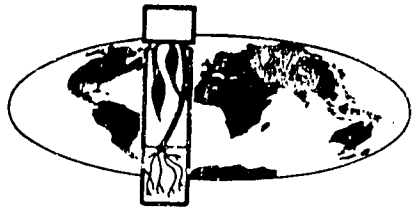

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1 INTROGRESSION OF ROOT-KNOT NEMATODE RESISTANT GENES FROM LYCOPERSICON SPECIES INTO TOMATO THROUGH EMBRYO CULTURE

Mohamed Ammati, T. Murashige and I.J. Thomason. Department of Plant Pathology, Institut Agronomique et Veterinaire Hassan II, B.P. 121 Ait Melloul, Agadir, Morocco

Screening of accessions of exotic species of tomato for resistance to root-knot nematodes and high soil temperatures resulted in selected clones of Lycopersicon peruvianum and L. glandulosum with good levels of resistance. The present study was aimed at introgressing these exotic germplasm genes into tomato through embryo culture. M.S. medium was compared to another containing high salts, high sucrose and low vitamins and amino acids (HLH) to regenerate plantlets from hybrid embryos.

As many as 20 out of 20 excised embryos of some Lycopersicon sp. crosses survived and formed callus. Plantlets were not regenerated on any media. Another experiment was conducted to determine the age threshold for embryo culture. Twenty-five days after pollination appeared to be a suitable time to rescue the hybrid embryos in our incompatible crosses.

2 RAPID MULTIPLICATION OF TECTONA GRANDIS LINN. BY TISSUE CULTURE

Pimchai Apavatjrut[1] and Apichart Kaosa-ard[2]
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The micropropagation system for mass propagation of teak was demonstrated. Shootlets obtained from in vitro grown seedling derived from selected seeds or buds from mature elite clones can be used for rapid multiplication. Either the top or single-nodal explants from the shootlets were induced to form multiple shoots on an agar medium containing Schenk and Hildebrandt (1972) macro nutrients + Murashige and Skoog (1962) micro nutrients + BAP and IBA at 1 mg and 0.3 mg/l respectively. The rate of multiplication was increased generally up to 15 folds at every 8 week intervals. Clonal variation was also found. Shootlets having two pairs of leaves were successfully rooted either in vitro or in vivo. The roots obtained had tap-root like system.

3 MICROPROPAGATION OF TEA (CAMELLIA SINENSIS L.)

Paul V. Arulpragasam and Razia Latiff, Tissue Culture Project, Tea Research Institute, Talawakele, Sri Lanka.

Although there is a very successful and efficient method for the vegetative propagation of tea, it has its own limitations and is unable to cope with the present day need to replant large extents of old debilitated old seed tea, with high yielding quality clones, to increase rapidly the production and the productivity of our tea lands.

Work was therefore initiated recently at the TRI for the micropropagation of tea. Since, only a few successful attempts have been made so far in the micropropagation of tea, our immediate objective was to develop a suitable culture technique.

Shoot tips and nodal segments with axillary buds were used as explants. Contamination and browning of the explants in culture were major problems in the early stages. Contamination was overcome to a great extent by double sterilization with chlorox (15%) and mercuric chloride (0.2%). Browning was prevented by using low concentration of salts in the establishment medium. Shoot multiplication was successful in two media, both consisting of Murashige and Skoog salts supplemented with modified organic and hormonal components. Micro-cuttings of shoots produced in culture have been subcultured successfully for three generations for further shoot production. Attempts are now being made to root these micro-cuttings.

Callus formation has also been induced in cotyledon and stem tissues. A good rate of multiplication of the calli has been obtained and initial attempts to regenerate plants from callus tissue have been successful.

Results obtained so far indicate that the micropropagation of tea is a distinct possibility in the future.

4 PROPAGATION OF CARNATION BY TISSUE CULTURE

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*Department of Horticulture, Faculty of Agriculture, Zagazig University, Egypt.

Propagation of carnation by lateral shoots in EGYPT is still a problem, because it is carried out only during February to April.

Terminal and lateral buds have been explanted on Murashige and Skoog medium (1962), after some modifications.

Lateral shoots showed more rooting and shooting formation than terminal shoots. The balance between auxin (IBA or NAA) and cytokinin (BA or 2 ip) showed clear trend in affecting the rooting and shooting formation.

Subjecting lateral shoots to different doses of gamma-irradiation before the explanting resulted in enhancing the rooting and shooting formation, especially with the lower doses.

5 ROLE OF CULTURE MEDIUM IN MICROSPORE EMBRYOGENESIS
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The experiments conducted on *Datura metel* and *Petunia hybrida* to identify the cause(s) of promotory effect of cold pretreatment on *in vitro* androgenic response, revealed that although this pretreatment lowers the mortality rates of induced as well as uninduced microspores but the enhancement in response seems to be due to the increased incidence of androgenesis and advanced induction. The unexpected observation emanating from these investigations was induction of androgenesis by cold pretreatment *without* any culture medium. The prechilling of floral buds, in some undiscernible manner, induces the microspores to switch over to sporophytic mode of development which is manifested in the form of nuclear or cell divisions when buds are incubated at relatively higher temperatures (12-25 °C). The significant point is that for such a transition no external supply of nutrients is required. This observation unequivocally proves that for induction of androgenesis and its early expression culture medium is *not* at all required. Rather, the observed enhanced plantlet yield in the cultures raised from buds kept at higher temperatures for a day subsequent to the cold pretreatment as compared to that of those raised from only the cold pretreated buds, is indicative of its inhibitory effect during the initial stages of androgenesis. However, since the somatic tissues of anther cannot support the growth of induced units indefinitely, a suitable milieu in the form of a culture medium has to be provided for their further development.

6 *IN VITRO* VIRUS ELIMINATION AND IDENTIFICATION OF VIRUS CONTAMINATION IN TAMARILLO (*CYPHOMANDRA BETACEAE*)
M. Barakchi and G. Balasingam. Plant Physiology Division, Department of Scientific and Industrial Research, Private Bag, Palmerston North, New Zealand

Almost all tamarillo plantations in New Zealand are infected with viruses. CMV, ArMV, TaMV, and an unidentified virus (XMV) all infect tamarillo; however, the last two viruses are most common. The infected plants do not show signs of infection at temperatures above 20°C; however, fruit quality and quantity are reduced in the infected plants.

Nodal bud segments from infected tamarillo plants were established in MS medium containing 0.25-1.00 mg/l BAP at incubation temperatures of 16-32°C. The effect of incubation temperature and its duration on shoot growth and virus concentration in the shoots were studied. When regenerated shoots were transferred to 16°C only shoots incubated at high temperatures for certain periods did not produce the typical mosaic virus infection sign and the rest did, indicating that virus elimination could have occurred and *in vivo* tests confirmed this. Electron microscopy and serological experiments are also underway to confirm the presence or absence of virus contamination in response to *in vitro* temperature treatments. The possibility of a single mild thermotherapy during micropropagation and a reliable visual identification of virus-infected plants in cultures by incubation at lower temperatures will be attractive and economical to the industry.

7 IN VITRO REGENERATION, REJUVENATION, AND COLD STORAGE OF TAMARILLO (CYPHOMANDRA BETACEAE)

M. Barghchi. Plant Physiology Division, Department of Scientific and Industrial Research, Private Bag, Palmerston North, New Zealand

Tamarillo is a perennial sub-tropical fruit belonging to Solonaceae. It is planted in South and Central America, Sri Lanka, India, South East Asia, New Zealand and Australia. Various explants (i.e. nodal bud segments, internodes, leaves, inflorescence, anther) from mature tamarillo plants were established in vitro in MS medium supplemented with plant growth regulators. Shoot regeneration was achieved from all explants except from anther culture. However, callus culture was established from anther culture. The produced shoots rooted readily in MS medium supplemented with 1-2 mg/l IBA. By reducing humidity plantlets were hardened off in culture, reducing the need for maintenance of high humidity at transplanting stage to soil. Maturity of cultured explants could be maintained in vitro for a considerable time; however, they eventually returned to juvenile stage. Rejuvenation of mature explants in vitro was studied and the more frequent regeneration cycle induced earlier rejuvenation. Adventitiously produced shoots were rejuvenated in the first generation. The performance and period before plantlets were able to flower in vivo were studied. A method for in vitro cold storage of tamarillo is established and various factors such as preconditioning of explant, morphology of explant and cold storage conditions were studied.

8 PLANT TRANSFORMATION FOR CROP IMPROVEMENT: VIRUS DISEASE AND HERBICIDE RESISTANCE AS USEFUL TRAITS

Roger N. Beachy, Patricia Powell Abel, Richard E. Nelson, Steven G. Rogers, Dilip Shah and Robert T. Fraley. Dept. of Biology, Washington University, St. Louis, MO 63130, and Monsanto Company, Chesterfield, MO 63198

Development of techniques to introduce foreign genes into plants and subsequent regeneration to whole plants provide agriculturalists with an additional source of genetic material. Recently these techniques have been used to produce plants that 1) are cross-protected against virus infection, and 2) resistant to the herbicide glyphosate. In the first example a nuclear gene that encoded the coat protein of TMV was expressed in transgenic tomato and tobacco plants. Plants expressing this gene were cross-protected against infection by several strains of TMV and tomato mosaic virus. The implications of these research findings for applications to field agriculture will be discussed. We will also present the results of experiments demonstrating that transgenic plants that express a gene for EPSP synthase, an enzyme in the shikimic acid biosynthesis pathway, are tolerant to application of the herbicide Roundup . The active component in Roundup is glyphosate, a molecule whose mode of action is well described. Transgenic plants that overproduce EPSP 20 to 40 fold are tolerant to glyphosate levels that are damaging to normal plants. Plants that express either the viral coat protein gene or the EPSP synthase gene appear normal throughout development.

9 SOMATIC EMBRYOGENESIS IN TISSUE CULTURES OF SOYBEAN (GLYCINE MAX. I.) AND BRASSICA SP.

H.K. Cheema, Botany Department, Panjab University, Chandigarh -160014, India.

Tissue culture studies have been aimed at establishing conditions controlling morphogenesis i.e. to bring about maximum high frequency of somatic embryo genesis and plant regeneration. This report describes the development of morphogenetically competent cultures of Soybean and Brassica with the induction and development of embryogenic callus. Embryoids could be observed in juvenile leaf and stem callus of soybean induced on MS + S (2 %) + 2, 4-D (4-8 ppm). The embryoids remained only at the globular stage on 2,4-D enriched medium but when 2,4-D was omitted from the medium, the embryoids developed further to torpedo and heart-shaped stages. Globular, torpedo and heart-shaped embryoids could be observed in cotyledonary leaf, stem and bud callus of Brassica induced on 2,4-D containing medium. Although somatic embryogenesis was initiated on Murashige and Skoog's medium supplemented with sucrose and 2,4-D but the development of embryoids occured after the cordinate removal of 2,4-D from the medium. No regeneration of plantlet was, however, observed on this medium. In order to initiate differentiation of a complete plant, the embryogenic callus, was transferred to variously modified MS medium. After 4 month of cutting on the medium containing succrose alone, shoots regenerated which were followed by the differentiations of roots.

10 THE STUDY OF A SWEET POTATO VIRUS AND ITS ELIMINATION BY APEX CULTURE IN MOROCCO

R'kia Cheick, Benham E.L. Lockhart and Mohamed Aaouine. Complexe Horticole d'Agadir, B.P. 121 Ait Melloul, Agadir, Morocco

Sweet potato in Morocco was frequently found with virus-like mosaic symptoms. Such plants were found to be infected with a flexuous filamentous virus approximately 900 nm long. The virus was transmissible mechanically and by Myzus persicae following a brief acquisition feeding period. The virus was not eliminated by heat treatment for 7 months at 38-40 C. However, the virus was eliminated by culturing shoot apices on a modified MS medium containing, in mg/l: thiamine.HCl 20; i-inositol 100; sucrose 30,000; gelrite 2,000; kinetin 100; and IAA 30.

Incorporation of activated charcoal in the medium prevented callus formation and enhanced explant growth.

The regenerated plants were indexed by electron mecroscopy and were found to be virus-free.

11 SOMACLONAL VARIATION: A COMMERCIAL PLANT BREEDER'S PERSPECTIVE.

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The recognition that stable variation can be expressed in plants regenerated from tissue culture has led to the rapid interjection of this technology into plant improvement programs. Manipulating this variation in cell cultures poses the same type of challenge to biologists that the variation within a segregating population of plants poses to plant breeders. Effective management of this variation should be given priority consideration even though the causal mechanisms of somaclonal variation (SCV) are not yet understood.

Recommendations for improving the management of SCV could be placed into three major categories. First, each research program should include an explanation as to why a particular phenotype is expected to be recovered from SCV within the time constraints placed upon commercial plant breeders for varietal release. The second category prescribes the culture conditions needed to maximize the production of embryogenic callus which is often cultivar specific. The third category specifies the amount of time the callus is to be left in culture until regeneration is initiated. Within these categories the following items should be considered: the number of embryos selected from which cultures will be initiated, choice of germplasm, selection schemes to be used in culture and the way in which the somaclones will be integrated into a breeding program for agronomic evaluation.

12 INTERSPECIFIC HYBRIDIZATION THROUGH EMBRYO RESCUE IN COWPEA

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Cowpea (Vigna unguiculata (L.) Walp.) is an important food legume throughout the tropics covering Asia, Africa and Central and Southern America. However, the average yield of local varieties is low ranging from 200 to 300 kg/ha due to their susceptibility to a number of diseases and insect pests. The International Institute of Tropical Agriculture (IITA) which has worldwide mandate for cowpea improvement, has made significant progress in developing improved cowpea varieties which combine multiple disease and insect resistance. However, these are still susceptible to Maruca pod borer and coreid bugs because good sources of resistance to these insects are not available within the cultivated and crossable wild germplasm collection of cowpeas. Several wild species of cowpea such as Vigna vexillata and Vigna pubescens exist which have pubescence and other traits which may confer partial protection from these insects. However, these species are difficult to cross with Vigna unguiculata through conventional methods. The hybrid pods between V. pubescens and V. unguiculata develop normally but the seeds are extremely shrivelled and do not germinate. Attempts to rescue hybrid embryos of V. unguiculata x V. pubescens have been successful using in vitro culture and the F1 plants raised to maturity. These plants are partially sterile and cytogenetic studies are underway to elucidate chromosonal behaviour. Backcrosses are also being made to transfer hairy character from V. pubescens to V. unguiculata. Meanwhile concerted efforts are being made to rescue the hybrid embryos between V. vexillata and V. unguiculata.

13 ELIMINATION OF P.L.R.V. AND P.V.Y. POTATO VIRUSES BY APEX CULTURE IN MOROCCO

Zahra Ferji, Mohamed Aaouine and Benham E.L. Lockhart. Complexe Horticole d'Agadir, B.P. 121, Ait Melloul, Agadir, Morocco

Shoot apices of some potato clones were cultured on a modified MS medium containing, in mg/l: 0.1 thiamine.HCl; 0.5 pyridoxine.HCl; 0.5 nicotinic acid; 30,000 sucrose; 30 AIA; and 20 kinetin.

The incorporation of charcoal in the medium was necessary to prevent callus formation, to avoid explant oxidation and to ensure plant regeneration. The regenerated plants were tested for freedom from P.L.R.V. and P.V.Y. using "ELISA" technique. The viruses were eliminated in 83% of cases for P.L.R.V. and 74% for P.V.Y.

14 RAPID PROPAGATION OF RED GINGER (Zingiber officinale Rosc.) THROUGH TISSUE CULTURE TECHNIQUE

Endang Gati[1], Ika Mariska[2], and Fathan Muhadjir[3]
1) and 2) Bogor Research Institute for Spice and Medicinal Crops
3) Bogor Research Institute for Food Crops, Bogor Indonesia.

Red ginger (Zingiber officinale Rosc.) is potentially used for essential oil. Almost 40% investation belongs to seedlings. Method of rapid propagation of red ginger through tissue culture is being explored in Indonesia.

Explants were derived from rhizomes. The data indicated that medium with kinetin does not induced bud formation. The use of 10 mg/l BAP with 1 mg/l auxin was found best for induction of compact callus, followed by fomation of 3-5 adventitions bud, and finally induced of rooting. Bacterial contimination can be protected by using 70% alcohol, $HgCl_2$ (0,5%), sodium hypochloride (50%) and finally is cleaned three times by sterilize aquadest.

15 MICROPROPAGATION, RESEARCH AND APPLICATION TO HORTICULTURAL CROPS AND PLANTATION SPECIES

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Researches on tissue culture micropropagation of a variety of horticultural crops and plantation species have been conducted in various government agencies, research institutes as well as in the private sectors. However, only a few have been successfully multiplied for large scale production. Among these, ornamentals and flowers are the most classical examples. For instance, orchids in Thailand and Singapore, Spathiphyllum and Ferns in USA, Synogonium in Australia and carnations in Holland have been multiplied by tissue culture for commercial production.

Interests to introduce micropropagation to achieve rapid multiplication and crop improvement in fruit trees, nuts, palms, rubber and forestry have increased considerably in the commercial sector. Several private companies as well as government and research institutes have put their efforts on research and development of these crops. Some of them have been very successful and were able to multiply for large scale production. Researches are, however, still urgently needed to develop micropropagation protocols for others.

16 PLANT REGENERATION FROM LONG-TERM CULTURES DERIVED FROM MATURE SEEDS OF INDICA AND JAPONICA RICE CULTIVARS

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Long-term sustained regeneration was the first objective of this research. Secondly, with long-term culture conditions optimized, stress selection was investigated. Mature seeds of both Indica and Japonica rice produce embryogenic callus within the first monthly passage. The basal media used was LS with 2% sucrose and 0.5 to 1.0 mg/l 2,4-D and 0.2 to 0.5 mg/l Kinetin. Some varieties required 50-100 mg/l tryptophan for optimum embryogenic callus formation. Regeneration capability has been maintained for up to 30 passages for most varieties and up to 52 passages for Mahsuri. Regeneration on media with up to 9 g/l NaCl has been maintained for up to 20 passages. The most tolerant line, Giza-159, was grown for 5 passages on 13 g/l NaCl and retained the regeneration capability.

17 CLONAL PROPAGATION OF ARACHIS HYPOGAEA CV. NC-7

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Pakistan is basically an agricultural country with self suffiency in wheat, rice and cotton. However, she has to spend a lot of foreign exchange on the import of edible oil. Although peanuts are being cultivated since a long time, the production can satisfy the demand only required for use in confectionery and as roasted nuts. Therefore, nothing is left for oil extraction. Probablt the low production is due to shortage of area under cultivation and good quality seeds. Peanuts are usually sown in rainfed regions, therefore, drought-resistant varieties have to evolved which should also give high yields with minimum of moisture and financial inputs.

Pakistan Agricultural Research Council, Islamabad is carrying out trail experiments on various cultivars for introduction to our environmental conditions. NC-7 is an imported variety from U.S.A. which gave promising results under experimental studies. Unfortunately the seeds are in short supply. Therefore, tissue culture studies were initiated to mass propagate the plants of this cultivar for possible seed production on a large scale with the ultimate aim of distribution to the farmers. Plantlets have been induced from hypocotyl and mesocotyl segments on MS medium contianing various combinations of cytokinins. Furthermore, these plantlets regenerated on the callus without undergoing embrycgenesis. Addition of an auxin, e.g. 2,4-D, delayed plantlet formation, while promoting callus. Moreover, direct flower production has also been induced on cotyledon explants under the influence of BAP. These flowers are under observation for various morphogenetic studies.

18 POTENTIAL OF TISSUE CULTURE TECHNIQUES FOR THE DEVELOPMENT, ENHANCEMENT, AND EVALUATION OF CROPS RESISTANT TO INSECTS.

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The use of callus tissue as a screening procedure for evaluating crops for possible resistance to insect feeding is evaluated. Growth and development of insects feeding directly on callus tissue, fresh plant material, or these plant sources incorporated into artificial insect diets are evaluated. Ease and time requirements of each procedure are presented. The potential role of somaclonal variation for the development and/or enhancement of crop genotypes with resistant to attack by insects is also considered. Maize, Zea mays L., and alfalfa, Medicago sativa L., are the crops emphasized.

19 TEMPERATURE TOLERANT AND SALT RESISTANT STUDIES OF TRIGONELLA FOENUM-GRAECUM CULTURES

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Cell suspension cultures of Trigonella foenum-graecum Linn., were grown under heat, cold and salt ($NaCl$, Na_2SO_4, etc.) resistant media conditions. Cell lines capable to grow in the presence of such temperature variations and salt concentrations were subcultured on selective and control media. After a growth period of 6-8 weeks, biomass determination and the effect on secondary metabolites was carried out. During their growth on control media, most of these lines were characterised by slight variations in the metabolites concentrations. Efforts have been made to use the static cultures also for such stress-conditions. These studies have been undertaken to find out the cultural conditions for most optimum growth, particularly the medicinal and aromatic plants.

20 RAPID PROPAGATION OF CERTAIN FRUIT PLANTS OF THAR DESERT THROUGH TISSUE CULTURE, REGENERATION AND ESTABLISHMENT

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In vitro methods have been used widely for vegetative propagation of crop plants and techniques have been well developed for commercial exploitation. Reasons for choosing an in vitro system for clonal propagation include simplicity and efficiency of multiplication, maintenance of heterozygosity and overcoming sexual sterility or incompatibility problems. Production of sufficient number of plants of a unique genotype for evaluation and further development of the strain is the prime objective of clonal propagation, especially in cases where genetically stable seed production is not possible. The plants selected for presentation of results are: i. Zizyphus mauritiana, ii. Aegle marmelos, and iii. Phoenix dactylephera. Axillary buds and young leaves from inflorescence were used after surface disinfection with calcium/sodium hypochlorite solution (5-10% of commercial preparation) for 3-10 minutes. Explants produced multiple shoots on MS medium with appropriate concentration of auxin and cytokinins were excised and rooted on low nutrient media (1/4 strength MS or whites basal). The plantlets were transferred to pots containing vermiculite soil mixture and allowed to grow under high humidity and moderate natural light conditions in glass-house. After successful establishment the plants were transferred to field conditions.

21 MICROPROPAGATION OF A LEGUMINOUS SHRUB - SESBANIA BISPINOSA

Seema Kapoor and Shrish C. Gupta. Department of Botany, University of Delhi, Delhi 110007, India.

In vitro micropropagation of Sesbania bispinosa has been carried out employing tissues excised from seedlings as well as field grown plants. Multiple shoots differentiated from hypocotyl explants cultured on B_5 basal medium (BM) alone or in combination with BAP (10^{-7}-10^{-4}M) whereas for cotyledon explants BAP (10^{-6}-10^{-4}M) was necessary. The maximum shoots (10-12/explant) differentiated on both the explants in BM + 10^{-5}M BAP. The shoots rooted if cultured on BM + 10^{-5}M IBA. Plantlets thus raised were transferred to soil with 100% survival. They flowered and set fruits profusely. In vitro raised (one year old) plants served as the source material. The nodal explants (1 cm long) were cultured on the medium which proved best also for the juvenile tissues. Average number of shoots per nodal explant varied from 7 to 8. For further elongation, these shoots were transferred to BM + 10^{-5}M GA_3. Finally, they were excised and implanted on BM + 10^{-5}M IBA medium for rhizogenesis. Ultimately, they were shifted to the field.

22 MERISTEM CULTURE IN PLANT PRODUCTION AND IMPROVEMENT

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The term 'meristem culture' denotes the in vitro culture of the apical dome along with a portion of the subjacent tissue containing one to several young leaf primordia. These meristems referred to as 'meristem-tips' when cultured in vitro on a suitable nutrient medium and under optimal environmental and hormonal conditions, grow and differentiate into whole plants of their own type. In some species, each meristem in culture grows into one plant, while in others, the growth results in the production of a number of plants thereby providing an efficient means of mass propagation. The meristem culture is routinely used for clonal propagation, production of disease-free stocks and in vitro storage of germplasm. Recently, meristems have been identified to be excellent candidates for long-term preservation of germplasm through cryogenic techniques.

An exciting development in recent years on the biotechnological application of meristem culture is in the induction of somaclonal variation in meristem-derived progeny. Work along these lines carried out in the author's laboratory has resulted in the selection of strawberry plants with improved agronomic traits. It was found that the basis of somaclonal variation lies in the production of multiple buds of adventitous origin. Similarly, through in vitro selection strategy utilizing bean (Phaseolus vulgaris L.) meristems and the toxin (phaseolotoxin) produced by Pseudomonas syringae pv. phaseolicola, the etiological agent of 'Halo-Blight' disease, we were able to regenerate bean plants with resistance to the toxin. Here again, the level of resistance could be induced through somaclonal variation alone and without in vitro selection.

23 RAPID PROPAGATION OF A GYNOAECIOUS MUSKMELON

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The gynoaecious muskmelon (Cucumis melo) line 998 produces pistilated flowers only and therefore may be of great value for muskmelon hybrids production. Hermaphrodite flowers may be induced by repeated sprays with 100 ppm of $AgNO_3$. A rapid vegetative propagation was achieved using the following procedure: One-node cuttings were taken from 20-leaf plants, placed in vermiculite-peat (1:1) mixture and watered with 5 ppm solution of IBA or 10 ppm solution of NAA. Cuttings were then placed in humid atmosphere in the greenhouse for 2-3 weeks. A 10 ppm benzyl-adenin lanolin paste was then applied to auxilary buds to stimulate growth. Rooting was noted at about 3 weeks and new leaf growth at about 5 weeks. Desired genes for disease resistance were incorporated into line 998.

24 IN VITRO PROPAGATION OF BANANA (MUSA SP CV GEANT CAVENDISH) IN MOROCCO

Lahcen Kenny and Mohamed Aaouine. Complexe Horticole d'Agadir, B.P. 121 Ait Melloul, Agadir, Morocco

Since 1978 banana acreage in Morocco has been increasing drastically and the demand for nursery plants has rocketed. Tissue culture was suggested as a means of mass propagation of high quality cultivars.

Lateral and terminal buds were cultured on a medium the basal constituents of which were Murashige and Skoog salts and, in mg/l: i-inositol, 100; adenine sulfate, 160; Thiamine.HCl, 0.4; thyrosine, 100; sucrose, 30,000; and gelrite, 2,000. The multiplication of propagules was accomplished on this basal medium with 2 mg/l IAA and 3 mg/l BA. Rooting of the shoots was obtained on the same basal medium with 3 mg/l kinetin and 10 mg/l IAA.

A 98% survival was obtained upon transferring the rooted plants in a greenhouse and our results with producing plants showed no more than 3-5% off-types.

25 IN VITRO FLOWER INDUCTION AND POLLEN EMBRYOGENESIS IN ANTHERS DERIVED FROM INDUCED FLOWER BUDS OF NICOTIANA TABACUM CV. VIRGINICA.

Khalida Khatoon. Department of Botany, University of Karachi, Karachi-32, Pakistan.

Flower buds were induced in vitro on superficial thin cell layers of Nicotiana tabacum cv. Virginica excised from stem segments. The sporogenous cells in the young developing anthers passed through normal stages of pollen development. Mature anthers contained a high percentage of viable pollen grains.

Pollen embryogenesis in anthers taken from glasshouse grown plants and in anthers induced in vitro under lab. conditions was compared. Anthers from glasshouse grown plants were less prone to browning in culture irrespective of the hormone composition of the medium. The extent of browning of the in vitro induced anthers, on the other hand, was sensitive to the presence or absence of hormones in the medium. Both anther response and anther prodictivity decreased by exogenous hormones, in anthers of both types. Anther response was somewhat more dependent on anther stage in in vitro induced anthers than in anthers taken from glasshouse grown plants.

26 PLANT REGENERATION OF SUGARCANE TISSUE CULTURES

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The young leaves of sugarcane (Saccharum officinarum L. cv.Q_{83}) were cultured in Murashige and Skoog's medium supplemented with 4 mg/l 2,4-D or NAA in the presence of 15% coconut water. The induced calli were transferred to a fresh medium without 2,4-D. Regeneration of roots and shoots was observed within 4-6 weeks. Plantlets showed interestingly different longitudinal banded leaves indicating some promising morphological variation in the sugarcane tissue cultures.

27 VARIATIONS IN SUGARCANE SUBCLONAL POPULATIONS FOR BIOCHEMICAL TRAITS.

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Variation in Sugarcane Subclonal Populations for Biochemical Traits. Sugarcane (Saccharum officinarum) subclonal populations from 8 donors were studied to determine levels of variability for sucrose, purity of juice, ash in juice and fibre percent fresh weight. It was found that while all the characters were genetically control some showed wider variation than the others regardless of the environment. It was generally believed that when cane was grown on soils with high potassium or in saline soils the ash in juice will be high. The current studies show that ash in juice is independent of levels of potassium in the soils. Juice purity has been found to show minimum variability distribution in the subclones.

28 ORGANOGENESIS IN ANTHER CULTURE OF SOME CUCURBITS

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Anther culture studies have been carried out in five important Cucurbitaceous species Luffa echinata, L. cylindrica, L. acutanrula, Trichosanthes dioica and Coccinia indica. The study aimed to explore the potentiality of pollen in culture for callus formation and differentiation. The Murashige and Skoog's (1962) medium was used as basal medium supplemented with coconut milk, Kinetin, Benzyl amino purine, 2,4-dichlorophenoxy acetic acid, Indole acetic acid, Indole butyric acid, Nephthyl acetic acid and Gibberellic acid either singly or in different combinations. The effect of pollen stage and age of the doner plant on callus formation were studied. Addition of coconut milk to the basal medium was essential for all the species. 2,4-D was more favourable for callus formation than NAA and IAA. Cytokinin alone was less effective, but combinations of auxin and cytokinin gave best results. Media with high cytokinin favoured differentiation of embryoids, whereas high auxin favoured root differentiation. L. echinata was most responsive for organogenesis followed by L. cylindrica, L. acutanula, C. indica and T. dioica. The pathways of embryoid formation from pollen were traced. Cytological and histological studies of differentiated organs were done to determine their ploidy status, extent of vascular differentiation, site and stages of organ formation.

29 SOMATIC EMBRYOGENESIS FOR EFFICIENT PLANTLET REGENERATION FROM SEED CULTURE IN *ORYZA SATIVA* L. VARIETY IR 50

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Calli developed from germinating seeds of IR.50 in the M.S. medium added with 2,4-D and kinetin formed the source for plantlet regeneration. When transferred to the regenerati n medium supplemented with kinetin and NAA, meristamoid embryo initials occurred in 10 to 15 days and the initials organised into somatic embryoids within 20-25 days. The somatic embryogenesis pathway studied through histological examination revealed that initially single to few cells take part in embryoid formation. The initials later develop into globular embryos and get organised into shoot and root axis. The proliferation of somatic embryoids in a callus is owing to the simultaneous onset of embryogenesis over large number of cells of epidermal to subepidermal in origin. The results could be repeated in further experiments also. Studies made during different stages of morphogenesis indicated that zymogram pattern of esterase varied with species and varieties investigated. It was also evident that increase in peroxidase activity and alterations of its isoenzymes had a correlation with morphogenesis.

30 ANTHER CULTURE AND SOMATIC EMBRYOGENESIS IN RICE HYBRID

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Anther response to callusing in tissue cultures was evaluated in varieties and hybrids of rice. Induction of embryoids in a repeatable manner was achieved in anther calli with hormonal combinations of IAA and BAP. Numerous embryoids developed from each callus could be easily developed into adult plants.

The induced embryoids of pollen calli are examined for frequency of haploids and diploids. The diploids are stable and homozygous as revealed by progeny tests in the fields. The anthers from F1s of crosses between improved rice cultivar with pest and disease resistant parent could be cultured and doubled haploids are obtained through embryoids. They are now field tested for yield, maturity duration and resistance to pests and diseases.

Anther cultured plants from *indica* rice variety, Ponni gave rise to plants which are doubled haploids, but were distinctly different from parent for plant type while retaining panicle and the grain type. Spontaneous regenerants from some of the haploid lines is of significance to get homodiploid lines. Variation within and between the haploids and diploids also being assessed.

31 TISSUE CULTURE TECHNIQUES OF *Angelica acutiloba* Kitagawa

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Angelica acutiloba Kitagawa originated from Japan. By using tissue culture techniques, it is expected to get easier propagation than conventional method. Explants were isolated from apical bud of tuber. Results of the experiment showed that medium without plant growth regulator slowly induced bud formation. The use combination of 2,0 mg/l IAA + 0,5 mg/l BAP + 0,5 mg/l Kinetin + 1,0 mg/l GA_3 and vitamine from group B, was found best for rapid growth of bud, and three weeks after planting induced of rooting formation.

32 SELECTION FOR WILT RESISTANCE FROM ANTHER DERIVED HAPLOIDS AND LEAF DERIVED REGENERENTS IN TOBACCO

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Somaclonal variation provides an important source for plant improvement. The present report deals with wilt resistant variations occurring in anther derived haploid plants and regenerents from leaf callus of tobacco.

Anthers with uninucleate pollen grains of *Nicotiana tabacum* var. A2 were inoculated on Nitsch medium. Plantlets that emerged after 30 days were transplanted to pots. They were tested for disease resistance by measuring the growth of leaf discs inoculated on a medium containing culture filtrate (CF) of the wilt fungus *Fusarium oxysporum* f. sp. *nicotianae*. Growth of leaf discs was reduced and ranged from 10 to 45% of the control. This indicated variation in disease resistance of haploid regenerents, since our previous experiments have shown close correlation in sensitivity to CF from the cellular to whole plant levels.

In the other study, cell suspension cultures from leaf derived callus were maintained in liquid MS medium. Cells at a density of 6.5×10^4/ml were plated on MS medium containing various concentrations of filter sterilized CF of the fungus and plating efficiency was determined. Lethal dose 50 was found to be at 30% of CF. Plants were regenerated from surviving colonies in the continued presence of the same level of CF and are being tested for their disease resistance.

33 INITATION OF ANDROGENESIS IN RICE (ORYZA SATIVA L.) VAR. TAIPEI309

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Initiation of androgenesis was studied in rice variety Taipei 309 in liquid medium N6. The pollen exhibited trimorphism of response to culture with three types of pollen, i.e. highly enlarged empty grains, medium enlarged responsive grains filled with cytoplasm and unenlarged and unresponsive grains appearing within 48 hours of culture. Three pathways of initiation were observed. Pathway I, where initiation occurred in the uninucleate grain, was the most frequent. Pathways II and III occurred in binucleate grains. In the former, only the vegetative nucleus took part in the formation of the pollen callus while in Pathway III, the generative nucleus divided first followed by the division of the vegetative nucleus and both contributed to the development of the callus. Wall formation occurred after 16 or more free nuclei were formed. Callus was released by the gradual disintegration of the exine. Establishment of suspensor-like outgrowth between developing pollen grain and anther wall was not observed. Early fragmentation of calli and their independent development indicated that all calli recovered after 35-40 days from culture plates may not be arising from individual pollen grains and this suggested the possibility of distortion of progeny ratios. Polyploidization of haploid cells was observed very early in the development of the callus but in the competition for growth during later development, the faster growing haploid and diploid cells might overcome the possibly slower growing polyploid cells resulting in a very low percentage of polyploids among regenerated plants.

34 DEVELOPMENT OF IMPROVED AND SALT TOLERANT RICE VARIETIES THROUGH TISSUE CULTURE TECHNIQUES

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Of the five rice varieties, namely, BR 3, BR 11, IRATOM 24, IRATOM 38 and IR 36 selected as test materials, BR 3 callused best on MS medium(Agar 8g/l) supplemented with 30g/l sucrose, 3g/l yeast extract and 2mg/l 2,4-D. Some vigorously growing calli of BR 3 produced multiple shoots on MS medium (8g/l agar) supplemented with $2X10^{-6}$ M NAA, $5X10^{-5}$ M Kinetin, 70g/l sucrose, 3g/l yeast extract and 3g/l casein hydrolysate. Using modified techniques, nine regenerants from a single callus were transferred to pots for establishment. These nine plants differed among themselves in height, flowering date, grain size and shape, some individuals showing highly promising characters such as fine grain quality and early flowering. The offspring from these nine regenerated plants are being tested for the above important agronomic traits as well as for salt tolerance along with BR 3 and salt tolerant check variety Pokkali.

35 ANTHER CULTURE CONSTRAINTS AND PROSPECTS IN PLANT IMPROVEMENT

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The need of selection for new plants better adapted to specific soil and climatic conditions is becoming more and more obvious.
Now a days, the tool of in vitro tissue culture is taken a greater importance in the mind of plant breeders. It is also due to the fact that it does not only save time to produce homozygous lines but it allows at the first generation the observation of recessive traits usely hidden in crossing. Placing the breeder in front of all different traits available in a combination.

The most important factors to succed in producing plants from the male gametophyte lie in :

- The physiological state of gamete producing plants namely the donor plant (mitotic cycle, flowering or fruit set...).
- The developmental state of pollen; microspore uninucleate, in mitosis or early binucleate.
- The culture condition which allows the shift from gamatogenesis to vegetative development of the microspore.
- The ability of a specie to survive at the haploid level and or at the homozygous state.

Other proposition will be made to obtain homozygous line with the female gametophyte. Inter specific or intergeneric crosses have been successful by rescuing, in situ, non viable embryos which have been grown to plants in vitro. An example of wide cross between Zea mays and Pennisetum americanum will be discussed.
It brings a new opportunity to introduce, via the natural process, some caracters from one specie to another.

36 IN VITRO MUTATION INDUCTION IN MERISTEM-TIP CULTURE OF BANANA AND PLANTAIN

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The sexual breeding of banana and plantain is limited only to Gross Michel triploids and for diploid clones which produce few seeds when pollinated. Mutation induction is the only alternative for breeding the most important Cavendish group of banana and Horn plantain. The meristem-tip culture was established in nine clones of banana and plantain with different ploidy levels. Mutagenic gamma irradiation was applied on isolated meristem-tips. The influence of gamma rays on in vitro growth and shoot regeneration was assessed. For triploid banana and plantain a 50% decrease was observed after doses of 30-45 Gy. The diploid clones are more sensitive. Ethylmethanesulfonate (EMS) was applied under aseptic conditions on isolated shoot meristems. A 50% decrease of in vitro growth was obtained after EMS concentrations of 0.75%. The potential of the in vitro system for disease resistance selection is discussed. In vitro mutation breeding technology for banana and plantain is proposed.

37 ORGANOGENETIC INDUCTION OF VICIA FABA IN VITRO

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Different tissue explants of Vicia faba seedling were cultured on modified SH basal medium (Mitchell and Gildow, 1975) supplemented with 0.1 mg/l kinetin, 2.0 mg/l pCPA and 2.0 mg/l glycine. The results showed that leaf explants produced callus with roots, petiole and stem explants proliferated only callus tissues while those of cataphyll could regenerate callus and whole plantlets.

Reference Mitchell, J.P. and Gildow, F.E. 1975. Physiol. Plant. 34,250-253.

38 IN VITRO CULTURE OF RATTAN (Calamus manilensis H. Wendl.), A TECHNIQUE FOR ITS RAPID PROPAGATION

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The requirements for in vitro culture of rattan (Calamus manilensis H. Wendl.) were investigated to determine its applicability for rapid propagation. A hundred percent seed germination was obtained using Murashige and Skoog's (MS) basal medium. After one month, shoots (1 cm long) were excised and subcultured in MS medium with 5 mg/l benzyladenine. A four-fold increase in the number of shoots per explant was observed in 2 months. Using Linsmaier and Skoog's medium with benzyladenine (5 mg/l), a six-fold increase in the number of shoots per explant can be obtained. However, the frequency of shoot proliferation per explant is variable. Considering a four-fold increase in the number of shoots per explant, one million shoots can be obtained using 480 seeds in a period of 13 months. The thirteen-months propagation period includes one month of seed germination and 6 cycles (2 months per cycle) of shoot proliferation. This scheme provides a rapid means of propagating rattan. To determine the requirements for rooting, single shoots were excised and transferred to MS medium with varying levels of indolebutyric acid (IBA). Rooting was found to be best at 5 mg/l IBA. Plantlets were then transferred to potting mix and allowed to harden for 3 months before transplanting to the forest, rattan's natural habitat. With this technique, a high survival rate (90%) was obtained. Similar experiments using 2 commercially important species gave similar results except for slower response in shoot proliferation, rooting and plant growth.

39 PLANT REGENERATION AND STRESS TOLERANT VARIANTS FROM LONG-TERM CULTURES OF SORGHUM BICOLOR.

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Tissue cultures of Sorghum bicolor were studied for their ability to regenerate after long-term culturing and selection for NaCl tolerance. Mature embryos of sorghum were excised from seeds and plated on LS media containing 2 mg/l 2,4-D, 0.5 mg/l Kinetin and 2% sucrose. Embryogenic callus was isolated after 3 to 4 monthly passages and has been maintained for up to 23 passages with continued plant regeneration. Selection of embryogenic callus for transfer to fresh media is a key factor for long-term cultures which retain the regenerative capability. Regeneration was achieved after 7 passages when the calli were grown on media containing 9 g/l NaCl and 2 passages when the NaCl level was 15 g/l. Somaclonal variants have been observed in field tests. These variants expressed increased tolerance to toxic levels of aluminum on acidic soils.

40 PLANT REGENERATION FROM ANTHER CALLUS OF RICE UNDER SALT STRESS CONDITION

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Callus production was initiated from anthers of rice (Oryza sativa L.cv. IR 1552) cultured on modified Miller medium. Plant regenerations were undertaken in various salt stress culture conditions. Frequency of regeneration decreased as the NaCl percentage increased from 0.5 % up to 1.25 %. No plant was obtained from callus cultured on medium containing 1.5 % of NaCl.

41 RICE TISSUE CULTURE STUDIES AT THE IARI

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Anther culture techniques in rice have been under investigation in our laboratory for many years. Several hundred pollen-derived lines have been raised and selection of promising recombinants made from certain potentially desirable rice hybrids. Some of the selections are undergoing multilocation performance trials in different parts of the country. Currently, anther culture technique is being extended to wild rices and interspecific hybrids with the main objective of developing alein addition/substitution lines possessing desirable traits of resistance to disease and pests. Besides, somatic cell culture studies are being pursued mainly in the tall fine grain aromatic rice variety Basmati-370, for isolation of desirable semi-dwarfs.

The paper will present a state of the art covering our achievements, problems and future prospects.

42 MERISTEM CULTURE FOR GENE INTROGRESSION BETWEEN INDIAN (Desi) and MEDETERANIAN (Kabuli) TYPES OF CHICKPEA.

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Investigations on in vitro culture of chickpea (Cicer arietinum) is relevant:(i)To augment the limited available genetic variability for attributes of economic value and (ii) To combine characteristics of the local and Mediteranian types, an objective not easily achieved through conventional methods. Meristem culture was attempted because it can serve to release genetic variability when routed through callus phase and can preserve genetic fidelity when persued through direct shootlet regeneration. Our findings are:

1. Among the seven media (B_5,Blayedes, LS,MS, PC(L)-2,SH and Whites)tested, B_5, MS and PC(L)-2 are better for callusing, caulogenesis and rhizogenesis.
2. Evaluation of hormone effects revealed that (i)2,4-D, either alone or in combination with cytokinin (BAP/Kinetin) produced light brown callus,(ii)IAA alone, or in combination with cytokinin, failed to induce callus; (iii)NAA with or without cytokinin, induced callus, shoot and root growth (iv) Combination of NAA and BAP in low concentration range (0.1 to 2.0 mg/l) was efficient in callus shoot and root formation. Moderate (3.0 to 6.0 mg/l) and high (7.0 to 10.0 mg/l) concentration ranges, induced light green and light brown calli respectively.
3. Shoot regeneration from callus was strongly influenced by medium used for callusing. Regeneration media with very high BAP(7.5 or 10.0 mg/l) and low NAA (0.1 or 10.0mg/l) were more favourable for shoot regeneration.
4. Direct plantlets were obtained on MS + NAA (2.0 mg/l) + BAP (0.1 mg/l).

43 GENETIC SEGGREGATION IN INTERSPECIFIC AND INTERGENETIC SACCHARUM HYBRIDS.

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Subclonal populations derived from crosses between Saccharum officinarum var Badila and var Korpi with S. spontaneum and Narenga porphyrocoma were studied for seggregation and varietions in morphological, biochemical and cytological characters. The intergenetic hybrids do not produce fertile flowers whereas the interspecific crosses when selfed do not show any wide variations in the form of seggregation. It has been found that tissue culture is a noble technique to seggregation and variations.

44 RICE IMPROVEMENT THROUGH SOMACULTURE.

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A system was developed that allowed efficient regeneration from U.S. rice cultivars and several introduced cultivars through somaculture. This system is based on regenerating plants from callus from immature panicles. We have regenerated and transplanted to the greenhouse more than 6000 plants. About 700 lines (R_1) from seed produced on regenerated plants (R_0) were grown in the field at the Rice Research Station at Crowley, LA in 1985. More than 1100 additional lines were grown in the field in 1986. These lines were evaluated for morphological variation, yield, and disease resistance. Line to line variation was observed in lines from the same cultivar for differences in height, days to heading, grain type, tiller number, tillering habit, leaf shape, leaf color, panicle size, and panicle weight. Within line differences were observed for dwarfing, height, apiculus color, pubescence, awn production, hull color, and other characters. We observed apparent resistance in the field to the narrow brown spot, blast, and sheath blight diseases in several lines regenerated from a susceptible cultivar. Several variations that were segregating in the R_1 generation were carried to the R_2 generation in the field. Segregating lines gave segregation ratios characteristic of single gene characters, or in one case, of two complementary genes. Several lines that appeared uniformly taller than the parent cultivar did not give segregation in the F_2 population when crossed back to the parent cultivar.

The number of plants regenerated has only been limited by the time required to grow the material in culture and by our ability to transfer and maintain plants in the greenhouse. Many lines from plants regenerated from certain cultivars show variation. A few cultivars gave lines with little or no variation. We believe that somaculture will produce variants in rice that will be useful for genetic studies and that may contribute useful germplasms to the breeding program.

45 RAPID MULTIPLICATION AND IMPROVEMENT OF BANANA THROUCH TISSUE CULTURE

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New cultivar of banana' Giant Kai Phra Tabong' developed from in vitro mutation breeding technique was used for rapid propagation. Shoot tip was aseptic cultured on MS medium supplemented with 15% coconut water ans 5 mg/1 BA. It was found that shoot initiated from axillary bud can be developed into plantlets. When divided the plantlets and subcultured on new growing medium at every 4 weeks, 1,025 plantlets could be produced in 20 weeks.

For induction of salt tolerant and acid tolerant mutants, plantlets grown in vitro were irradiated twice with gamma rays at the doze of 2 Krad at a time. Mutation characters observed were variations in leaf shape, leaf color, leaf arrangement and dwarf shoots with slow growth. Some banana plantlets were cultured onto MS medium supplemented with 15% coconut water, 5 mg/lBA and 0, 1, 2, 3, 4, or 5% NaCl for salt tolerant selection. The others were cultured onto MS medium supplemented with 15% coconut water and 5 mg/lBA with various pH: 4, 5, 5.6, 6, 7 and 8 for acid toterant selection.

46 CLONAL PROPAGATION OF ELEPHANT YAM (Amorphophallus kerrii) THROUGH TISSUE CULTURE

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Shoot tips of elephant yam were aseptic cultured in MS medium supplemented with 15% coconut water. Shoot formation occurred within 2 months. Then shoot tips, petioles and leaves were sectioned and subcultured onto MS medium with addition of various concentrations of 2, 4-D (0, 0.25, 0.50, 0.75 1.0, 2.0, 4.0 and 6.0 mg/1), pH of the medium was adjusted to 5.7 - 5.8 and cultures were kept at 25 - 28° C in dark condition. After 8 weeks, the appearance of shoot formation was observed from shoot tips, basal part of the leaves and all parts of the petioles, especially occurred best in the basal part.

Callus induction and later growth were best in 0.25 mg/1 2, 4-D but in case of the percentage of callus induction, there was no difference among levels of 2, 4-D. In case of leaf, same as petiole, callus could be induced best from the basal part, and the middle part was better than tipped part.

Calli from leaves and petioles cultured in 0.50 mg/1 2, 4-D in the past experiment were divided into the size of 0.8 cm^3, and planted in the same basal medium supplement with the combination of NAA and kinetin 0, 0.5, 1.0 2.0, 3.0 and 4.0 mg/1. After cultured 8 weeks in the light, the survival percentage of calli was about 50%. Slow callus growth was observed when there was only NAA in the concentration of 4.0 mg/1 or kinetin 1.0 mg/1 and in the combination of NAA and kinetin at the level of 4.0 mg/1. Shoot initiation occurred in the medium supplemented with kinetin in all concentrations and the concentration of NAA was lower than 1.0 mg/1, however, occurred best when there was only kinetin in the concentration of 1.0 mg/1. Plantlets were completely developed from calli in MS medium and were successfully grown in soil.

47 CLONAL PROPAGATION OF DATE PALM (Phoenix dactylifera L.) THROUGH TISSUE CULTURE

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Date palm calli were multiplied by culturing in the agar medium composed of MS salt, 100 mg/l meso-inositol, 0.4 mg/l thiamine HCl, 4.0 mg/l NAA, 0.4 mg/l kinetin, 30 g/l sucrose and subcultured in every 8 weeks. In the same medium embryoids could develop from calli, and became seedlings in both MS and Y_3 media supplemented with 45 g/l sucrose and 3 g/l activated charcoal. The 2-3 foliage-stage seedlings were divided into shoot tips, basal, middle and tipped parts of leaves and roots, then cultured in MS and y_3 media supplemented with 2, 4-D at the concentrations of 0, 2, 4, 8, mg/l. Callus induction from shoot tips was best at 2 mg/l 2, 4-D in Y_3 medium. Roots formed callus best in both media at the same concentration of 2, 4-D as shoot tips. Only the basal part of the leaves produced callus. MS medium supplemented with 8 mg/l 2, 4-D was found to be the optimum for callus induction from the basal part of the leaves.

Various concentrations of growth regulators (NAA and IAA 0, 2, 4, 6, 10 mg/l; kinetin and BA 0, 0.25, 0.50, 0.75, 1.0 mg/l) and other factors namely sucrose (0, 15, 30, 45, 60 g/l) and pH (4, 5, 6, 7, 8) were studied. The best callus multiplication was found in the medium supplemented with 6.0 mg/l NAA, 54 g/l sucrose and pH 8. However, shoot development was found best in the medium supplemented with 0.5 mg/l kinetin. Whereas the growth of embryoids was best in 0.25 mg/l BA, 45 g/l sucrose and pH 5. Adventitious roots could be induced from seedlings by NAA, especially at the concentration of 0.05 mg/l. Seedlings with adventitious roots at the basal part of the stem had better survival rate after transplanting than seedlings with primary and secondary roots.

48 SHOOT AND ROOT DIFFERENTIATION IN DIFFERENT VARIETIES OF ELEUSINE CORACANA GAERTN.

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Ragi or fingermillet (Eleusine coracana Gaertn.) is highly nutritive and is used in the form of vegetable,malt,cake etc. It has high calcium and fiber content mostly preferred by field labourers and diabetics.

Callus of ragi seed varieties viz., I_4,I_5,I_8, etc., were obtained on MS medium with different concentration of 2,4-D (1 ppm to 10 ppm). I_5 showed white nodular callus and that I_8 was pale brown and nodular . 1 ppm 2,4-D was more conducive for callus growth. On transfer of a portion of callus on MS + 0.5 ppm 2,4-D resulted in root initiation. Further subculturing these callus in lower concentrations of 2,4-D enhanced the rapid growth of roots.

The callus was subcultured on MS, supplemented with different concentration of BAP, IAA and NAA either individually or in combination. On MS + IAA (2 ppm) callus turned green and nodular which was found to give multiple shoots on further subculturing. Transfer of the same to higher concentrations of BAP + IAA (5 ppm) exhibited rapid shoot growth.

49 SCREENING SOYBEAN GERMPLASM FOR ALUMINIUM TOLERANCE, USING SOLUTION AND SOIL CULTURES

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A total of 150 soybean varieties were screened for aluminium tolerance, planted in solution containing 0.4 mM $AlCl_3.6\ H_2O$, and in soil with high aluminium content in pot. Root length was measured at four days after planting for the solution culture, and at 15 days after planting for the pot culture. Relative root length between the two methods were in disagreement, and the correlation did not significantly differ from zero, Pot culture using soil containing high in aluminium was more reliable for screening aluminium tolerance in soybean, because other agronomic traits could be observed and used as tolerance criteria. Wide range of reactions were observed among the 150 varieties planted in pot, including 27 varieties which were tolerant and 41 varieties which were susceptible or highly susceptible. Visual scoring to the plant growth at flowering stage, using scores from 1 = normal green plant, to 5 = very stunted, browning leaf, was effective to differentiate the soybean reaction to aluminium toxicity. The correlation between visual score and pods per plant was significant.

50 MULTIPLE SHOOT FORMATION ON COWPEA COTYLEDONARY NODE SEGMENTS IN CULTURES

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Cotyledonary node segments of cowpea (Vigna unquiculata) were cultured in Murashige and Skoog's medium supplemented with various concentrations of benzyladenine (BA) and napthaleneacetic acid (NAA) alone or in combination. Multiple shoot formation was induced from these segments on medium containing only the cytokinin, BA. The effect of explant pre-treatment in order to stimulate multiple shoot formation from cotyledonary node segments will also be discussed.

51 MICROPROPAGATION OF BOUGAINVILLEA THROUGH SHOOT TIP CULTURE

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Shoots tips of Bougainvillea were excised from mature field grown shrubs and induced to regenerate multiple shoots on MS medium supplemented with BA. This was due to cessation of apical dominance and enhancement of axillary branching. The shootlets upon excision and transfer to fresh MS medium supplemented with BA continued to produce a new crop of multiple shoots. Transferring the shootlets either to MS medium containing auxins and agar as gelling agent or liquid MS medium supplemented with auxins and filter paper bridge support was of little help in inducing rooting. However,MS liquid medium supplemented with NAA with acid washed sand as a support induced rooting in 47.61% of the cultures. Thus,fully developed plantlets could be established in vitro. On an average 77.11% of the in vitro produced plantlets survived transplantation to soil. These plants have been established and grown to the stage of flowering. The shootlets produced in vitro are being subcultured for the past five years and successive generations have been established. There has been no loss of vigour and no apparent variants have been noticed in the successive subcultures.Thus,it has been possible to establish a viable biotechnology for micropropagation of Bougainvillea.

52 INDUCTION OF SMUT RESISTANCE IN SUGARCANE DURING CALLUS STAGE

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An attempt to incorporate EMS at different concentrations and times into the liquid media to induce somatic variation was made. Plantlets regenerated from treated and untreated calli were found to vary in size and morphology appreciably. It was found that the frequency of variants arising in the EMS-treated and untreated populations were not different.

Two smut resistant plants were obtained from these treatments and both clones are being subjected to field testing.

53 TISSUE CULTURE OF SUGARCANE FROM YOUNG LEAF

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Young leaf sections with creamy white color were cultured in the dark for seven days on the MS medium supplemented with 10% coconut water and 3 ppm 2,4-D. Some calli appeared at or near cut surfaces in the dark but most of them were observed after five days in the light. These grew into compact mass, composting mostly of isodiametric cells, in forms of white and green nodules in the next five weeks. Differentiation of shoot apex began two weeks after a well grown callus was transferred into medium without 2,4-D and coconut water, and roots grew out of a differentiated stem later indicating organogenesis.

54 INITIATION AND MAINTENANCE OF EMBRYOGENIC CALLUS FROM UPLAND RICE SEEDS AND CORN EMBRYOS

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The research aims to the eventual regeneration of drought- and acid soil-tolerant rice and corn plants to suit the upland conditions in the Visayas, Philippines.

Mature seeds of five cultivars/lines of upland rice namely, Kinadong Patong, UPLRi7, IR 43, IR 13754-5-2 and IR 12721-5-1-1-3 and immature embryos of corn cv. Improved Tiniguib, IPB 2, and ViSCA 8311 were used for callus initiation.

In rice, difference in genotypic response to callus initiation medium was observed among the five cultivars/lines with IR 13754-5-2, UPLRi 7 and IR 43 showing significantly higher percent embryogenic (E) callus. On the maintenance medium, the E callus doubled weight in less than 4 weeks.

In corn, embryos of 1.5 - 2.0 mm length appeared to be responsive to callus initiation medium. Nodular callus was observed on the surface of the scutellum at about 10 days after inoculation. Type I and type II callus were observed in the cultures initiated with 1.5 mm long embryos, with type II being noted at high frequencies. MS medium supplemented with 1 mg/l 2,4-D and 5 uM proline was superior than N6 medium incorporated with the same additives in initiating E callus. Preliminary observations indicate a rapid loss of regenerative ability of the E callus after 3 passages at 3 week-interval.

55 HIGH FREQUENCY SOMATIC EMBRYOGENESIS IN A LEGUMINOUS TREE - ALBIZIA RICHARDIANA KING.

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Large amounts of green compact as well as some light green fragile calli were obtained on MS + 10^{-6}M BAP medium by culturing 1 mm thick discs of hypocotyl explants excised from aseptically raised 12 day old seedlings of Albizia richardiana. The green compact calli have been cultured repeatedly at a five week passage on MS medium containing 3% sucrose for more than a year. In the first passage, 8% of calli turned embryogenic on MS + 10^{-6}M BAP medium and produced 2-20 embryos per culture. The percentage of cultures producing somatic embryos could be increased to 50 by selective sub-culturing of the embryogenic calli. The isolated somatic embryos mostly turned into green embryogenic calli on MS medium with 2% sucrose, but ultimately developed somatic embryos once again. Some of the somatic embryos organised into complete plants as well. In still other cultures, the compact green calli directly gave rise to shoots on the same medium (MS + 10^{-6}M BAP). Addition of higher concentration of $CaCl_2.2H_2O$(13.2 gm/l) to the inductive medium enhanced the development of shoot buds on green compact calli.

56 HIGH FREQUENCY PLANT REGENERATION THROUGH IMMATURE LEAF CULTURE OF SUGARCANE

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Immature leaf segments of sugarcane (Saccharum officinarum Linn.) were cultured on MS medium suplemented with 10% coconut water, 3ppm 2,4-D and 0.2% sucrose in the dark for one week. Then the flasks were transfered to 16:8 light-dark period. Numerous calli were initiated within 2 weeks from the wounded surfaces. These calli were of creamy color, composted majority of cells were isodiametric. The calli were transfered to MS medium without auxin and cytokinin and were kept in the light as stated above. Many white and green nodules were observed within two weeks. At this period shoots and roots began to appear and later they became complete plants.

57 TOWARD ESTABLISHING SALT TOLERANT LINES OF RICE.

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Salt tolerant plants were obtained from both NaCl-treated and un-treated calli. Seedlings of plant regenerated under salt stress demonstrated definite salt tolerant characteristic, but the seedlings of the other group were quite variable. Among the untreated calli, it was found that the second generation was more tolerant than the first generation, and the variability was high in both generations.

The results obtained from these experiments indicate that both methods can be used for establishing salt tolerant line through spontaneous somatic mutation arising in callus stage.

58 EFFECTS OF MACROELEMENT ON REGENERATION OF PLANT FROM RICE CALLUS

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Effects of ammonium, nitrate, phosphate potassium, calcium and magnesium ions on plant regeneration from rice callus were studied. A siginificant increase in shoot formation was observed in media containing 3.2 mM anmonium. The increase of nitrate nitrogen and phosphorus in White's formula induced more shoot formation, however, the change in potassium, calcium and magnesium levels showed no significant differences.

The levels of macroelements that gave reliable result in shoot formation contained 3.2 mM ammonium, 34.2 mM nitrate, 1.9mM potassium, 6.6 mM potassium, 5.1 mM calcium, and 2.9 mM magnesium.

59 THE APPLICATION OF MERISTEM CULTURE IN THE IMPROVEMENT OF PLANTAIN AND COOKING BANANA.

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Increased production of plantains and several other cooking bananas (Musa sp.) is seriously threathened in Africa by the rapid spread of the Black Sigatoka disease. Plantains are a major staple food in the humid lowlands of Africa and no resistant plantain cultivars are known to exist. Action coordinated by the International Network for the Improvement of Banana and Plantain focusses on the selection, exchange and rapid diffusion of resistant non-plantain cooking cultivars (Musa ABB group) and on the creation of resistant plantains.
Resistant germplasm, which exists in the major collections of Jamaica, Honduras and the Philippines, is vegetatively propagated. This creates problems with respect to the rapid multiplication, the international exchange and the in situ conservation of this valuable germplasm.
A well-established in vitro meristem culture technique is described and its role as an appropriate solution for rapid multiplication, disease elimination, germplasm exchange and conservation, and mutation induction is discussed in relation to the problem of somaclonal variation.

60 ANTHER CULTURE OF *HEVEA BRASILIENSIS*

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The available verieties of rubber tree are highly heterogeneous in nature. The incidence of inbreeding during seed set is usually only 0.02-0.03%. By using conventional breeding, it is impossible to obtain pure lines with desirable characters by means of successive inbreeding. The establishment of anther culture technique of *Hevea* will enable us to obtain pure lines of different genotype in a short time. Anther culture was first tried with rubber trees grown in the Songkla Rubber Research Center using method of Chen et. al.. Pistillate flowers located at the top of the central axis and lateral branch were collected. Flowers buds (3-3.5 mm) in which the majority of anther are at uninucleate stage, are excised and used for callusing in a dedifferentiation media. We observed calli of yellowish cell masses after 7 weeks of innoculation. Subculturing into differentiation and plantlet forming media to induce embryoid and plantlet formation is in progress.

Chen, Z., Chen, F., Chien, C., Wang, C., Chang S., Hsu, H., Ou, H., Ho, Y., and Lu, T. (1979) A process of obtaining pollen plant of *Hevea brasiliensis* Muell-Arg Sci.Sin. *22*:81-90.

61 TRANSFORMATION OF THE MONOCOT *Dioscorea opposita* USING *Agrobacterium tumefaciens*

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Monocots are widely considered to be naturally resistant to the infection with *Agrobacterium*, possibly because of no binding of agrobacteria to the cell walls of monocots or because of an abnormal phytochormone balance in monocot cells. It has been reported , however , that T-DNA- specified opines were detected in *Narcissus* and *Chlorophytum capense* as well as in *Asparagus officinalis*.And recently we indicated the T-DNA transfer and expression in monocotyledonous plants of *Hippeastrum rutilum* and *Chlorophytum comosum*. We described the transformation of *Dioscorea opposita* , a member of the family *Dioscoreaceae*, using leaf disc transformation system or *in planta* infection by an A208 strain of *A. tumefaciens* carrying pTiT37 plasmid. We consequently established an *Agrobacterium*-transformed cell culture from such species. This data shows the potentiality in using *Agrobacterium tumefaciens* for transformation of cereals.

62 ROLE OF THAILAND NATIONAL CENTER FOR GENETIC ENGINEERING AND BIOTECHNOLOGY IN PLANT RESEARCH FOR CROP DEVELOPMENT

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The National Center for Genetic Engineering and Biotechnology (NCGEB), established in 1983, has put emphasis on plant biotechnology by setting up the Plant Genetic Engineering Laboratory at Kasetsart University, Kampaeng Saen Campus, and by supporting a number of R&D projects at various institutions. These include tissue culture propagation of oil palm, production of disease-free potato seeds, development of flowering and ornamental plants production, association between rice and nitrogen-fixing bacteria, and enzyme markers for rubber production. Recently the NCGEB in association with the Science and Technology for Development Project, and with support from USAID and BOSTID, has launched a Network for Plant Tissue Culture Technology, in which the major institutions throughout the country are participating in extensive R&D and information transfer activities for tissue culture and other areas of plant biotechnology with a major emphasis on crop development in Thailand.