were mass-propagated and allowed to produce microtubers at high temperature.

1. INTRODUCTION

The main objectives of potato breeding in India include high yield, late blight resistance, virus resistance and early maturity. 'Kufri Jyoti' and 'Kufri Chandramukhi', the two most popular varieties are susceptible to late blight. Also the cropping pattern in North India demands an early planting variety in September so that it matures by November and a normal crop of wheat can be raised. The temperature during this period ranges between 20 to 32°C. The present project was initiated with the objective to induce *in vitro* variation for resistance to late blight and heat tolerance in cvs. 'Kufri Jyoti' and 'Kufri Chandramukhi' through radiation.

2. MATERIALS AND METHODS

Cv. 'Kufri Jyoti' and 'Kufri Chandramukhi' were used as the experimental material. 'Kufri Jyoti' is a medium late maturing (100-110 days) and 'Kufri Chandramukhi' an early maturing (90-100 days) variety. Both are high yielding with slow rate of viral degeneration, wide adaptability and desirable commercial attributes, but are susceptible to late blight and are not suitable for early planting being heat susceptible.

Micropropagation:

Micropropagation allows rapid multiplication of clones in a short duration under disease free, controlled environment and on a year round basis. For producing *in vitro* plantlets, healthy potato tubers were rinsed with 10% ethanol and incubated at 20°C in dark. When the sprouts had 6-10 nodes, the tubers were transferred to light for 7 days to harden the sprouts. The

sprouts were surface sterilized in a mixture of 0.1% $HgCl_2$ and 0.1% sodium laurel sulphate for 5 minutes, and rinsed three times with sterilized distilled water. The nodal sections (0.5-1.0 cm long) were cultured on Murashige and Skoog [1] medium without vitamins and hormones. The above methodology was being used for producing *in vitro* plants of potato in this laboratory, but it did not work satisfactorily with 'Kufri Chandramukhi' and 'Kufri Jyoti', because the cultures showed very high incidence of fungal infection. It was probably from internal contamination of the explants, which could not be removed by surface sterilization. To overcome this, the sprouted tubers were planted in pots, and nodal sections from the stems of the plants were used as explants which gave better results than the nodal sections from sprouts. The cultures were kept under 16 hr photoperiod (3000-4000 lux, at 28 ± 2°C) and 8 hr dark, (25 ± 2°C). After 5 to 6 weeks, the plantlets were cut into 0.5-1.0 cm long segments. Each segment carried one axillary bud and usually one leaf. They were cultured on fresh medium and allowed to produced a second generation of plantlets. The *in vitro* grown plants with 5 to 6 nodes were irradiated with 20 and 40 Gy gamma-rays. The irradiated plants were cut into nodal sections, and cultured on MS medium for 4 to 5 weeks.

Microtuberisation

Plantlets, 4 to 5 week old, were used for microtuberisation. The plants were cut into 0.5-1.0 cm long segments, each with one leaf and an axillary bud, and transferred for microtuberisation to MS medium with 8% (w/v) sucrose. The cuttings were placed in jam bottles (40 ml medium per 300 ml bottle) or Watson Module containers (25 ml medium per 120 ml container). The Watson Module system consists of disposable pre-sterilized clear plastic containers with snap on lids. Cultures were kept at 28 ± 2°C (16 hr light) and 25 ± 2°C (8 hr dark) for 60 days. They were then transferred to dark conditions at 20 ± 1°C after pouring liquid MS medium containing 10 mg/l BAP and sucrose 8% sucrose. This protocol was tested for 25 cultivars, and was found suitable for all except 3 to 4 varieties (data not shown).

Callus induction

Leaf cuttings from *in vitro* plants were cultured on Linsmaier and Skoog [2] modified medium by omitting cytokinins and adding 5 mg/l NAA. Callus initiation and subsequent proliferation was obtained in dark at 28°C. The calli thus obtained were irradiated with 20 and 40 Gy gamma-rays. Regeneration was obtained by transferring calli to modified LS medium containing 50 ml coconut milk, 0.1 mg NAA and 5 mg BAP per litre. Young regenerated sprouts were transferred to B-5 medium [3].

Screening procedures

To screen for late blight resistance, microtubers were sown in pots with mixture of soil and farm yard manure in the ratio of 1:1. The microtubers were planted at a depth of ½ to 1 inch. The leaves from plants so produced were surface sterilized with 0.1% $HgCl_2$ and 0.1% Sodium laurel sulphate for 7 minutes. Plantlets were also raised *in vitro* from these plants. The sterile leaves were then placed on the toxic medium containing culture fluid of one pathotype of *Phytophthora infestans* and normal ingredients of Gamborg medium [3]. The culture filtrate was prepared by inoculating the fungal cultures on the Pea's extract medium. The cultures were checked for infection by microscopical observations and afterwards cultured in fluid Pea's extract. The culture fluid was filtered after 3 weeks to remove sporangia. Batches of 20 pieces

of calli (ca. 2 mm) were placed on the toxic media in a Petri dish. In the other case, young sprouts were transferred to culture medium containing filtrate. During selection, cultures were illuminated 14 hr a day by white light ca. 3000-4000 lux.

To screen for heat tolerance, the plantlets were grown at 28 ± 2°C, and allowed to microtuberise at this temperature. Plantlets were produced from irradiated calli cultured on normal B5 medium, and transferred to MS medium, and allowed to produce microtubers at 28-30°C.

3. RESULTS AND DISCUSSION

Micropropagation protocol had broad applicability as all the 25 genotypes tested responded well. The nodal explants from the plants obtained from sprouted tubers planted in soil gave better results than those from sprouted tubers. A total of 501 *in vitro* plantlets (253 in 'Kufri Chandramukhi' and 248 in 'Kufri Jyoti') were irradiated. The plantlets after irradiation with 2 and 4 kr gamma rays were used for microtuberisation. The number of microtubers per plant varied for different genotypes (data not shown). In case of 'Kufri Jyoti' and 'Kufri Chandramukhi', the total number of microtubers produced till now was 517 (Table I). Microtubers were usually 2 to 10 mm in diameters and originated as aerial structures from microstems, although, a few were also formed in the medium. All microtubers had cream to white skin.

TABLE I. MICROTUBERIZATION OF IRRADIATED *IN VITRO* PLANTS

Variety	Dose (Gy)	No. of plants irradiated	No. of plants for micro-tuberisation	No. of microtubers
Kufri Chandramukhi	20	133	384	129
	40	120	278	110
Kufri Jyoti	20	133	315	152
	40	115	350	126

Callus was induced on two media: 1. MS + 5 mg/l NAA + 5 mg/l IAA + Kin 0.5 mg/l KIN, and 2. LS + 5 mg/l NAA + 2 mg/l IAA + 0.3 mg/l KIN. The efficiency of callus induction was much more on the second medium. In case of Linsmaier and Skoog medium the success was 83.9 and 86.7% for 'Kufri Chandramukhi' and 'Kufri Jyoti', respectively; on MS medium, the success was 42.8 and 39.2% for 'Kufri Chandramukhi' and 'Kufri Jyoti', respectively (Table II).

TABLE II. CALLUS IRRADIATION AND REGENERATION

Variety	Dose (Gy)	No. of tubers irradiated	No. of plants regenerated
Kufri	20	25	18/56
Chandramukhi	40	36	0/62
Kufri	20	38	10/62
Jyoti	40	22	1/40

The microtubers obtained from the *in vitro* plantlets were planted on two different dates. Data were obtained on plant morphology and tuber characteristics to study genetic variation. The data were recorded on the irradiated as well as the control (uniradiated) plants. Variation was present for different morphological characters such as number of nodes, leaf ratio, lateral leaf ratio, tuber number and tuber weight (data not shown).

Screening for resistance to late blight was done by using the detached-leaf method. The leaves of plants obtained from microtubers were sterilized and put on the toxic medium containing the fungal-culture filtrate. Plants gave different reaction. There was yellowing and browning of leaves in most cases, but some leaves remained unaffected. The leaves of the *in vitro* produced plants were also placed on the toxic medium and disease reaction was observed. Lesions appeared on the leaves. The number of lesions varied for different plants. The plants were graded on the scale of 1-10 with 10 being the most susceptible plant. The plants were thus classified into resistant, moderately resistant and susceptible types (Table III). All control plants were susceptible. Resistance was observed in both 'Kufri Chandramukhi' and 'Kufri Jyoti', irradiated with 40 Gy and moderate resistance was observed at 20 Gy dose (Table III).

TABLE III. DISEASE REACTION OF THE *IN VITRO* SCREENED PLANTLETS FOR LATE BLIGHT

Variety	Treatment	Plants with disease reaction			
		R (%)	MR (%)	S (%)	Total plants listed
KCM	50 Gy	36	20	44	39
	30 Gy	12	8	80	40
	Control	-	-	100	6
KJ	40 Gy	20	20	60	40
	20 Gy	8.6	30.4	61	47
	Control	-	-	100	8

The irradiated calli were also placed in Petri dishes containing the toxic medium; but all the calli turned brown and died. When organogenic calli were irradiated and placed on the toxic medium, all the calli from 40 Gy dose died after turning brown/black. However, a few calli from 20 Gy survived and showed greenish sectors. For heat tolerance, 29% of the irradiated calli showed regeneration.

Future work plan

Screening for late blight

a) The plantlets regenerated from resistant calli will be screened for late blight resistance by detached leaf method.

b) The tubers obtained from potted plants will be used in field trial to select for late blight resistance and agronomically important traits.

Screening for heat tolerance

a) Microtubers obtained at 28 ± 2°C and 20°C will be planted in field at different times (September 1, 15, 30 and 15th October 1995).

b) Plantlets regenerated from irradiated calli will be used for microtuber formation at 28 ± 2°C, and tubers thus obtained will be planted in the field.

REFERENCES

[1] MURASHIGE, T., SKOOG, F. A revised medium for rapid growth and bioassay with tobacco tissue. Physiologia Planta. 15 (1962) 473-497.

[2] LINSMAIER, E.M., SKOOG, F. Organic growth factor requirements of tobacco tissue cultures. Physiol. Plant. 18 (1965) 100-127.

[3] GAMBORG, O.L., MILLER, R.A., OJIMA, K. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50 (1968) 151-158.

EFFECT OF GAMMA RADIATION ON DIFFERENT EXPLANTS OF SWEET POTATO (*Ipomoea batatas* L. (Lam)) TO INDUCE NaCl TOLERANCE

A. GUTIERREZ-ROSATI
Biology Department
Genetic Resources and Biotechnology Center
National Agriculture University
La Molina, Lima, PERU

Abstract

Clonal lines tolerant to NaCl were obtained by combining *in vitro* culture and gamma radiation in two Peruvian varieties 'Amarillo de Quillabamba' and 'Nemañete'. The most suitable explants were pedicel sections and leaf blades. Embryogenic callus was induced on basal MS basal medium containing 0.5 ppm 2,4-D. The embryogenic calli were irradiated with 5 Gy from a ^{137}Cs source. Several putative mutants appeared to be stable.

1. INTRODUCTION

Sweet potato is an important tuber crop in many African and Asian countries. It is used as food, feed and for industrial purposes. Among the root and tuber crops, sweet potato is second in importance after white potato. Sweet potato is basically an Asian crop. With increasing urbanization in South-East Asia, growing of sweet potato in home gardens has a high potential in nutrition of urban population and food security. In Africa, Uganda is the largest producer of sweet potato.

Of the major sources of starch foods, root and tuber crops such as potato, sweet potato, yam, cocoyam (or taro) and cassava are very important specially in tropical countries. However, being vegetatively propagated or difficult to reproduce by seeds, these crops have remained in a relatively primitive state in terms of plant breeding. For these crops induced mutations can be expected to broaden their genetic variation, thus facilitating selection for desired characters [1]. Hence, it is important to use tissue and cell culture techniques [2,3,4,5,6] in combination with mutations [7] in sweet potato breeding. This would allow selection of mutants in a short time and limited space. The main goal of the present research is to develop a protocol that combines *in vitro* techniques [2,3] and mutagenic treatment, and demonstrate the usefulness of mutation in sweet potato breeding and selection of NaCl tolerant genotypes.

2. MATERIALS AND METHODS

Two local varieties 'Amarillo de Quillabamba' (AQ) and 'Nemañete' (N) were selected for this study. These varieties are extensively grown in the coastal part of Peru because of their good agronomical characteristics; however, these varieties do not produce well in soils with high NaCl content. Using material already established as mother plants under *in vitro* conditions, the following explants were taken for the experiments: 1. bud meristems, 2. axillary buds, 3. protoplasts, 4. embryogenic calli derived from leaf petioles and leaf blades (without petioles).

Radio-sensitivity test

Both, protoplast and callus cultures were irradiated with gamma rays from a ^{137}Cs source. LD_{50} was determined from 50% reduction of growth or regeneration of *in vitro* materials compared with the control. The explants, axillary buds, meristems, nodal pieces and leaf blades were placed in small Petri dishes for irradiation. The protoplasts were irradiated during digestion with cellulase. The dose delivery rate was 1.86 Gy/min. To determine optimal dose, explants were irradiated with a range of gamma rays 0, 5, 10, 15, 20, 30, 50, 80, 100 Gy [7]. Each experiment was repeated twice with the same number of explants each time.

Method for in vitro screening

Following irradiation, the explants were cultured either on growth and regeneration media or media containing different amounts of NaCl in two groups: Group A - selection pressure was applied from the beginning of the experiment. Group B - after irradiation, the explants were cultured on media without selection pressure, and the selection pressure was applied after the formation and propagation of the regenerants. During selection, the response of *in vitro* plantlets, callus formation and callus growth to different concentrations of NaCl (0, 5, 8, 10, 12, 15 ppm.) was evaluated.

3. RESULTS

3.1. In vitro culture of non-irradiated explants

The results of *in vitro* culture are shown in Table I. Regeneration was obtained from different explants, e.g. leaf blades, leaf petioles and calli; however, it was genotype-dependent, and occurred at a low frequency.

3.2. Effect of radiation dose

The effect of radiation on the response of different explants for differentiation and regeneration is summarized in Table II.

TABLE I. RESPONSE OF NON-IRRADIATED EXPLANTS ON REGENERATION MEDIA.

Type of explant	Medium and growth regulators(mg/l)	Explant response	Var.	Res. (%)
Bud meristems	B5+AG_3 (20 mg/l) + L-Arginine (100 mg/l) + Ca Panth (2.0 mg/l)	Plant propagation	AQ N	100 100
Axillary buds.	MS+AG_3 (10.0 mg/l) + IAA (0.05 mg/l)	Plant propagation Multiple shoot	AQ N	100 >50 100 >30
Protoplasts	VKM+0.1% BSA.	Cell division Root derived from calli Plant regeneration. from roots	AQ N >10	>15 >20 >2 >15 >1
Leaf petioles	MS+2,4-D (0.5 mg/l) MS+zeatin (1.0 mg/l)	Embryogenic callus Plant regeneration	AQ N	>90 >50 >90 >30
Leaf blades	MS+2,4-D (0.5 mg/l) MS+zeatin (1.0 mg/l)	 Embryogenic callus Plant regeneration	AQ N	>90 >50 >90 >30

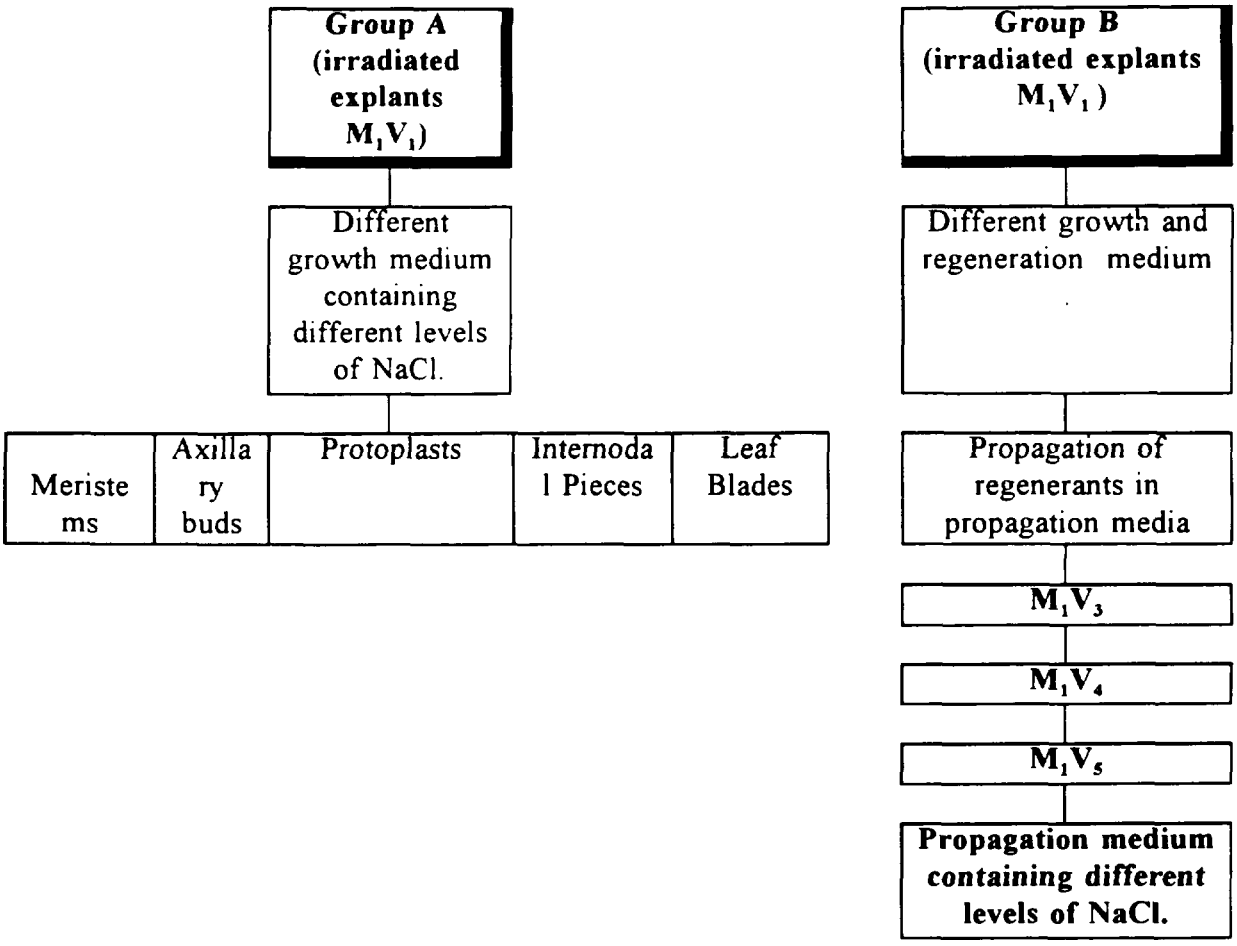
MS = Murashige & Skoog medium [8]
B5 = Gamborg *et al.* [9]

TABLE II. EFFECT OF GAMMA RAYS ON DIFFERENT EXPLANTS OF SWEET POTATO.

Explant	Evaluation		Gamma ray dose (Gy)								
		Var	0	5	10	15	20	30	50	80	100
Meristems	Growth after 30 days (%)	AQ	100	85	63	27	0	0	0	0	0
		N	100	70	45	15	0	0	0	0	0
Axillary Buds	Height of plantlets after 30 days (cm)	AQ	9.6	8.5	6.4	3.7	1.7	0.9	0	0	0
		N	8.5	7.0	5.2	2.1	0.8	0.2	0	0	0
Protoplasts	Cell division (%)	AQ	>15	>5	0	0	0	0	0	0	0
		N	>15	>5	0	0	0	0	0	0	0
	Root derived from calli (%)	AQ	>20	>1	0	0	0	0	0	0	0
		N	>17	>1	0	0	0	0	0	0	0
	Plants coming from roots (%)	AQ	>12	>0.5	0	0	0	0	0	0	0
		N	>10	>0.5	0	0	0	0	0	0	0
Leaf petioles	Embryogenic callus (%)	AQ	>86	>32	>9	0	0	0	0	0	0
		N	>84	>29	>7	0	0	0	0	0	0
	Embryo development (%)	AQ	>50	>26	>5	0	0	0	0	0	0
		N	>30	>20	>5	0	0	0	0	0	0
Leaf blades	Embryogenic callus (%)	AQ	>90	>30	>2	0	0	0	0	0	0
		N	>85	>22	>5	0	0	0	0	0	0
	Embryo development (%)	AQ	>50	>30	>1 0	0	0	0	0	0	0
		N	>30	>17	>6	0	0	0	0	0	0

Response of explants to selection for NaCl tolerance

The following doses were used in the subsequent experiments: meristems and axillary buds 10 Gy; protoplasts and embryogenic callus 5 Gy. After irradiation, the materials were divided in two groups as below:



From the two methods of selection, it was clear that the second method in Group B was more suitable, because in Group A no regenerants were obtained from protoplasts, embryoids or meristems because of osmotic problems during the first stage of culture. Only very few axillary buds grew at the concentration of 5 ppm NaCl. The plantlets obtained after irradiation from Group B were subsequently micropropagated up to M_1V_5, using the following codes: plantlets coming from meristem $M_1V_5m_1$; plantlets coming from axillary bud $M_1V_5ab_1$ regenerants from root derived protoplasts $M_1V_5pr_1$; regenerants from calli derived protoplasts $M_1V_5pc_1$; plantlets from internode derived embryoids, $M_1V_5el_1$; plantlets from leaf blade derived embryoids $M_1V_5el_1$.

The surviving plants were selected as tolerant to NaCl and are now being micropropagated to increase their number to carry out a second selection experiment to screen the material, and identify the probable chimerism which was observed in the initial experiments among the plantlets obtained from axillary buds. No chimeras were observed among plantlets which originated from embryoids.

In the second phase of the work, it is planned to develop a method to confirm by molecular techniques the putative mutant. It is also planned to use these mutants as a source of material to initiate a series of experiments for *in vitro* mutagenesis for tolerance to NaCl. Table III shows the time Schedule for the planned experiments.

TABLE III. PLANNED TIME SCHEDULE OF THE EXPERIMENTS.

Step	Duration
1995	
Determination of LD_{50} dose	3 months
M_1V_1	2 months
M_1V_2	1 month
M_1V_3	1 month
M_1V_4	1 month
M_1V_5	1 month
First selection for NaCl tolerance	1-2 months
1996	
Second selection for NaCl tolerance	1-2 months
Molecular characterization	6-8 months

CONCLUSIONS

It is concluded that gamma radiation in combination with *in vitro* culture offers the possibility to select sweet potato plants tolerant to NaCl. The radiation dose for meristems and buds was 10 Gy, and for protoplasts and embryogenic callus 5 Gy. Of the various explants used, the best results were obtained from embryogenic calli, which gave more and stable plants than those obtained from axillary buds or meristems.

REFERENCES

[1] DONINI, B., MICKE, A. Use of induced mutations in improvement of vegetatively propagated crops. Proc. Regional Seminar on the Utilization of Induced Mutations for Crop Improvement for Countries in Latin America. IAEA-TECDOC 305 (1984) 78-98.

[2] BIDNEY, D. L., SHEPARD, J.F. Colony development from sweet potato petiole protoplast and mesophyll cells. Plant Science Letters, 18 (1980) 335-342.

[3] CARSWELL, G.K., LOCY, R. D. Root and Shoot initiation by leaf, stem and storage root explants of sweet potato. Plan Cell Tissue Organ Culture 3 (1984) 229-236.

[4] JARRET, R.L., SALAZAR, S., FERNANDEZ, Z. Somatic embryogenesis in sweet potato. Hort. Sci. 19 (1984) 395-397.

[5] SALINAS, M., GUTIERREZ-ROSATI, A. Regeneración de plantas a partir de embriones somáticos de camote (*Ipomoea batatas* L.). ACEVIV, 3 (3) (1991), Colombia

[6] SALINAS, M., GUTIERREZ-ROSATI, A. Análisis del desarrollo de embriones somáticos en camote (*Ipomoea batatas* L.). ACEVIV, 3 (3) (1991), Colombia

[7] MANUAL ON MUTATION BREEDING. 2nd Edition. Tec. Rep. Ser. No. 199, (1977), IAEA, Vienna.

[8] MURASHIGE, T., SKOOG, F. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiology Plant. 15 (1992) 473-497.

[9] GAMBORG, O.L., MILLER, R.A., OJIMA, K. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50 (1968) 151-158.

INDUCTION OF SOMACLONAL VARIATION AND MUTATIONS IN SUGARCANE CALLI FOR SELECTING MUTANTS WITH RESISTANCE TO RED-ROT AND TOLERANCE TO WATER-LOGGED CONDITIONS

M.A.Q. SHAIKH, S. BEGUM,
M.A. SAMAD, K.M. SHAMSUZZAMAN
Bangladesh Institute of Nuclear Agriculture
Mymensingh, Bangladesh

Abstract

Immature leaves of cv. 'Isd-16' of sugarcane were cultured on modified MS medium supplemented with 3.0 mg/l 2,4-D for callus induction. The calli were transferred to MS medium supplemented with 5.0 mg/l IAA and 2.0 mg/l KIN for shoot regeneration. The shoots were rooted on MS medium supplemented with 5.0 mg/lNAA and 70 g/l sucrose. The regenerated plants were screened against red-rot disease and water-logged condition in a field. Of the 368 plants inoculated with red-rot pathogen, only one was moderately resistant and two were moderately susceptible. In another set of 500 R_1 plants, six clones were tolerant to water-logged condition. Four week-old callus cultures were irradiated with doses of 2, 3, 4, 5, 6, 7, 8 and 10 Gy gamma-rays. Survival of calli decreased with increase in radiation dose and ranged from 58 to 91%. Regenerated shoots were obtained from all irradiated calli except those treated with 8 and 10 Gy. Shoot regeneration from the irradiated calli ranged from 8 to 50%, and gave 768 R_1 plants. The highest regeneration of plants was obtained from calli treated with 3 Gy. These plants are being grown in a field for screening against red-rot and water-logged conditions.

1. INTRODUCTION

During the last two decades the techniques of plant cell and tissue culture have become an important tool in crop improvement [1]. In many crops, regenerants obtained from callus cultures have shown a wide variation [2,3,4]. The application of mutagens may play an important role for inducing genetic variability in tissue cultures. In China, two high yielding and high sugar content sugarcane lines were selected by gamma-ray irradiation of callus [5]. Ahloowalia [6] reported that it should be possible to regenerate desired genotypes through either somaclonal variation or through *in vitro* mutagenesis in case of vegetatively propagated plants like sugarcane. Sugarcane is an important industrial crop of Bangladesh but its cane yield per hectare is much lower than other countries. During the rainy season, nearly 50% sugarcane growing area of the country is submerged under water, which causes decrease in cane yield and sugar content as well as it increases disease infection specially with red-rot (*Colletotrichum falcatum)*. There is no natural source of resistance against this disease in the available germplasm. The present study was undertaken to determine the effect of gamma rays on plant regeneration from sugarcane callus with the ultimate objective to produce variants with resistance to the red-rot disease and tolerance to water-logged condition.

2. MATERIALS AND METHODS

Explants were taken from the leaf sheaths of cv. 'Isd.16', and surface sterilized with 70% ethyl alcohol. The materials were cultured on modified MS medium [7] supplemented with 3.0 mg/l 2,4-D and 10% coconut water to induce callus. For shoot induction, MS medium was supplemented with 5 mg/l IAA + 2 mg/l KIN. Shoots were cultured for rooting on MS medium containing 5 mg/l NAA and 70 g/l of sucrose. The regenerated plants (R_1) were grown in a field for screening against red-rot disease and water-logged condition. In one set, 936 hills were grown at BINA, Mymensingh farm for screening against the red-rot disease during 1994-95. The experiment was planted in plant-progeny rows with distance between rows and hills of 100 and 30 cm, respectively. Artificial inoculation with spore suspension of the red-rot pathogen was done on 368 canes by using the Plug method [8]. After two months, the canes were split open, and graded using a scale of 0-9 [9]. In the second set, 500 R_1 hills were grown at BINA, Mymensingh to select for water-logging tolerant variants. The experimental plots were irrigated from time-to-time to maintain water-logged conditions at least for 3 months. Hills showing green leaves and moderate growth under these conditions were selected. In a third set, 3-4 week old calli were irradiated with 2, 3, 4, 5, 6, 7, 8 and 10 Gy dose of gamma rays to create variation and to observe the mutagenic effects on the regenerated plants. Data on survival of calli, frequency of shoots obtained from the irradiated calli and number of plants regenerated from shoots were recorded. Regenerated plant were first transferred to small pots for establishment; afterwards they were planted dose wise in the field for artificial inoculation tests.

3. RESULTS AND DISCUSSION

In the first batch, 368 canes from 936 hills were inoculated with red-rot pathogen. Of these, only one clone was found to be moderately resistant and two were moderately susceptible (Table I). In the second set, 6 hills were tolerant to water-logged condition. Selected canes have been grown for further screening against red-rot disease and water-logged conditions. In the third set, it was observed that after irradiation the colour of calli changed from white to blackish. Change in colour of calli following irradiation has been also reported in barley [10]. Survival of the calli decreased with increase in radiation dose (Table II) which ranged from 58 to 91%. Shoots were regenerated from calli irradiated with all doses except those from 8 and 10 Gy. Shoot regeneration of the irradiated calli ranged from 8 to 50%. The highest shoot regeneration was observed from calli irradiated with 3 Gy. Lu [5] obtained a wide variation following irradiation of sugarcane callus with ca. 2 Gy. Frequency of shoot regeneration in calli decreased with increase in radiation dose. A total of 768 R_1 plants from all the treatments survived; these are growing in a field for screening against red-rot and water-logged conditions.

TABLE I. NUMBER OF RED-ROT RESISTANT PLANTS SELECTED IN R_1 GENERATION OF SUGARCANE CV. 'ISD-16' GROWN AT MYMENSINGH.

Line No.	No. of hills	No. of plants inoculated	Moderately Susceptible	Moderately resistant	Resistant
1	142	70	2	1	-
2	156	75	-	-	-
3	137	52	-	-	-
4	165	50	-	-	-
5	174	73	-	-	-
6	162	48	-	-	-
Total	936	368	2	1	-

TABLE II. EFFECT OF GAMMA RAYS ON CALLUS INDUCTION AND REGENERATION OF PLANTS IN SUGARCANE VARIETY, ISD-16.

Doses (Gy)	Calli treated (No.)	Survival after after 21 days (%)	Shoot regeneration from survived calli (%)	Plants obtained (No.)
2	90	9	45	202
3	90	87	50	255
4	90	78	28	140
5	90	71	23	92
6	90	70	11	44
7	90	66	08	35
8	90	62	-	-
10	90	58	-	-

REFERENCES

[1] RAZDAN, M.K., COCKING, E.C. Improvement of legumes by exploring extra-specific genetic variation. Euphytica. 30 (1981) 819-833.

[2] LARKIN, P.J., SCOWCROFT, W.R. Somaclonal variation - a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60 (1985) 197-214.

[3] KRISHNAMURTHY, M. 1982. Disease resistant sugarcane developed through tissue culture. Plant Tissue Culture. Proc. Japan Assoc. Plant Tissue Cult., Tokyo (1982) 769-770.

[4] METHEWS, H.V., RAO, P.S., BHATIA, C.R. Somaclonal variation in cotyledonary plants of mungbean. Z. Pflanzenphysiol. 96 (1986) 169-173.

[5] LU, Y.B. Mutagenic effects of irradiation with ^{60}Co gamma-rays on sugarcane callus. Acta Agriculturae-Nucleatae-Sinica 4 (1990) 65-70.

[6] AHLOOWALIA, B.S. *In vitro* mutagenesis for the improvement of vegetatively propagated plants. Induced Mutations and Molecular Techniques for Crop Improvement, Proc. IAEA/FAO International Symposium on the Use of induced Mutations and Molecular Techniques for Crop Improvement. IAEA, Vienna (1995) 531-541.

[7] HEINZ, D.J., MEE, G.W. 1969. Plant differentiation from callus tissue of *Saccharum* species. Crop Sci. 9 (1969) 346-348.

[8] HUTCHINSON, P.B. A note on disease resistance ratings for sugarcane varieties. Proc. Intnatl. Soc. Sugarcane-Tech. 13 (1978) 1087-1089.

[9] ALEXANDER, K.C. Technical Report on Plant Disease of All India Coordinated Research Project on Sugarcane. Indian Council of Agricultural Research, Indian Institute of Sugarcane Research, Lucknow, U.P. (1990) 20-23.

[10] UKAI, Y. Effect of gamma-ray irradiation on growth of calli derived from mature embryos or seeds of barley. Japan J. Breed. 38 (1988) 292-300.

SELECTION OF SUGARCANE MUTANTS WITH RESISTANCE TO RED-ROT DISEASE, WATER-LOGGING AND DELAYED/NON-FLOWERING

M.A.Q. SHAIKH, K.M. SHAMSUZZAMAN, M.A. MAJID
M.A.R. HOWLIDER, M.M. ISLAM
Bangladesh Institute of Nuclear Agriculture
Mymensingh, Bangladesh

Abstract

Three batches of sugarcane cuttings were irradiated with gamma-rays in three different years for isolating mutants for delayed flowering, resistance to red-rot disease and water-logged conditions. In the first batch cuttings of cvs. 'Isd-2/54', 'Latarijaba' and 'Nagarbari' were irradiated with 20-40 Gy gamma-rays. In M_1V_4 generation, 2,114 canes selected from inoculated M_1V_3 generation, were re-inoculated with red-rot pathogen. Of these, four canes were resistant and 64 canes were moderately resistant to the disease. The M_1V_5 generation of the selected clones was grown at two locations for selection. In the second batch, cuttings of cvs. 'Isd-16', 'Isd-2/54', 'Nagarbari' and 'Latarijaba' were irradiated with 20-60 Gy gamma-rays. The irradiated material was divided into three lots and each lot was put under different selection pressure. For isolating mutants with resistance to red-rot disease, 15,104 canes were artificially inoculated in the M_1V_3. Among these, one clone was resistant and 16 were moderately resistant. Of the 10,000 M_1V_3 canes, grown under water-logged condition and selected for greenness of leaf at harvest, 38 canes were reasonably tolerant. For selecting late flowering mutants, about 8,500 canes were left in a field for a month after normal harvest; of these five showed late flowering. These mutants were grown for further selection in the M_1V_4. To screen out non-flowering canes, cvs. 'I-291/87', an early flowering type, and 'I525/85', a late flowering type were irradiated with 20-40 Gy gamma rays. M_1V_1 generation has been grown in the field.

1. INTRODUCTION

Sugarcane is one of the most important cash crops in Bangladesh, but its yield is much lower than in some other sugarcane growing countries. The average yield of sugarcane in Bangladesh is about 40-50 t/ha [1]. One reason for poor yield is the susceptibility of existing varieties to diseases [2]. The red-rot disease, *Colletotrichum falcatum* alone causes an yield reduction of 10-15%. The disease can cause total crop loss in some plots [3]. The problem of red-rot disease is further compounded under water-logged conditions [4, 5]. Water-logged condition has become an integral part of sugarcane culture because the crop has been slowly pushed towards the marginal lands in the lower elevations due to increased demand of land for growing upland rice. Secondly, the excessive rain during June-October normally creates water-logged situation, thereby decreasing the cane yield considerably.

Although, flowering of canes has a beneficial effect on yield and there is no adverse effect on juice quality up to 60 days [6, 7], it is not possible to crush all the canes within this period. Crushing in some mills continues up to 3-4 months beyond the 60 days limit. As a result, there is deterioration in both yield of cane and sugar recovery. All the existing cultivars are susceptible to the red-rot disease and water-logged condition and have the tendency to deteriorate in yield and quality after 60 days of flowering. Hence, a breeding programme was

initiated to induce and select mutants with resistance to red-rot disease, tolerance to water-logged condition and delayed or non-flowering characteristics. To achieve these goals, three batches of sugarcane cuttings were irradiated with gamma rays in three different years.

2. MATERIALS AND METHODS

Batch I

In this batch, cuttings of cvs. 'Isd-2/54', 'Latarijaba' and 'Nagarbari' were irradiated with 20, 30 and 40 Gy gamma rays in 1990. The results of radio-sensitivity studies in M_1V_1 and the selection procedures followed in M_1V_2 and M_1V_3 generation have been reported elsewhere [8]. The M_1V_4 generation of the selected material of 5268 canes was grown at the BINA farm, Mymensingh during 1993-94. This generation comprised of plant-progeny rows of the resistant (R) and moderately resistant (MR) canes selected from the M_1V_3 generation. Distances between rows and hills within rows were 100 and 30 cm, respectively. Normal cultural practices were followed. Data on the number of tillers per hill, height and diameter of canes were recorded for preliminary assessment of yield. In addition, two centrally located canes of each of the 2114 hills were artificially inoculated with red-rot spore suspension. Standard plug method [9] of inoculation was carried out on 10-months old canes using spore suspension of 10-15 days old culture of *Colletotrichum falcatum* grown on PDA (Potato Dextrose Agar). The inoculum consisted of ca. one million spores/ml in water and 0.2 ml was injected into each cane. The canes were split open after two months and scored for severity index (0-9) developed by the All India Coordinated Research Programme for Red-rot of Sugarcane [10]. Canes showing disease severity indices of 0.0 to 2.0 were selected as resistant, and 2.1 to 4.0 moderately resistant.

Batch II

In this batch, four varieties, 'Isd-2/54', 'Isd-16', 'Nagarbari' and 'Latarijaba' were irradiated with 20, 40 and 60 Gy gamma-rays, and M_1V_1 was grown during 1991-92. Two central canes from each M_1V_1 hill were taken and only about one-third portion from the middle of each cane was cut for growing in M_1V_2. Rows of red-rot infected canes were planted in between the rows containing treated materials for field screening. One to two canes were taken from the central portion of the field tolerant M_1V_2 hills again and only the middle one-third part of each cane was used for planting in the M_1V_3 generation. In the M_1V_3 generation, there were 7052 hills with 35,204 canes. Only two central canes from each hill numbering 15,104 canes, were artificially inoculated with red-rot spore suspension, and selected as described for batch I. About 10,000 M_1V_3 canes were grown in low-lying plots for selecting resistant/tolerant canes to water-logged condition. After 180 days of planting (July), the plot was irrigated from time to time to maintain the water level up to 30-50 cm and it was continued till harvest (November). The canes remaining green were selected as tolerant to water-logging. About 8,500 M_1V_3 canes were grown in a separate plot to select non-flowering/ delayed-flowering canes. Canes not flowering even after one months from the flowering date of the controls were selected. Normal cultural practices were followed during the entire growing period. Agronomic data on the number of tillers per hill, cane height, diameter of cane (base, middle and top) were taken from the selected lines along with the control varieties in all experiments.

Batch III

Two sugarcane varieties 'I-291/87' (early flowering) and 'I-525/85' (late flowering) were irradiated with 20, 30 and 40 Gy gamma rays in 1994. Treated buds were grown in seed beds and scored for germination. The M_1V_1 seedlings have been grown at BINA, Mymensingh for further propagation and selection for delayed/non-flowering in M_1V_2 generation.

3. RESULTS AND DISCUSSION

Batch I (M_1 V_4 generation)

Only 4 canes were found to be resistant (R) and 64 canes were moderately resistant (MR) to red rot disease (Table I). Ranges, standard deviation, coefficient of variation (%) of the 68 selected canes are presented in Table II. The data show that the number of tillers per hill had the highest coefficient of variation (28.8) followed by cane height (15.8), and diameter of the middle (15.7), top (13.9) and basal portion (6.2) of the cane. Some variants were taller than the tallest parental variety, 'Isd-2/54'. Some tall variants also showed increase in the number of tillers per hill. Previously, induced mutations have been successfully used by other workers to increase cane yield, sugar content, improve growth rate and resistance to diseases [11, 12, 13].

TABLE 1. NUMBER OF RED ROT RESISTANT CANES SELECTED FROM M_1V_4 GENERATION OF SUGARCANE.

Variety	Doses (Gy)	Total no. of tillers	Inoculated canes	Moderately resistant (MR)	Resistant (R)
Isd-2/54	20	652	248	24	1
	30	812	326	14	3
	40	993	414	11	-
Control	0	120	20	-	-
Latarijaba	20	430	216	1	-
	30	422	202	6	-
	40	413	196	2	-
Control	0	132	20	-	-
Nagarbari	20	375	140	2	-
	30	520	210	1	-
	40	275	102	3	-
Control	0	124	20	-	-

TABLE II. FIELD PERFORMANCE OF SELECTED M_1V_4 CANES AND PARENTS DURING 1993-94.

Statistical parameters	No. of tillers (cm)	Cane height	Diameter of cane (cm)		
			Base	Middle	Top
Range					
High	8.0	284	3.2	3.9	3.0
Low	2.0	119	1.6	1.5	1.5
Standard deviation	1.4	36.8	0.1	0.3	0.3
Coefficient of variation (%)	28.9	15.8	6.2	15.7	13.9
Isd-2/54 (Cont.)	4.3	2.0	2.0	2.0	2.0
L. Jaba (Cont.)	3.8	211	2.1	1.9	2.0
N. bari (Cont.)	6.7	209	2.0	2.0	2.1

Batch II (M_1V_3 generation)

Out of the 15,104 artificially inoculated canes, only one cane was resistant (R) which was selected from the cv. 'Nagarbari' treated with 60 Gy gamma-rays (Table III). Sixteen canes were moderately resistant (MR). There were no resistant variants among the varieties 'Isd-2/54' and 'Latarijaba'.

TABLE III. SELECTION OF RED ROT RESISTANT CANES IN M_1V_3 GENERATION.

Variety	Doses (Gy)	Total no. of tillers	Inoculated canes	Moderately resistant (MR)	Resistant (R)
Isd-2/54	Control	100	20	-	-
	20	3104	1308	-	-
	40	2886	1276		
	60	2712	1182	-	-
Isd-16	Control	86	20	-	-
	20	3300	1282	2	-
	40	2796	1244	3	-
	60	2980	1180	3	-
Nagarbari	Control	92	20	-	-
	20	3208	1318	-	-
	40	2866	1216	2	-
	60	2896	1248	6	1
L. Jaba	Control	76	20	-	-
	20	2892	1298	-	-
	40	2900	1242	-	-
	60	2310	1230	-	-
Total		35204	15104	16	1

Agronomic data on these selected lines are presented in Table IV. Number of tillers showed the highest coefficient of variation (CV%) followed by cane height and diameter. Inoculation will be done again in the next generation, and selection will be made on the basis of disease severity, number of tillers per hill and cane height.

TABLE IV. PERFORMANCE OF SELECTED M_1V_3 LINES OF SUGARCANE DURING 1993-94.

Varieties/ Mutant	Doses (Gy)	No. of tillers/ hill	Cane height (cm)	Diameter of cane (cm)		
				Base	Middle	Top
NB(P1) MR	40	4	188	2.10	1.95	1.75
NB(P2) MR	40	5	159	2.05	1.95	1.85
NB(P11) MR	60	5	226	1.95	1.90	1.71
NB(P12) MR	60	6	214	1.65	1.65	1.40
NB(P13) MR	60	4	192	1.85	1.80	1.65
NB(P14) MR	60	5	197	1.65	1.50	1.45
NB(P15) MR	60	4	185	1.90	1.80	1.65
NB(P16) MR	60	5	203	2.00	1.80	1.70
NB(P17) MR	60	4	198	1.90	1.80	1.70
Isd-16(P3) MR	20	5	222	2.40	2.25	2.15
Isd-16(P4) MR	20	6	254	2.20	2.00	2.00
Isd-16(P5) MR	40	4	253	2.30	2.20	2.05
Isd-16(P6) MR	40	4	278	2.50	2.20	2.10
Isd-16(P7) MR	40	3	178	1.80	1.70	1.65
Isd-16(P8) MR	60	6	213	2.30	2.00	1.90
Isd-16(P9) MR	60	4	227	2.15	2.25	2.10
Isd-16(P10) MR	60	4	184	1.90	1.90	1.70
Nagarbari Control		4	188	2.10	1.95	1.81
Isd-16 Control		4	225	2.20	2.12	1.88
Isd-2/54 Control		5	168	2.02	1.89	1.78
Latarijaba Control		4	188	1.98	1.89	1.83
SED		0.8	29.9	0.20	0.20	0.20
CV (%)		18.0	14.5	11.00	10.30	11.20

Out of 10,000 (M_1V_3) canes, only 38 canes showed tolerance to water-logged condition. These canes withstood water-logged condition and remained green till harvest. The range, standard deviations, coefficient of variations of these selected canes are shown in Table V. Number of tillers/hill showed the highest CV% (26.8) followed by cane height (16.0) and diameter of canes.

TABLE V. PERFORMANCE OF M_1V_3 CANES SELECTED FOR WATER-LOGGING TOLERANCE, 1993-94.

Statistical parameters	No. of tillers hill	Cane height (cm)	Diameter of cane (cm)		
			Base	Middle	Top
SD	1.73	35.50	0.22	0.18	0.20
Range					
High	8.00	304	2.40	2.30	2.10
Low	2.00	135	1.70	1.60	1.20
CV(%)	26.90	16	10.90	9.50	11.70
Isd-16 Cont.	3	276	1.80	2.00	1.70
Isd-2/54 Cont.	5	211	1.90	2.00	1.90
L. Jaba Cont.	4	275	1.90	2.00	1.80

Five delayed flowering hills were selected from M_1V_3 generation. Agronomic data on these selected hills and canes and controls are presented in Table VI. The selected hills from 'Isd-16/20' (P1) showed better performance than the controls.

TABLE VI. AGRONOMIC CHARACTERISTICS OF SELECTED LATE FLOWERING CANES, 1993-94.

Selected lines	No. of tillers hill	Cane height (cm)	Diameter of cane (cm)		
			Base	Middle	Top
Isd-16-20 (P1)	6	304	2.4	2.2	2.0
Isd-16-40 (P2)	5	269	2.9	2.1	1.9
L.Jaba-20 (P3)	6	188	1.8	1.6	1.5
Isd-2/54-60 (P4)	2	247	2.3	1.9	1.8
Isd-2/54-60 (P5)	5	261	2.2	2.0	1.9
Isd-16 Cont.	5	285	1.9	2.1	1.8
Isd-2/54 Cont.	5	221	2.1	2.0	2.0
L. Jaba Cont.	5	285	1.9	2.1	1.8

REFERENCES

[1] IQBAL. M., MALEK, M.A., RAHMAN, S. Incidence of sugarcane diseases at Sugarcane Research and Training Institute Farm. Bangladesh J. Sugarcane, 8 (1986) 72-74.

[2] AHMED, H.U., KHAN, M.A., MIAH, I.H., Sugarcane diseases in Bangladesh. SPN. 15/16 (1976) 51.

[3] ANONYMOUS, 1979. Annual Report for the period 1973-78 (Special issue). Sugarcane Research Institute, Ishurdi, Pabna, Bangladesh, (1979) 95.

[4] ABBOTT, E.V. Red rot of sugarcane. USDA. Tech. Bull. 641 (1938) 96.

[5] ABBOTT, E.V., HUGHES, C.G. Sugarcane Diseases of the World. (MARTIN, J.P., ABBOTT, E.V., HUGHES, C.G. Eds.), Elsevier, Amsterdam, Vol.1 (1961) 262-287.

[6] SARKAR, M.A.A., MIAH, M.A.S. Study on the effect of flowering of cane on the quality of juice. Bangladesh J. Sugarcane, 2 (1980) 33-36.

[7] MIAH, M.A.S., SARKAR, M.A.A. Effect of flowering of cane on the quality of sugarcane. Bangladesh J. Sugarcane, 3 (1982) 25-28.

[8] SHAIKH, M.A.Q., SHAMSUZZAMAN, K.M., MAJID, M.A., HOWLIDER, M. A.R. 1996. Radiation sensitivity studies in sugarcane and selection of mutants with resistance/tolerance to red-rot disease. Bangladesh J. Nuclear Agric. (1996) (in press).

[9] HUTCHINSON, P.B. A note on disease resistance ratings for sugarcane varieties. Proc. Intnal. Soc. Sugarcane Tech. 13 (1978) 1087-1089.

[10] ALEXANDER, K.C. Technical Report 1989/90 on Plant Disease of all India Coordinated Research Project on Sugarcane. Indian Council of Agricultural Research, Indian Institute of Sugarcane Research, Lucknow (UP), (1990) 20-23.

[11] DARMODOJO, J.R., SEEDJONO, M. Induction of mosaic disease resistance in sugarcane by gamma ray irradiation. ISSCT Sugarcane Breeders' Newsletter, 39 (1977) 4.

[12] JAGATHESAN, D., BALASUNDARAM, N., ALEXANDER, K.C. 1974. Induced mutations for disease resistance in sugarcane. Induced Mutations for Disease Resistance in Crop Plants, Proc. Research Co-ordination Meeting, Novi Sad, (1973) IAEA, Vienna (1974) 151.

[13] SRIVASTAVA, B.D., BHATT, S.R., PANDEY, S., TRIPATHI, B.K., SAXENA, V.K. Mutation breeding for red rot resistance in sugarcane. Sugarcane 5 (1986) 131-135.

USE OF RADIATION FOR INDUCING MUTANTS IN POTATOES THROUGH TISSUE CULTURE TECHNIQUE

M. T. SHARABASH
National Center for Research
and Radiation Technology
Atomic Energy Authority
Nasr City
Cairo, Egypt

Abstract

Meristem-tips obtained from sprouts of potato tubers, cv. 'Diamant' (*Solanum tuberosum*) were cultured on MS-medium and multiplied into plantlets through micropropagation. After 2-3 weeks, the micropropagated plantlets had 5-6 nodes each. The plantlets were irradiated with 20 to 40 Gy gamma rays at 27.7 rad/sec. Irradiated plantlets were cut into single nodes and cultured on MS-medium supplemented with 2 g NaCl. Salt resistant plantlets were transferred to MS-liquid medium supplemented with 2g NaCl/l 5.2, and microtubers were collected after 6 weeks. Minitubers were produced under the same level of salinity.

1. INTRODUCTION

Potato *(Solanum tuberosum* L.*)* is an important food crop in Egypt. It ranks second after onions among the exported vegetable crops. An intensive tissue culture programme has been on-going in Egypt to produce virus-free seed potato to reduce the quantity of annual seed imports which may save more than 20 million Egyptian pounds spent every year to import virus-free seed tubers. The aim of this project is to obtain mutants of potatoes suited for growing in the newly reclaimed desert, where potato crop is irrigated with underground saline water.

2. MATERIAL AND METHODS

Material

Potato tubers of cv. 'Diamant' *Solanum tuberosum* L. were obtained from Potato Research Department, Agriculture Research Center.

Meristem isolation

Potato sprouts were taken and cut into single-nod segments. Each segment had one axillary bud from which leaves were removed. The segments were sterilized by soaking in 70% ethanol for 30 seconds, followed by soaking in 25% Clorox for 20 minutes. They were then washed thoroughly with sterile double distilled water to remove the excess of Clorox. The sterilized meristem-tips consisted of a dome surrounded by leaflets and a few leaf primordia.

Explant culture

Isolated meristem-tips were cultured on sterilized solid MS-medium, pH 5.7, and were kept at 22°C under 16 hr day-length, 3000 Lux light intensity. After 6 to 8 weeks, when the plantlets were 5 to 7 cm tall, micropropagation was initiated.

Micropropagation

Plant populations was increased to sufficient number for the experiments. Plantlets were cut into single nodes, and large leaves were removed. Each node was inoculated onto solid MS-medium, pH 5.7, and incubated at 20-22°C, under 16 hr day light with intensity of 3000 Lux. After 2-3 weeks, each plantlet developed 5 to 6 nodes. Plantlets were trimmed by cutting off the roots and large leaves, and transferred to liquid MS-medium. Cultures were divided in 3 groups to study the effect of radiation, salinity and interaction between irradiation and salinity.

1. Irradiation: Culture vessels containing plantlets were exposed to 20 and 40 Gy from a ^{60}Co source at a dose rate of 0.277 Gy/sec.

2. Salinity treatment: Trimmed plantlets were transferred to liquid MS-medium supplemented with 0, 2000 and 4000 ppm NaCl.

3. Gamma irradiation x Salinity treatment: Trimmed irradiated plantlets were transferred to liquid MS-medium, supplemented with 0, 2000 and 4000 ppm NaCl.

Production of microtubers

Irradiated plantlets were cut into single node pieces after trimming the large leaves. They were placed in 300 ml jars containing 60 ml liquid MS-medium, pH 5.7, and supplemented with 0, 2000, 4000 ppm NaCl, and incubated for 2 weeks under the same conditions as above. Then, the plantlets were transferred to tuberization liquid medium supplemented with 0, 2000 and 4000 ppm NaCl. The microtubers were collected after 6 to 8 weeks.

Minituber production

Microtubers were preserved in a refrigerator at 10°C for approximately 3 months. The sprouted microtubers were sown in 20 cm pots containing a mixture of cleaned sand, peat moss, and granules of plastic foam in a ratio 1:1:1, and irrigated with a solution 0, 2000 or 4000 ppm NaCl.

RESULTS AND DISCUSSION

It seems that the promising variants may occur among the minitubers obtained from plantlets irradiated with 20 Gy and grown on 2000 ppm NaCl. Under these treatments, the mean fresh weight of microtubers was 0.47g, while the mean fresh weight of the minituber was 22.3g.

The mother tuber and the minitubers, obtained after 20 Gy irradiation and grown on 2000 ppm NaCl, were analyzed for total soluble solids, reducing sugars, non-reducing sugars, starch, phenolic compounds, crude protein, total soluble protein and amino acids. The results showed that the differences in biochemical composition between mother tubers and the obtained minitubers were not significant (Tables I, II).

TABLE I. BIO-CHEMICAL COMPOSITION OF MOTHER TUBERS AND MINITUBERS.

	Mother tuber*	Minituber*
Total soluble solids	0.86	0.69
Reducing sugars	0.37	0.29
Non-reducing sugars	0.47	0.41
Starch	17.00	15.90
Phenolic compounds	0.24	0.33
Crude protein	2.45	3.00
Total soluble protein	0.12	0.10

*value are g/100g fresh weight.

TABLE II. AMINO ACID CONTENT OF MOTHER TUBERS AND MINITUBERS.

	Mother tuber*	Minituber*
Aspartic acid	23.09	20.86
Threonine	3.80	2.73
Serine	3.69	2.86
Glutamic acid	14.40	18.20
Proline	5.35	5.20
Glycine	2.97	2.27
Alanine	3.00	2.70
Valine	4.40	3.13
Isoleucine	2.85	2.20
Leucine	5.59	4.50
Tyrosine	2.60	2.00
Phenylalanine	3.69	2.27
Histidine	1.40	1.33
Lysine	4.64	3.33
Arginine	3.45	2.47

*values are g amino acid/100 g protein.

Future strategies

It is planned that the future research shall include: 1. production of macro tubers showing resistance to salinity, 2. evaluation of the macro tubers for chemical composition and cooking quality, 3. induction of new mutants adapted to high concentration of NaCl and resistance to drought and viruses.

Acknowledgements

The author is grateful to the International Atomic Energy Agency, Vienna for financial support of the project through Research Contract No.7623.

IN VITRO SELECTION OF INDUCED MUTANTS TO SALT-TOLERANCE: INDUCIBLE GENE REGULATION FOR SALT TOLERANCE

ILGA WINICOV
Department of Microbiology and Biochemistry
University of Nevada-Reno
Reno, NV 89557, USA

Abstract

A selection protocol to obtain salt tolerant calli, followed by regeneration and progeny-test of the regenerated plants for salt tolerance in rice was investigated. Callus cultures were initiated from salt-sensitive US elite rice lines and cv. 'Pokkali'. Salt-tolerant cell lines were selected from these by a single step selection procedure. The selected salt-tolerant lines grew well on medium with ± 0.5% or 1% NaCl, while the parent lines occasionally survived, but did not grow at these salt concentrations. Plants were regenerated from these cell lines through different passages on medium containing salt. Seed was collected from the regenerated plants and salt tolerance of R2 seedlings was compared with those regenerated without salt selection. Salt-tolerance was measured by survival and productive growth of newly germinated seedlings in Hoagland solution with 0.3% and 0.5% NaCl for 4 weeks. Heritable improvement in salt tolerance was obtained in R2 seedlings from one plant regenerated after 5 months selection. Survival and growth of these seedlings was equivalent to that from 'Pokkali' seedlings. These results show that cellular tolerance can provide salt-tolerance in rice plants.

1. INTRODUCTION

We have previously selected salt-tolerant alfalfa cell lines and regenerated salt-tolerant plants from these lines [1]. The regenerated plants were considerably more salt tolerant than the parent salt-sensitive plants. The trait for salt-tolerance was inherited through the seed in a semi-dominant fashion. We have extended these studies to rice using a single step *in vitro* selection protocol to select salt tolerant calli, followed by regeneration and progeny-test of the regenerated plants.

Salt tolerance in crop plants has been limited in significant improvement by breeding. Selection for salt tolerance might be possible in cell cultures. Plants, with improved salt tolerance, have been regenerated from salt-tolerant callus, showing that cellular tolerance can play an important role at the whole plant level. Isogenic salt-tolerant and salt-sensitive cell lines and plants allow us to identify genes that may contribute to improved salt tolerance and/or serve as molecular markers for tolerance

2. MATERIALS AND METHODS

Callus cultures were initiated from seeds of two elite US rice lines (L-202 and M-202) and *Indica* rice varieties 'Pokkali', 'IR 28' and 'IR 42'. Cv. 'Pokkali' has been classified as relatively salt resistant and served as a positive control in all experiments. The protocol for plant regeneration and selection is presented in Table I.

TABLE I. SELECTION FOR SALT TOLERANCE AND REGENERATION IN RICE.

Cell Culture Medium	Selection	Regeneration
Linsmaer & Skoog agar	+	+
0.3 µg/l kinetin	+	-
1.0 mg/l 2,4-D	+	-
100 mg/l tryptophan	+	+
1% NaCl (171 mM)	+	-

Culture conditions: 28°C in continuous light (30 µmol quanta/m^2/s).
Regeneration: roots and shoots in 4-8 weeks.

3. RESULTS AND DISCUSSION

Cell lines tolerant to 1% (0.171 M) NaCl were obtained from all calli. Several plants were regenerated from salt tolerant lines of L-202, M-202 and 'Pokkali'. The selection process and regeneration of the embryogenic callus had minimal adverse effect on the rate and extent of plant growth, since the regenerated plants were similar to those initiated from the parental seed stock. All plants flowered and in most cases were fertile. Seed was collected from the regenerated plants and the germinated R2 seedlings were tested for tolerance to salt. The salt tolerance of R2 seedlings was compared with plants regenerated from salt tolerant calli of L-202 that had been maintained on 1% NaCl for up to 5 months after selection. Heritable improvement in salt tolerance was obtained in R2 seedlings from one L-202(R4) plant that had been regenerated after 5 months of selection on salt-containing medium. The salt tolerance of these seedlings was comparable to that obtained with seedlings from 'Pokkali' under conditions where the unselected L-202 seedlings died.

Selection for salt tolerance and regeneration

Our finding of the apparent time dependence of selection in culture that yielded the L-202 regenerated plant with improved trait for salt tolerance provides practical information that may be useful for selection of other cell lines. It was apparent that rice cell cultures continue to maintain a mixed population of salt-tolerant and salt-sensitive cells even under conditions of a single step increase in lethal salt concentration in the culture medium. Under these conditions, short-term salt selected rice callus contains many embryogenic calli that do not carry heritable changes for the salt tolerance phenotype, and therefore necessitate screening for the tolerance in the progeny of the regenerated plants. However, the successful recovery of at least one regenerated plant with improved salt tolerance inherited in the R2 generation shows that the method of selection for salt tolerance at the callus level, followed by regeneration is applicable to selection in rice [2, 3]. However, we find that prolonging the selection process *in vitro* for rice improves the likelihood of regenerating plants with improved salt tolerance. Since prolonged growth in tissue culture is generally undesirable because of accumulation of potentially undesirable mutations, mutagenesis of the initial culture followed by a relatively shorter selection process might increase the yield of regenerated mutants with improved salt tolerance.

Studies on gene regulation

We have also focused on the characterization of altered gene expression in the salt tolerant cells and plants in order to identify genes that are likely to contribute to acquired salt tolerance in normally salt sensitive plants by identifying genes that are "up-regulated" by salt in our system of salt-tolerant alfalfa (Table II).

TABLE II. GENES UP-REGULATED BY SALT IN SALT-TOLERANT ALFALFA.

Gene	Characters
MsPRP-2	Encodes a root specific proline rich cell wall protein with a hydrophobic region in carboxyl terminus
Alfin-1	Putative zinc finger transcription factor
pA18	cDNA, unknown function
rbcS	
Cab	Nuclear genes for photosynthesis functions in chloroplast
rbcL	
psbA	
psbD etc.	Chloroplast genes for photosynthesis
H3	Cell cycle dependent and independent histone H3

We have concentrated our studies on two cloned genes in alfalfa that are induced at the mRNA level in salt tolerant alfalfa grown in salt solution. One of these genes (*MsPRP2*) encodes a root specific transcript, that accumulates dramatically in presence of salt. Translation of the coding segment of *MsPRP2*, suggested that it encodes a chimeric 40,569 kDa cell wall protein consisting of an amino-terminal signal sequence, a repetitive proline-rich sequence, and a cysteine-rich carboxyl-terminal sequence homologous to nonspecific phospholipid transfer proteins [4]. We have subcloned a 2.5 kb fragment of the promoter of this gene and are currently sequencing it to identify potentially important regulatory regions in this gene.

Another gene that we had identified in our differential screen of salt tolerant cell cultures is *Alfin1*, which encodes a putative zinc finger transcription factor. We are continuing work on this gene and have utilized an expression vector to engineer a construct for expression of this protein in *Escherichia coli*. We have finally achieved successful production of this protein in bacteria and are in the process of purifying it for DNA binding studies as well as preparation of antibodies.

The selected salt tolerant cell lines showed an association of chloroplast activation and increased mRNA accumulation for photosynthesis related genes with the salt tolerant phenotype. We have previously shown that at least some of these mRNAs are translated into functional products. Current on-going experiments show both transcriptional and post-transcriptional regulation of a number of genes in this system [5,6]. These results indicate that in the salt tolerant cells, post-transcriptional stabilization plays an active role in photosynthesis gene transcript accumulation. We also plan to investigate this aspect of gene regulation at the whole

plant level, especially in the roots of the salt tolerant plants grown in presence of salt.

REFERENCES

[1] WINICOV, I. Characterization of salt tolerant alfalfa (*Medicago sativa* L.) plants regenerated from salt tolerant cell lines. Plant Cell Rep. 10 (1991) 561-564.

[2] WINICOV, I. Characterization of rice (*Oryza sativa* L.) plants regenerated from salt tolerant cell lines. Plant Sci. 113 (1996)105-111.

[3] WINICOV, I. Improving salt tolerance of crop plants through tissue culture and molecular biology". Proc. Latvian Acad. Sci. 5/6 (1995) pp. A37.

[4] DEUTCH, C.E., WINICOV, I. Post-transcriptional regulation of a salt-inducible alfalfa gene encoding a putative chimeric proline-rich cell wall protein. Plant Mol. Biol. 27 (1995) 411-418.

[5] WINICOV, I., KRISHNAN, M. Transcriptional and post-transcriptional regulation of mRNA accumulation in salt tolerant alfalfa cells. Plant Physiol. Sup. 108 (1995) pp. 43a.

[6] SUN-HOFFMAN, L., WINICOV, I. Nuclear proteins that interact. Molec. and Cellular Biochem. 145 (1995) 159-168.

EVALUATION OF GENETIC VARIABILITY IN POTATO CV. 'PARDA-PASTUSA' OBTAINED THROUGH PHYSICAL MUTAGENIC AGENTS

A. ANGARITA-ZERDA, T. MOSQUERA, C. ÑUSTEZ
Tissue Culture Laboratory
Agronomy School
National University of Colombia
Santafé de Bogotá, Colombia

Abstract

'Parda-Pastusa' is probably the most important variety of potato in Colombia. Its susceptibility to frost and *Phytophthora infestans* can cause important losses in the crop. In order to induce genetic variability to select clones resistant to frost and late blight, disease-free micropropagated plantlets, obtained from meristem culture of indexed tubers were irradiated with gamma rays from a ^{60}Co source. Virus-free mericlones were initially irradiated with 0, 25, 50, 75 and 100 Gy or with 0, 10, 20 and 30 Gy. The optimal radiation dose was found to be 20 Gy. A mass propagation was carried out, and plantlets were irradiated with the optimal radiation dose. Clones derived from irradiated material were propagated for selection under simulated frost conditions (-7°C for 360 minutes) and co-cultured with extracts of fungus, *Phytophthora infestans*. The selected clones will be transferred to field conditions for evaluation of agronomic and genetic characteristics.

1. INTRODUCTION

Breeding for resistance to late blight and frost injury are important in potato improvement in Colombia. We have chosen the most important variety of potato in Colombia cv. 'Parda-Pastusa', which is very susceptible to frost and *Phytophthora infestans* (Mont.) de Bary. Considering the difficulties in selection for more than one character at one time, we have decided to select first for resistance to *Phytophthora infestans*. Previous research in our laboratory proved that *in vitro* selection against *Phytophthora infestans* using autoclaved mycelium extracts could be used for selection of callus and plantlets. We also improved protocols for isolation and multiplication of the fungus. However, after recent discussions with phytopathologists at our university, we have decided to select for resistance to *P. infestans* after adaptation of plants to *ex vitro* conditions, and mature plants, because of the variability in virulence of some strains of the pathogen. We had initially selected *in vitro* 81 variants, but most of them showed abnormal black leaves and very poor vigour probably due to the large number of micropropagations. The resultant seventeen variants will be adapted to field conditions in order to evaluate the resistance to the different strains of *Phytophthora infestans*.

We have planned the following protocol in this project. 1. Pre-germination of tubers and establishment of meristem-tip cultures. 2. Micropropagation of the mericlones obtained. 3. Selection of virus and viroid-free mericlones by ELISA test for PVX, PVY and PLRV, and molecular hybridization test for PSTV. 4. Micropropagation of virus and viroid-free mericlones. 5. Evaluation for gamma radio-sensitivity curve, and determination of the optimal radiation dose. 6. Micropropagation of the non-irradiated controls and mass irradiation with the optimal

dose. 7. Micropropagation of the irradiated node-derived clones. 8. Adaptation to *ex vitro* conditions and microtuber production. 9. Germination of microtubers and inoculation under controlled conditions with different isolates of *Phytophthora infestans*. 10. Evaluation of phenotypic and agronomic characters (including frost resistance) of variant clones.

2. MATERIALS AND METHODS

Plant material

Potato tubers of cv. 'Parda-Pastusa', obtained from the Colombian Central Potato Collection were pre-germinated by treating them with 200 ppm gibberellic acid (GA3) for 30 minutes.

Pre-germination and meristem tip culture

Tubers were kept under pre-germination conditions in pots, and after 4 weeks, shoots ca. 8 to 10 cm were used to establish aseptic meristem cultures. The leaves of shoots were removed, and nodal sections were surface sterilized with 70% ethanol for 30 seconds, then with 20% commercial clorox for 5 minutes, and finally rinsed three times with sterile distilled water. More than 120 meristems were excised and cultured on a medium consisting of MS salts [1], 100 ppm myo-inositol, 2 ppm calcium pantothenate, 1 ppm gibberellic acid, 3% sucrose, and 0.6% oxoid agar, with pH 5.8 After six weeks of exposure to the incubation condition (temperature 22+/-2°C; photoperiod 16 hr light), each derived plant was considered as a mericlone, and indexed individually to select for virus and viroid free condition..

Micropropagation of mericlones and indexing procedures

The mericlones obtained were micropropagated three times to obtain enough material for ELISA and molecular hybridization tests. Shoots with four to five nodes were incubated on the same medium but without agar and GA3. Culture conditions were similar as for meristem culture. Ninety clones were tested for PVX, PVY and PLRV, the most common viruses in Colombia, by ELISA test and the virus free mericlones tested for PSTV by molecular hybridization procedures as described by AGDIA Inc.

Evaluation of different doses of gamma radiation to determine optimal radiation dose

Initially, we evaluated effect of five doses of gamma radiation (^{60}Co), 0, 25, 50, 75 and 100 Gy on 40 virus and viroid-free plantlets each per treatment in a complete randomized design. We recorded the number of shoots and roots as parameters for morphogenetic potential. In the second assay, we evaluated the non-detrimental doses of 0, 10, 20 and 30 Gy to determine the optimal radiation dose. Analysis of variance and tests of contrast means (orthogonal polynomials) through general linear model procedure on the number of shoots and roots after each irradiation dose were carried out, and finally the optimal radiation dose was defined.

Irradiation dose and micropropagation

One hundred plantlets were irradiated with the optimal dose (20 Gy) and immediately transferred to fresh medium. Each node was considered as a different potential variant, and micropropagated with a given code. Three micropropagations have been carried till April 1996.

3. RESULTS AND DISCUSSION

Meristem culture

From 120 meristem cultured, only 90 produced plantlets. After three micropropagations, each mericlone was tested for PVX, PVY and PLRV; of these 16 mericlones were discarded because of their contamination with PVX or PVY or both. PLRV was not present in any of our mericlones tested. This is probably because this virus is easily eliminated through meristem culture. Similarly, PSTV molecular hybridization test showed no contamination in any of the 74 mericlones tested.

Determination of optimal radiation dose

In the initial study on irradiation with 0, 25, 50, 75 and 100 Gy, doses of 50, 75 and 100 Gy were either detrimental to growth or lethal. In the second assay, gamma radiation doses of 0, 10, 20 and 30 were tested. The analysis of variance and means (orthogonal polynomials) based on the general linear model procedure on the number of shoots and roots after the first and second micropropagation, showed 20 Gy as the optimal dose. After irradiation of a large number of *in vitro* plants with optimal dose, most of the 81 node derived clones were abnormal with black leaves and very poor vigor; the surviving 17 clones were propagated and rooted *in vitro* for the further studies.

Future prospects

It is proposed that the selected 17 variants clones will be cultured on the rooting medium of half- strength MS salts, 100 ppm myo-inositol, 2 ppm calcium pantothenate, 1 ppm IAA, 0.6% oxoid agar, with pH 5.8. The clones will be transferred in an aphid-proof greenhouse, and allowed to form microtubers, which will be pre-germinated for inoculation under controlled conditions with several isolates of *Phytophthora infestans*. Controls of non-inoculated variants will be transferred to field conditions to study their characters and frost resistance during the coldest months of December 1996 and January 1997.

Acknowledgements

Financial support for this project from the International Atomic Energy Agency, under Research Contract No. 7622/RB is gratefully acknowledged.

REFERENCES

[1] MURASHIGE, T., SKOOG, F. A revised medium for rapid growth and bioassay with tobacco tissue. Physiologia Planta. 15 (1962) 473-497.

INDUCTION OF MUTATIONS IN GARLIC BY COMBINED USE OF GAMMA-RAYS AND TISSUE CULTURE

ZHEN HAI ROU
Horticultural Research Institute
Shanghai Academy of Agricultural Sciences
Shanghai, China P.R. 201106

Abstract

Callus cultures were initiated from leaf explants of garlic on MS medium supplemented with 1.0 mg/l KIN + 1.0 mg/l IAA + 2.0 mg/l 2,4-D. Plantlets were induced from leaf calli on MS medium with 2.0 mg/l BA + 2.0 mg/l IAA. Bulblets were induced from plantlets on MS medium containing 3.0 mg/l ITA. Callus growth and plantlets induction were remarkably inhibited with irradiation doses of 8 and 10 Gy. It was found that doses of 3 and 5 Gy were suitable to induce variation. Somatic embryos were induced on MS medium supplemented with 2.0 mg/l 2,4-D + 500 mg/l casein, 1000 mg/l yeast extract and 3 to 5% sucrose.

1. INTRODUCTION

Garlic is an important crop in China. The objective of this research project is to induce mutants for tolerance to high temperature stress and resistance to garlic mosaic virus (GMV) by using *in vitro* techniques and radiation. For this purpose, *in vitro* regeneration of garlic plantlets and bulblets was studied from leaf explants, and different media were tested to obtain somatic embryogenesis. The effect of different doses of gamma-rays on callus growth of irradiated garlic leaves was investigated.

2. MATERIALS AND METHODS

The local variety of garlic, cv. 'Ga ding' was used in the experiments. Young leaves from germinating bulbs were used as explants. The protective leaves and storage leaves were taken from cloves, surface-sterilized in a solution of 70% alcohol for 10 sec., then in a solution of 0.1% mercuric chloride for 8 min., and rinsed 3 times with sterile distilled water. The lower parts of leaves were cut into 2 mm long pieces and used as explants. The explants were cultured on basal MS medium supplemented with various combinations of growth regulators such as kinetin (KIN), NAA, IAA, and 2,4-D, and maintained at 25°C under photoperiod of 16/8 hr light/dark with light intensity of 3000 lux. Nine different media, A1 to A9 (Table I) were used for callus induction from leaf explants. Eight media, S1 to S8 were used for plantlet induction from callus cultures (Table II). Three media R1-R3 were used for bulblet induction from plantlets. These were R1: MS + 1 mg/l ITA, R2: MS + 2 mg/l ITA. and R3: MS + 3 mg/l ITA. In addition, media G1-G5 and E1-E7 were tested for induction of somatic embryos from calli (Table III and IV).

^{60}Co was used as radiation source. The distance from the radiation source to material was 2.8 m and the radiation rate was 0.258 Gy/min. Doses of 1, 3, 5, 8 and 10 Gy were used in the present experiments.

TABLE I. MEDIA FOR CALLUS INDUCTION FROM LEAF EXPLANTS.

Medium	KIN mg/l	NAA mg/l	IAA mg/l	2,4-D mg/l
A1	1	-	-	2.0
A2	2	0.5	-	-
A3	2	-	10	2.0
A4	-	-	-	3.0
A5	1	-	1	-
A6	2	-	2	1.0
A7	-	-	-	2.2
A8	2	1.5	5	-
A9	1	-	1	2.0

TABLE II. MEDIA USED FOR PLANTLET INDUCTION FROM CALLI OF GARLIC.

Medium	KIN (mg/l)	BA (mg/l)	IAA (mg/l)	NAA (mg/l)
S1	-	-	-	-
S2	-	2.3	-	-
S3	2	-	2	-
S4	6	-	2	-
S5	2	-	6	-
S6	2	-	-	2
S7	-	2	-	2
S8	-	2	2	-

TABLE III. MEDIA USED FOR THE INDUCTION OF SOMATIC EMBRYOS FROM CALLI OF GARLIC.

Medium	P-CPA (mg/l)	2,4-D (mg/l)	Kinetin (mg/l)	IAA (mg/l)
G1	0.95	0.45	0.1	-
G2	0.95	0.90	0.1	-
G3	1.90	0.45	0.1	-
G4	1.90	0.90	0.1	-
G5	-	-	4.3	1.8

TABLE IV. MEDIA FOR THE INDUCTION OF SOMATIC EMBRYOS FROM CALLI OF GARLIC.

Medium	2,4-D (mg/l)	6-BA (mg/l)	Casein hydrolysate (mg/l)	Yeast extract (mg/l)
E1	1	-	-	-
E2	1	0.5	-	-
E3	1	1	-	-
E4	2	-	-	-
E5	2	0.5	-	-
E6	2	1	-	-
E7	2	-	500	1,000

3. RESULTS AND DISCUSSION

Callus induction from leaf explants

The results showed that all the six media A1, A3, A4, A6, A7, A9, were able to induce callus from the leaf explants. All media contained 2,4-D. This suggested that 2,4-D was effective in inducing calli from leaf explants. Among nine media, the medium A9 was the best to induce callus. The callus growth was rapid, callus size was about twice the size of explant after 45 days culture.

TABLE V. EFFECT OF VARIOUS MEDIA ON CALLUS INDUCTION FROM LEAF EXPLANTS.

Medium	Explant number	Callus induction frequency (%)
A1	20	100
A2	20	-
A3	20	-
A4	20	85
A5	20	-
A6	20	100
A7	20	90
A8	20	-
A9	20	100

To obtain calli with rapid growth and excellent texture, it was necessary to supplement MS medium containing 2,4-D with other auxins and kinetin. The medium A9 (MS medium containing 2 mg/l 2,4-D, 1 mg/l IAA and 1 mg/l KIN) was very effective in inducing calli, and the frequency of callus induction was 100%. The calli were characterized by rapid growth and excellent texture (Table V).

Plantlet induction from leaf callus

The results showed that five media combinations (A6-S7, A6-S8, A7-S2, A7-S7, A9-S4) were effective in inducing plantlets from calli. The frequency of induction in all cases was more than 80%. This suggested that the calli induced on the MS medium containing 2,4-D when transferred to MS medium lacking 2,4-D, produced plantlets. The plantlet induction frequency was affected by the composition of medium used for callus induction. For example, when the calli were induced on the media A6, A7, A9, respectively, and were transferred to the medium S8, the frequency of plantlet induction was 82.8, 57.1 and 77.1 per cent, respectively. This suggested that the plantlet induction was influenced by callus induction medium indirectly. Improved frequency of plantlets and number of plantlets per callus were obtained on media A6-S7, A7-S2. Two media, S2 and S7 which contained BA were effective in inducing plantlet formation (Table VI).

TABLE VI. EFFECT OF MEDIA ON PLANTLET INDUCTION FROM GARLIC LEAF CALLI.

Media		Plantlet		Mean No. of Plantlets per callus
medium for inducing callus	medium for inducing plantlets	No.	Frequency (%)	
	S1	1	2.8	0.1
	S2	14	40.0	1.5
	S3	19	54.3	1.4
	S4	8	22.8	0.5
A6	S5	16	45.7	0.6
	S6	20	57.1	1.9
	S7	31	88.6	3.9
	S8	29	82.8	2.9
	S1	-	-	-
	S2	31	89.6	4.5
	S3	23	65.7	1.9
	S4	9	25.7	0.6
A7	S5	14	40.0	1.6
	S6	16	45.7	1.6
	S7	29	82.8	2.5
	S8	20	57.8	1.8
	S1	1	2.8	0.1
	S2	17	48.6	1.6
	S3	23	65.7	3.5
	S4	30	85.7	3.0
A9	S5	20	57.1	1.2
	S6	25	71.4	2.7
	S7	25	71.4	2.9
	S8	27	77.1	3.7

The bulblet induction from plantlets

The results showed that plantlets cultured on media S1, R1, R2, R3 were able to produce bulblets *in vitro*. ITA in the media R1, R2, R3 was effective in accelerating bulblet formation.

TABLE VII. EFFECT OF VARIOUS LEVELS OF ITA ON BULBLET INDUCTION.

Medium	Number of explants	Bulblet induction frequency (%)	Number of bulblets per callus
S1	60	58.3	1.7
R1	60	40.0	0.8
R2	60	78.3	1.9
R3	60	71.7	2.3

The bulblet number increased with the increase of ITA in medium. The number of bulblets induced on medium R3 (containing 3 mg/l ITA) was four times more than that on the medium R1 (containing 1 mg/l ITA). But the bulblets induced on the media containing ITA were smaller than those on MS (S1) medium without any auxin.

Somatic embryo induction from leaf calli

When leaf explants were cultured on media G1-G4 for 45 days, and then transferred onto the medium G5, or on media E1-E6 for 120 days, no somatic embryos were induced. Only E7 medium (MS medium containing 2 mg/l 2,4-D + 500 mg/l casein hydrolysate, 1000 mg/l yeast extract, with high sucrose, 3% to 5%) was effective in inducing somatic embryos. The calli formed after 14 days of culture, and the somatic embryos began to form after 40 days of culture. After 70 days of culture, surface of calli was covered by many small globules with smooth surface, some of these could be easily isolated from calli. After 110 days culture, all calli were able to produce somatic embryos which were easily isolated from calli (Table VIII).

TABLE VIII. INDUCTION OF EMBRYOGENIC CALLI AND SOMATIC EMBRYOS FROM LEAF CALLI.

Number of explants	Embryogenic calli		Number of somatic embryos	
	Number	Frequency (%)	Total	Mean per callus
40	40	100	489	12.2

The mean number of somatic embrys per callus was 12.2 after 110 days culture. The number of somatic embryos on single callus ranged from 1 to 60.

Effect of gamma rays on growth of calli from irradiated leaves

Leaves were irradiated with ^{60}Co gamma rays, with doses 1, 3, 5, 8 and 10 Gy. The leaves were cut into 4 x 4 mm pieces and used as explants and cultured on MS medium containing 1 mg/l KIN, 1 mg/l IAA, 2 mg/l 2,4-D. After 50 days culture, the formed calli were weighed. Analysis of variance gave an F value of 5.707, and at 0.01 per cent level (5,195) of 3.11; the differences between the treatments were highly significant. LSD test showed that the callus growth was promoted with 1 and 3 Gy, and inhibited with 8 and 10 Gy. Especially, the mean weight of individual calli in the treatment with 10 Gy was 76.3% less than that in the non-irradiated control (CK). Therefore, we used 5 Gy dose to induce mutants from irradiated leaves.

TABLE IX. EFFECT OF GAMMA RAYS ON GROWTH OF CALLI FROM IRRADIATED LEAVES.

Doses (Gy)	Number of explants	Mean weight of single callus (mg)	In comparison with CK (%)
CK	40	0.0885	
1	40	0.0975	+ 10.17
3	40	0.0965	+ 9.04
5	40	0.0858	- 0.03
8	40	0.0600	- 32.20
10	40	0.0210	- 76.27

Effects of different doses of gamma rays on plantlet differentiation

Leaves were irradiated with ^{60}Co gamma rays with 1, 3, 5, 8 and 10 Gy. The irradiated leaves were cut into 4 x 4 mm piece and used as explants. And then the explants were cultured on the MS medium containing 1 mg/l KIN, 1 mg/l IAA and 2 mg/l 2,4-D for inducing calli. After 35 days, the calli were cultured on the MS medium containing 6 mg/l KIN and 0.5 mg/l IAA for 70 days to induce plantlet. The results showed that the frequency of plantlet induction in the non-irradiated control (CK), 1 and 3 Gy dose were 85.5, 83.3 and 77.7%, respectively, but those in the higher dose (5, 8 and 10 Gy) treatments were only 75.9, 64.3 and 73.5%, respectively. The number of plantlets per callus in the non-irradiated control (CK), 1 and 3 Gy treatments were 3.9, 3.8 and 3.1, respectively, but those in the higher dose (5, 8 and 10 Gy) treatments were 2.2, 2.4 and 2.5, respectively. Therefore, we could induce garlic variant lines by irradiating garlic leaves at 5 GY dose and using tissue culture method.

TABLE X. EFFECTS OF GAMMA RAYS ON SEEDLING DIFFERENTIATION FROM IRRADIATED GARLIC LEAVES.

Dose (GY)	Number of explants	Calli with seedlings		Number of differentiated	
		Number	Frequency (%)	Total	Per explant
CK	28	24	85.7	108	3.9
1	30	25	83.3	113	3.8
3	27	21	77.7	83	3.1
5	29	22	75.9	65	2.2
8	28	18	64.3	68	2.4
10	34	25	73.5	85	2.5

Effect of gamma rays on plantlet differentiation from garlic leaf calli.

Leaf explants were cultured to initiate callus on the MS medium containing 1 mg/l KIN, 1 mg/l IAA and 2 mg/l 2,4-D. After 35 days culture, the calli were irradiated with 1, 3, 5, 8 and 10 Gy gamma rays, and the irradiated calli were cultured on the MS medium containing 6 mg/l KIN and 0.5 mg/l IAA for 70 days to regenerate plants. The results showed that plant induction was inhibited in the treatments with irradiation at all doses. In case of higher gamma ray doses, the frequencies of plant regeneration was markedly reduced. The frequency of plant induction in the non-irradiated control (CK) and 1 and 3 Gy dose was 100, 90 and 95%, respectively; but in those give higher doses, 5, 8 and 10 Gy, it was 55, 55 and 30%, respectively. The mean number of plants per callus in the non-irradiated control (CK), 1 and 3 Gy was 10.3, 5.4 and 6.5, respectively; but in the treatments with higher doses, 5, 8 and 10 Gy was 2.5, 2.6 and 1.3, respectively. Therefore, 3 or 5 Gy dose appeared to be optimal to induce mutant from irradiated leaf calli.

TABLE XI. EFFECT OF GAMMA RAYS ON PLANT REGENERATION FROM GARLIC LEAF CALLI.

Irradiation dose (Gy)	Number of explants	Calli with seedlings		Mean No. of seedlings differentiated	
		Number	Frequency (%)	Total	Per explant
CK	20	20	100	205	10.3
1	20	18	90	107	5.4
3	20	19	95	123	6.5
5	20	11	55	49	2.5
8	20	11	55	52	2.6
10	20	6	30	25	1.3

MUTAGENESIS IN SWEET POTATO

ZHEN HAI ROU
Horticultural Research Institute
Shanghai Academy of Agricultural Sciences
Shanghai, China P.R. 201106

Abstract

Stem explants of cv. 'Gao line 14' were cultured on the MS medium supplemented with 0.01 mg BA+1.0 mg NAA+2.0 mg IAA/l. The calli thus formed were irradiated with 5 Gy from a ^{60}Co gamma-ray. Irradiated calli were transferred to half-strength MS medium containing 2.0 mg KIN+2.0 mg IAA/l to induce plant regeneration. An early ripening mutant with high yield and low tuber number was selected among the regenerated plants grown in a field. Embryogenic calli were obtained from stem pieces, stem-tips and leaves on MS medium supplemented with 2,4-D.

1. INTRODUCTION

Sweet potato, cv. 'Gao line 14' (80514) was introduced from Japan in 1988. It is an excellent variety for food industry in Japan. At present 10,000 tons processed sweet potato are exported each year from Shanghai to Japan. However, the yield of this variety is low when it is planted in Shanghai and other areas of China, because of its late production of tubers. In Shanghai, the native sweet potato variety generally starts bearing tubers in the mid-August, but the 'Gao line 14' produces tubers in mid-September. The harvest of sweet potato is at the end of October in Shanghai.

2. MATERIALS AND METHODS

Since 1991, we have used the technique of tissue culture in combination with ^{60}Co gamma-ray irradiation to induce mutation in the cv. 'Gao line 14' to obtain an early tuber bearing mutant. The mutant was obtained as follows. Stem explants of cv. 'Gao line 14' were cultured on the MS medium [1] supplemented with 0.01 mg BA+1.0 mg NAA+2.0 mg IAA/l for inducing callus. After 14 days, the calli thus formed were irradiated with 5 Gy from a ^{60}Co gamma-rays (irradiation distance was 2 m, rate was 0.6825 Gy/min). Two weeks after irradiation,the calli were transferred to half-strength MS medium containing 2.0 mg KIN+2.0 mg IAA/l to induce plant regeneration. The plantlets formed were transplanted in soil, and the surviving plants were grown in field. Between 1991 to 1994, we cultured a total of 12,000 explants, and obtained 1585 plantlets.

3. RESULTS AND DISCUSSION

Selection of sweet potato mutant 91-C3-15

We have obtained a mutant plant from more than one thousand plantlets irradiated in October 1995. The plant yielded two tubers weighing 980 g, whereas the mean yield per plant in 'Gao line 14' is three tubers with 750 g. During 1996, we will observe several characters of

the mutant in the field including tuber-bearing stage, processing quality and other genetic traits.

Somatic embryogenesis in sweet potato

Leaves, petioles, stems, roots and stem-tips of sweet potato cv. 'Su.Su.No.1' were used as explants and cultured on the MS media containing different levels of 2,4-D to induce embryogenic calli. The frequency of embryogenic calli from leaf explants was 47 and 55% on MS media containing 0.02 and 0.5 mg 2,4-D/l, respectively. The frequency of embryogenic callus from petiole explants was 18.8, 30.0 and 33.3 on the MS media containing 0.01, 0.02 and 0.05 mg/l 2,4-D, respectively. The frequency of embryogenic calli from stem explants were 43.8, 62.5 and 33.3% on MS media containing 0.02, 0.05 and 0.1 mg 2,4-D/l, respectively. The induction frequency of embryogenic callus from stem-tip explants was 75% on the MS media containing 0.05 mg/l 2,4-D. We failed inducing embryogenic callus from root explants on all the MS media containing 2,4-D.

REFERENCES

[1] MURASHIGE, T., SKOOG, F. A revised medium for rapid growth and bioassay with tobacco tissue. Physiologia Planta. 15 (1962) 473-497.

CONCLUSIONS AND RECOMMENDATIONS

GENERAL CONCLUSIONS

1. Significant progress has been made in all research programmes since the last Research Coordination Meeting. Protocols for micropropagation have been standardized, the materials have been irradiated and multiplied, and in some cases are already in field tests. All research programs reported good regeneration and multiplication of the plant material following irradiation.

2. In radio-sensitivity tests, 20 Gy was found to be the optimal dose for irradiating *in vitro* grown potato plantlets. In sugarcane, the optimal dose for *in vivo* nodal cuttings was 20 Gy and 3 Gy for *in vitro* callus cultures. The optimal dose for *in vitro* irradiated shoot buds in pineapple was between 20 to 30 Gy.

GENERAL RECOMMENDATIONS

1. It was re-emphasized that for *in vitro* mutation induction, the initial material should be disease free, and established from indexed, virus and disease-free plants to avoid any inadvertent and unwanted mutations in the pathogens.

2. It was stressed that large populations need to be obtained for selection of mutants following established protocols.

3. As far as possible, the selected plants should be tested in more than one location.

4. It was suggested that during selection useful agronomic attributes and qualitative traits should be included (see specific recommendations).

5. Stringent selection methods should be used. However, if this is not feasible, great care should be taken in testing putative mutants to avoid false positives.

6. The selected mutants should be tested for the physiological basis of tolerance to salinity, i.e., whether these are salt uptakers or excluders.

7. Other investigations such as DNA fingerprinting, somatic hybridization, physiological analysis of variants should be done only after the desired variants have been obtained.

8. Use of molecular biology/biotechnology for mutant characterization may be included when specific markers become available.

9. In most cases, the population size was not sufficiently large for selection. It was again recommended that a large number of *in vitro* plants may be obtained, irradiated and multiplied. The target population size for testing for stress conditions should be ca. 10,000. Depending upon the facilities available, the plant populations should be produced and tested in batches.

SPECIFIC RECOMMENDATIONS

POTATO

To prevent losses during storage, the planting materials should be stored in suitable conditions; for example the storage of potato micro- and mini- tubers may be done at 4-6°C under diffused light conditions. The storage of mini-tubers could also be done at ambient temperature by placing the minitubers in a single layer under diffused light. To increase population size for selection, the available *in vitro* technology for the production of minitubers could be used. The minitubers can be then used for the production of normal seed potato tubers for field evaluation.

In Pakistan, to develop salt-tolerant potato mutants through *in vitro* techniques, larger populations of the irradiated material should be produced for field testing. Following *in vitro* selection, the material should be tested in saline soils.

In Egypt, additional irradiated plants should be micropropagated. These plants along with those already produced should be used for minituber production under normal (non-saline) conditions. The minitubers could be grown directly for field testing under saline conditions.

In Colombia, selection for frost resistance should be done *in vitro* at -7°C in the laboratory. The selected plants should be inoculated under controlled conditions in a greenhouse with several isolates of *Phytophthora infestans*. The surviving plants should then be grown under field conditions to test agronomic performance. The selected plants should be micropropagated, and used for the production of minitubers. These minitubers should be grown for field testing.

In India, microtuber formation should be carried out at 28°C under 12 hr day-length. Microtubers could be used for selection to screen heat tolerant variants. Microtubers should be grown in pots for the production of minitubers which should be then field tested.

SWEET POTATO

In China, the initial experiments suggest that the irradiation of callus produced non-chimeric mutant phenotypes. The calli should be irradiated with 5 Gy gamma rays and subsequent plant regeneration should be continued. Attempts should be made to increase the frequency of regenerated plants from irradiated calli through organogenesis and somatic embryogenesis. The material so produced should be multiplied and tested for drought tolerance, yield and quality.

In Peru, it is desirable to improve the *in vitro* methodology for large scale pre-selection for salt tolerance. The sources of explants should be reduced; auxiliary buds or apical meristem would be sufficient as explants. In addition to *in vitro* pre-selection for salt tolerance, testing of irradiated materials should be carried out under salt affected soils.

GARLIC

Sufficient material of garlic has been produced and is being multiplied in a greenhouse. The irradiated and *in vitro* produced materials should be multiplied further and evaluated under high heat (ca. 35°C) conditions and for resistance to garlic mosaic virus (GMV).

SUGARCANE

In Bangladesh, the plants selected as resistant to red-rot should be reinoculated to confirm their resistance. To determine stability of selected traits, progeny of selected variants should be tested in successive propagations. Useful agronomic and qualitative traits, e.g. yield and high sugar content, should be selected along with the desired traits. Performance trials on yield and quality evaluation should be initiated on the selected variants. Selection of plants for delayed flowering should be done 2-3 months after the normal flowering duration.

Both in Bangladesh and Pakistan, the populations of regenerated plants from *in vitro* mutation induction are too small to be meaningful for selection, hence population size should be increased. Micropropagation should be done to produce large numbers of variants for field selection.

ALFALFA

Selection pressures for salinity tolerance should be severe enough to reduce false positives by lowering survival to 1% or less after single shock to salt stress. Future efforts should be directed to elucidating the role of the cloned DNA binding factor (alfin-1) in gene regulation and its function under salt stress in salt-tolerant alfalfa.

PINEAPPLE

The M_1V_1 plants produced from radio-sensitivity tests should be planted in field for selection. Routine selection protocols for drought and heat tolerance need to be developed. Low humidity should be maintained during selection for drought and heat-tolerance. Useful agronomic and qualitative traits, e.g. spineless leaves and small fruit size, should be selected in addition to the targeted traits. Selection of variants should conform to the varietal requirement of the traditional pineapple growing areas in Ghana rather than the northern region.

LIST OF PARTICIPANTS

BANGLADESH

M.A.Q. Shaikh
Bangladesh Institute of Nuclear
Agriculture
P.O. Box No. 4
Mymensingh

CHINA, P.R.

H. Zhen
Horticultural Research Institute
Shanghai Academy of Agricultural Science
3000 Beidi Road
Shanghai

COLOMBIA

T. Mosquera
Faculty of Agriculture
National University of Colombia
Bogota

EGYPT

M. Sharabash
National Center for Radiation
Research and Technology
P.O. Box 29
Nasr City
Cairo

GHANA

Y. Lokko
Biotechnology and Nuclear
Agriculture Research Institute
P.O. Box 80
Legon
Accra

INDIA

J.L. Minocha
Biotechnology Centre
Punjab Agricultural University
Ludhiana
Punjab

PAKISTAN

Y. Zafar
Plant Biotechnology Division
National Institute for Biotechnology and
Genetic Engineering (NIBGE)
P.O. Box 577
Faisalabad

PERU	A. O. Gutierrez-Rosati National Agraricultural University Departamento de Biologia P.O. Box 456 La Molina Lima
USA	I. Winicov Department of Biochemistry and Microbiology University of Nevada Reno, NV 89557
SCIENTIFIC SECRETARY	B.S. Ahloowalia Joint FAO/IAEA Division International Atomic Energy Agency A-1400 Vienna