PLANT BREEDING AND GENETICS NEWSLETTER

Joint FAO/IAEA Division
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TO THE READER

The fifth issue of the Plant Breeding and Genetics Newsletter brings information on our activities in the first half of 2000. A new Co-ordinated Research Project (CRP) on "Mutational analysis of root characters in annual food plants related to plant performance" was initiated with the first Research Co-ordination Meeting (RCM) held in February 2000 in Vienna. Scientists participating in the RCM presented papers and discussed work plans on the use of mutants for genetic analysis of root system morphology, tolerance to soil stresses and mycorrhizal relationships. Mutated genes responsible for defined root characters will be incorporated to molecular markers based genetic maps by building their root systems to make them more adaptive to particular soil conditions. Preparation for initiation of another CRP on "Improvement of tropical and subtropical fruit trees through induced mutations and biotechnology" has been completed. We are expecting 14 participants at the first RCM, which will be held in Vienna in September 2000. It is expected that this CRP will make a real breakthrough in application of induced mutations for improvement of fruit trees. *In vitro*, especially somatic embryogenesis as well as conventional breeding methods will be used in combination with mutation techniques. Significant progress was noted, at the second RCM, on the application of biotechnology and mutation techniques for the improvement of local food crops in LIFDCs held in San Jose, Costa Rica, June 2000. The RCM was combined with a workshop on "*In vitro* culture techniques for the improvement of vegetatively propagated tropical fruit crops."

The Regional training course on "New frontiers of developing and handling mutants" was organized under the Technical Cooperation Project on "Mutational enhancement of genetic diversity in rice" and hosted by the Institute of Nuclear Agricultural Sciences, Zhejiang University, Hangzhou, China in June 2000. The course focus on current induced mutation techniques and their modifications through application of different biotechnologies including classical insertional mutagenesis and retrotransposons. Preparatory work is also very advanced for the organization of workshops under two regional projects for Africa dealing with development of new crop varieties with tolerance to abiotic stresses and with resistance to Bayoud disease in date palm.

The FAO/IAEA Mutant Varieties Database has more than 2200 accessions and during this year should be available to plant breeders through the internet. The idea to organize an efficient information system on crop mutants has been supported by many plant breeders and especially by molecular geneticists working on genomics. It was also suggested to organize a seed and tissue culture mutant's depository to stimulate free exchange of mutated germplasm. Molecular markers will be used for labeling of more promising mutants.

The first half of the year was also a very busy time for the Plant Breeding Unit of the FAO/IAEA Agriculture and Biotechnology Laboratory at Seibersdorf. Important results were obtained on increasing anther culture efficiency in rice using anthers from ratooned plants. The chimera dissociation after mutagenic treatment in three propagation systems of banana was monitored by flow cytometry.

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B. FORTHCOMING EVENTS

Research Co-ordination Meetings

Second RCM on "Application of biotechnology and mutation techniques for the improvement of local food crops in LIFDCs", San Jose, Costa Rica, 26-30 June 2000.

First RCM on "Improvement of tropical and subtropical fruit trees through induced mutations and biotechnology", Vienna, Austria, 25-29 September 2000.

Second RCM on "Molecular characterisation of mutated genes controlling important traits for seed crop improvement", Vienna, Austria, 2-6 October 2000.

Workshops and Training Courses

Regional Training Course on "New frontiers of developing and handling mutants", Hangzhou, China, 5-16 June 2000

FAO/IAEA/UCR Workshop on "*In vitro* culture techniques for the improvement of vegetatively propagated tropical food crops", San Jose, Costa Rica, 1-5 July 2000.

Regional (AFRA) Training Workshop on "Adoption of appropriate selection techniques for the development of drought tolerant germplasm" (RAF/5/042-007) of the project AFRA III-18, component 2 on "Development and evaluation of drought tolerant mutant germplasm of cereals and legumes", Kano, Nigeria, 9-13 October 2000

FAO/IAEA Workshop on "*In vitro* protocols and mutant selection using Bayoud toxin" in Morocco during 20-26, November 2000. The total number of participants will be eight (2 Tunisia, 4 Morocco, and 2 Algeria).

FAO/IAEA Workshop on "*In vitro* mutagenesis, tissue culture and molecular markers" in Bangkok, Thailand (THA/5/045) 17-23 December 2000.

C. PAST EVENTS

3rd RCM on "Cellular Biology and Biotechnology Including Mutation Techniques for Creation of New Useful Banana Genotypes", Colombo, Sri Lanka, 4-8 October 1999.

This CRP was established in 1994 and the third RCM was held in Colombo, 4-8 October 1999. The Belgium Government started supporting this CRP in 1999. A total of sixteen participants attended the RCM, from: Austria, Belgium, Colombia, Czech Republic, Cuba, Guyana, France, Germany, Israel, Malaysia, Mexico, Philippines, Sri Lanka and the USA. Conclusions and recommendations follow:

CONCLUSIONS

Participants highly appreciated the CRP as a unique opportunity to exchange information, organize collaboration and discuss common projects. In spite of limited funding significant progress has been made since the second RCM in Kuala Lumpur in 1997. Biotechnological tools, useful clones, screening protocols and linkages among participants have been developed. Desirable variants/putative mutants of banana have been identified for release or further confirmation trials. Examples are shown in the following table:

Country	Parent	Selected Traits	Technique	Place of induction
Cuba	(a) SH3436-L9 (AAAB) (b) 6.44 (Parecido al Rey) (AAA)	(a) Height reduction (b) Height reduction	Gamma rays irradiation	(a) Cuba (b) IAEA
Malaysia	Mutiara (Pisang Rastali, AAB) Novaria (AAA)	Tolerance to FOC race 4 Tolerance to FOC race 4	Somaclones Somaclones	United Plantations Bhd, Malaysia
Philippines	Lakatan (AAA) Latundan (AAB)	Height reduction Fruit size	Gamma rays irradiation	IAEA
Sri Lanka	Embul (AAB)	Earliness Height reduction	Gamma rays irradiation	Sri Lanka

Use of Biotechnological tools available for banana improvement

In vitro culture techniques:

- Somatic embryos obtained from anther/pollen culture
- *Agrobacterium* transformation has been achieved
- Co-transformation achieved through particle bombardment
- Availability of several technologies of banana meristem cryopreservation
- Dissociation of chimerism through repeated shoot-tip cultures whereby chimeric and non-chimeric plants are obtained beyond M_1V_4 generation

Molecular cytogenetic and cytometric techniques:

- Monitor cytochimera dissociation using flow cytometry
- Rapid estimation of the karyological status of embryogenic cell suspension cultures using flow cytometry
- Ploidy analysis of mutated tetraploids using flow cytometry. Most of these accessions were found to be triploids.

- Genomic *in situ* hybridization (GISH) for analysis of genomic constitution of hybrids
- Fluorescence *in situ* hybridization (FISH) providing first physical landmarks to physically map *Musa* chromosomes

Molecular marker applications:

- Twenty PCR-based simple sequence repeat (SSR) markers have been developed for *Musa* and found to display a high degree of polymorphism between genomes
- The 'long and narrow leaf' somaclonal mutation was linked to the *in vitro* activation of a retrotransposable element
- Several retroelement homologues have been identified in *Musa*. These retroelement sequences have been found to be enriched in the vicinity of rDNA in both A and B genomes
- The amplified fragment length polymorphism (AFLP) has successfully been used in distinguishing the A and B genomes and some known phenotypic somaclones and induced mutants
- The methylation sensitive amplified polymorphism (MSAP) has been shown to be a sensitive and reproducible technique to detect DNA methylation events.

Screening techniques for banana improvement

- Developed *in vitro* system for screening nematode resistance
- Developed culture technology to produce nematodes asceptically
- Developed protocol for purifying *Mycosphaerella fijiensis* toxic metabolites
- Demonstrated necrosis induction by purified metabolites on a susceptible cultivar
- Demonstrated modified chlorophyll fluorescence by purified metabolite (decrease of vitality index)
- Developed an innovative bioassay using isolated chloroplasts to detect resistance to black sigatoka toxin
- Developed an early screening technique for Fusarium wilt by using *in vitro* plants in a double-tray system
- Established guidelines *for Fusarium* wilt resistance evaluation published by INIBAP "Evaluation of *Musa* germplasm for resistance to sigatoka diseases and Fusarium wilt" INIBAP Technical Guidelines No. 3, 1998.

Forum and Linkage

- Strengthen and increase collaboration among the participating research groups of this CRP
- Enable the Catholic University Leuven (KUL) to get cell suspension derived plants being evaluated in the field through INIBAP and IAEA partners
- Provide technology transfer training. In the last two years, participants from Mexico, Cuba and Rwanda had been trained at KUL and scientists from Burundi and Cuba at FUSAGx

- Facilitate contacts that enable the University of Frankfurt, Germany to provide SSR primers to the Philippines

RECOMMENDATIONS

Characterization and evaluation of germplasm and selections

- Need to increase awareness and access to the "Descriptor for banana (*Musa* spp.)" published by INIBAP/IPGRI/CIRAD in 1996 and the INIBAP Technical Guidelines for the "Evaluation of *Musa* germplasm for resistance to Sigatoka and Fusarium wilt" published by INIBAP/IPGRI/CTA/PROMUSA in 1998
- The former will standardize the morphological evaluation of the selections and the latter will standardize the resistance evaluation for Black Sigatoka and *Fusarium* wilt
- The evaluation for resistance and agronomic performance of the selections under field conditions should continue in Cuba, Malaysia, Philippines and Sri Lanka (see achievements)
- In Guyana, Philippines and Malaysia local germplasm should be collected and characterized according to existing guidelines
- Continue ploidy analysis of ITC (INIBAP Transit Center) collections using flow cytometry
- Re-evaluate the genomic constitution of the accessions held at ITC using newly developed DNA techniques (GISH, SSR typing, AFLP fingerprinting)

Training

- Training at FUSAGx and KUL, both in Belgium, should proceed on the role of *Mycosphaerella fijiensis* purified toxins and embryogenic cell suspension cultures, cryopreservation and transformation. PROMUSA may also assist to disseminate information of these training opportunities
- A regional training course should be organized in which both scalp- and male flower-techniques will be taught. Another regional training course on marker analysis and flow cytometry should be organized. Such courses will make maximal use of limited resources and provide the opportunity for trainees to choose the technologies that are most applicable to their needs and technical capabilities
- Techniques for the initiation of embryogenic cell suspension cultures, either from male flowers or scalps, can be transferred to researchers having appropriate skills in tissue culture
- INIBAP may be consulted in finding an experienced partner in the region, and should follow up the performance of the former trainee under local conditions after his/her return

Technology transfer

- The technology to collect, culture and characterize *Pseudomonas* isolates should be transferred to Guyana

- Existing virus indexing methods should be transferred to Sri Lanka
- Existing flow cytometry methodology in Sri Lanka needs further optimization to allow ploidy measurements of local cultivars, wild types and *in vitro* multiplied plants
- Existing collaboration should continue and further strengthened, while new partnerships should be created within the CRP and with PROMUSA

Technology development

Production of haploid plants:
- Need to regenerate the haploid plants from anther and pollen cultures
- Need to distinguish diploid (anther wall origin) from di-haploid (pollen origin) cultures using a combination of flow cytometry and SSR markers

Somatic embryogenesis:
Production of somatic embryos from shoot tip cultures
- Investigation of different parameters influencing the growth behavior of cell suspension cultures with different regeneration capacity
- Cryopreservation of embryogenic cell suspension cultures with a low regeneration capacity
- Improved regeneration system of embryogenic cell suspensions with a low regeneration capacity
- Protein analyses of embryogenic and non-embryogenic cell suspension cultures and their correlation with the different growth phases

Flow cytometry:
- Use of flow cytometry to analyze ploidy in anther and pollen cultures
- Flow cytometric analysis of proliferating meristem cultures
- Evaluation of ploidy status of embryogenic cell suspension cultures
- Monitoring cytochimera dissociation of colchicine-treated cell suspension cultures
- Cell cycle analysis of embryogenic cell suspension cultures

Somaclonal variation:
- Development of tissue culture techniques with increased genetic stability
- Application of MSAP to investigate the relationship between methylation and somaclonal variation
- Further studies to investigate the relationship between retrotransposon activation and somaclonal variation
- Further studies to investigate whether other stress factors *(e.g.* irradiation, low temperature, pathogenesis) are able to induce retrotransposon activation

Genome analysis:
- Availability of large numbers of SSR markers from *M. acuminata*
- Development of SSR markers for *M. balbisiana*
- Detection of retrotransposon-like elements and extend this study to other genotypes

- Increase in number of physical markers
- Construction of large insert DNA libraries

Mutagenesis:
- Development of a protocol for irradiation of embryogenic cell suspension cultures

Nematodes:
- Biological, physiological and molecular analyses of the *Arabidopsis*/*Radopholus similis* interactions

Screening techniques

Sigatoka:
- Production of autotrophic plants under *in vitro* conditions, followed by early *in vitro* selection with *M. fijiensis* toxins
- Large scale comparative analyses of chloroplast and entire plants response to toxins using varieties of different genomic constitution
- Performing a more detailed analysis of the juglone action mechanisms on banana isolated chloroplasts
- Toxin testing of selections obtained through mutation induction and/or somaclonal variation
- Evaluation of somatic embryos for resistance after toxin application
- Resistance screening of transgenic plants by leaf-disc assays and glasshouse and field inoculation tests

Fusarium:
- Continue to use the double-tray method for early screening of somaclones ('Novaria', 'Pisang berangan' and 'Pisang Mas') and gamma-irradiated induced mutants ('Pisang berangan') for *Fusarium* tolerance

Nematodes:
- Optimization of *in vitro* screening for resistance by comparative studies with plant responses under pot and field conditions
- Systematic evaluation of cultivars, wild types, breeding lines and selections obtained through this CRP
- Studies with *Pratylenchus* spp. should be initiated following the protocols developed for *R. similis*

Moko disease:
- Screening of irradiated, field-established plantains for Moko disease resistance
  Complementary activities
- Establishment of embryogenic cell suspension cultures from Cuban plantains ('Platano Vianda') using the scalp-methodology

- Field establishment of cell suspension derived ABB cooking bananas in Cuba through a contract between INIBAP and INIVIT
- Production of embryogenic cell suspension cultures from Sri Lankan varieties ('Embul' and 'Kolikutu') using the scalp-methodology
- Karyological analysis of tissue culture derived plants
- Shoot tip irradiation of plantains from Guyana, Cuba ('CEMSA ¾' and 'Navolean') and Philippines ('Saba')

Linkage to PROMUSA

- INIBAP will continue to supply material required by researchers, provided it is classified as virus-indexed and 'available'
- INIBAP will develop segregating populations for in-depth genetic studies and make leaf samples/DNA samples available (starting 2001) for genetic improvement working group of PROMUSA. Meanwhile, PROMUSA secretary will explore accessibility to existing segregating populations
- Linkage between CRP research groups and PROMUSA in order to increase information exchange and enhance collaboration between scientists
- PROMUSA will facilitate the publication of any new technologies developed within this CRP in collaboration with FAO/IAEA
- PROMUSA will seek permission from FAO/IAEA for the publication of the abstracts of the Third RCM to enhance information dissemination
- The usefulness of having the next RCM 'back to back' with the PROMUSA genetic improvement working group meeting needs to be considered
- PROMUSA should facilitate field testing of transgenic plants
- Selections obtained through this CRP could be made available to the International Musa Testing Program (IMTP) through PROMUSA

Co-ordination Meeting of the Regional Project on the Control of Bayoud Disease of Date Palm (RAF/5/035), Sfax, Tunisia, 15-19 May 2000.

This meeting was hosted by the Biology Department, Faculty of Science, Sfax, Tunisia, and attended by Project Co-ordinators from Morocco, Tunisia and IAEA staff. The national co-ordinators from Morocco and Tunisia presented their reports highlighting achievements and constraints. The Agency Technical Officer also gave a presentation on major projects and recent advances in the field. During this meeting, an overall in-depth analysis of the project achievements was made since its inception in 1995. The following are the major outputs of this project: somatic embryogenesis and organogenesis of date palm have been well established, and are being routinely used for plant regeneration and multiplication; isolation and production of Bayoud toxin has been achieved in Morocco, (used on detached leaves and whole plantlet for selecting against Bayoud disease) molecular marker technology is being used; better techniques are available for selecting resistant lines during selection pressure of Bayoud toxin; gamma radiation treatment has for the first time improved the maintenance of embryogenic cultures up to 3 years without losing capacity of somatic embryogenesis and

plant regeneration; all three participating countries. Algeria, Morocco and Tunisia now have well trained manpower, tissue culture and molecular biology facilities. Close collaboration among concerned national institutions was established.

D. STATUS OF EXISTING CO-ORDINATED RESEARCH PROJECTS

Genetic Improvement of Underutilized and Neglected Crops in LIFDCs through Irradiation and Related Techniques

This CRP was initiated in 1998 with the objective to overcome major constraints to increase productivity of neglectecd and underutilized crops by genetic improvement, in order to enhance the economic viability and sustain crop species diversity, and in future to benefit small farmers. Mutation techniques in combination with biotechnology are applied for the improvement of variouos vetetatively and seed propagated crops: quinoa (*Chenopodium quinoa*), cocoyams (*Colosasia esculenta, Xanthosom*a spp.), yams (*Dioscorea* spp.), grain and vegetable amaranths (*Amaranthus* spp.), Bambara groundnut (*Vigna subterranea*), grasspea (*Lathyrus sativa*), okra (*Abelmoshus esculentus*), bitter potatoes (*Solanum jucepzukii*, *Solanum ajanhuiri*) and naranjilla (*Solanum quitoense*). At the present there are 18 participating institutes from Bolivia, Costa Rica, Ecuador, France, Germany, Ghana, India, Indonesia, Mexico, Slovakia, South Africa, Syria and Thailand including an agreement holder from IPGRI based at ICARDA. The next Research Co-ordination Meeting is being held in Costa Rica from 26-30 June 2000.

Cellular Biology and Biotechnology Including Mutation Techniques for Creation of New Useful Banana Genotypes

This CRP was initiated in 1994 with the general aim to integrate radiation induced mutations *in vitro* culture and molecular genetics methods into the conventional breeding of banana to induce desirable variation such as disease resistance, dwarfism and earliness, and also to promote the development of methods for large-scale and rapid multiplication of the mutants/segregants through micropropagation and somatic embryogenesis. Plants can be readily regenerated via somatic embryogenesis for large-scale plant production, which is ideal for *in vitro* mutagenesis to the selection of mutants with desirable agronomic traits. Flow cytometry analysis of nuclear DNA content in Musa showed that Musa A and B genomes differ in size. Since 1996, Belgium has become an important contributor to this CRP. Twenty institutions worldwide are involved. The 3rd RCM was held from 4-8 October 1999 in Colombo, Sri Lanka. Conclusions and Recommendations of this meeting are presented on pages 4-10.

Mutational Analysis of Root Characters in Annual Food Plants Related to Plant Performance

This CRP was initiated this year with the overall objective to assist Member States in the application of mutation techniques and related biotechnology to generate and utilise mutants for the identification of root properties and genes for improvement of crop plants. At the

present time there are 21 participating institutes in this project. The first RCM was held in Vienna from 14-18 February 2000.

Molecular Characterization of Mutated Genes Controlling Important Traits for Seed Crop Improvement

This CRP was initiated at the beginning of 1999 with the aim to assist Member States in the application of molecular genetics of mutated genes to improve production in both major cereals and related under-utilised crops. More specific objectives were to: (a) collectively develop, characterise and data-base mutant collections of key crops for application, in breeding programmes, of CRP members and the world scientific community; (b) molecular characterization of new or existing mutated genes affecting key agronomic traits in major crops using comparative approaches in under-utilized crops with a view to their eventual isolation. The Second RCM is planned from 2-6 October 2000, in Vienna.

E. NEW CO-ORDINATED RESEARCH PROJECTS

Improvement of Tropical and Subtropical Fruit Trees through Induced Mutations and Biotechnology

Tropical and subtropical fruit trees represent a group of important orchard and plantation crops produced mainly by developing countries. They include fresh fruits, fresh and processed juices, beverages, processed and dried fruits, spices, nuts and other fresh and processed raw materials. Tropical fruits are also becoming the targets for major supermarket outlets in developed countries so that quality and food safety targets are of increasing economic importance. Tropical fruits and nuts have great potential as valuable sources of nutrition. On a global scale, the produce can be consumed in either fresh or processed form and as such increases food security and sustainable crop production in developing countries. The tropical agro-products are sold on both local and international markets and represent significant foreign exchange earnings for many developing countries. The production, cultivation and maintenance of tree species provide highly sustainable production systems that conserve soils, micro-environments and biodiversity. Recent production figures show that in 1998 (FAO databases) global areas harvested (in millions of hectares) were for citrus 7.25; mango 2.73, cashew 1.91; datepalm 0.88; avocado 0.31; papaya 0.30; and other tropical fresh fruits (combined) 1.75. To date, genetic improvement of these crops has relied heavily upon the deployment of vegetative propagation techniques and some classical breeding.

Recent biotechnological techniques have the potential to provide efficient methods of vegetative propagation, screening techniques, genetic improvement through improved mutation and genetic transformation. The particular advances which hold revolutionary significance to the application of mutation technologies to tropical and subtropical fruit trees centre around plant tissue culture and plant molecular biology which can now be integrated with conventional techniques in generating new mutations in perennial tree crops. Plant tissue culture advances including somatic embryogenesis, micropropagation, micrografting, cryopreservation of embryos, *in vitro* selection of cells and tissues to fungal toxins, somatic hybridisation, embryo rescue have already been applied to tropical and subtropical fruit trees.

The importance of tropical and subtropical fruit trees to many developing countries and the fact that these crops are one example of a still untouched niche in terms of the massive efforts invested by commercial companies in genomics, means that these crops make a very

good candidate for the research proposed in the current CRP. Tissue culture and molecular biology technologies will lead to major breakthroughs in basic understanding of the genetic control of important biological processes such as phase change, *de novo* plant regeneration, flowering, sexual expression in floral tissues and adventitious rooting. These new developments complement previous achievements such as the generation and phenotypic characterization of mutants in various annual crops and underpin the large-scale clonal propagation and future genetic improvement of tropical fruit and nut trees. The production of haploids in highly heterozygous perennials could, for example, trap and stabilize potentially valuable genetic mutations in trees within a comparatively short time frame compared to decades of selection and backcrossing which would be required to stabilize such mutations in a perennial crop. The complementary approach to the use of chemical and physical mutagens is insertional mutagenesis which allows a connection to be made between an observed phenotype and a gene. Furthermore, the use of genes from various organisms such as *Arabidopsis* and others in which genomics have already reached an advanced stage can improve the genetic bases of tropical trees in many aspects such as to modify plant hormone metabolism and lead to real practical improvements in the adventitious rooting of stem cuttings of some difficult-to-root temperate trees. These approaches could lead to the tailoring of appropriate tree architectures for the modified types of orchard and plantation cropping systems now demanded under more intensive multiple cropping production systems located on fragile tropical soils.

Description of the problem:

Tropical and subtropical fruit trees are characterized by long juvenile period and large tree size. As a result, the available genetic information in these crops is very limited. Moreover, in many crops such as avocado, mango, lychee and others, control crosses are difficult to perform due to massive fruit drop. In addition, developing countries have limited funds available for research. Thus, tropical and subtropical trees are lagging behind other plants in terms of availability of genetic information and technologies for the improvement of the biological material. Each of these tree crops is facing major agronomic and horticultural problems in terms of propagation, yield, appearance, quality, diseases and pests control, abiotic stresses and shelf-life.

Specific problems in tropical crops:

The further use of these crops as a tool for economic development and political stability involves the removal of a number of obstacles. These include biotic and abiotic stresses, post-harvest losses, the supply of improved disease and virus-free plants coupled with efficient propagation techniques. In the developed countries most of these problems can be solved or limited with the application of agro-technologies. Most farmers in the developing world do not have the resources to invest in these capital and technologically intensive solutions.

Biotic stresses are probably one of the most important factors limiting the expansion of tropical and subtropical tree crops in the developing world. Diseases caused by *Fusarium, Phytophthora* and *Colletotrichum* combined with insect and nematode pests account for significant losses of produce, and/or trees. The capital value of the remaining crop is usually further reduced by the influence of the pests and diseases on the quality of the fruit. The presence of some of these diseases or pests on or in fruit can often prevent the export of produce, and hence the loss of much-needed foreign exchange. The use of chemicals to ameliorate these problems significantly reduces the profitability of these crops, but more importantly poses significant health and environmental risks. Many developing countries do

not have or enforce legislation that controls the use and disposal of these chemicals. Indiscriminate use of insecticides and pesticides usually results in the breakdown of biological control, resulting in an ever-increasing spiral of chemical dependence. Chemical residue standards in fruit imported into developed countries are increasingly stringent and if alternatives to agrochemicals are not found this may result in the loss of valuable foreign exchange earnings. The influence of abiotic stresses is exacerbated in developing countries because the infrastructure and equipment needed to moderate these influences are usually not available. Poor transport infrastructure and long distances to ports or airports, significantly exacerbate post-harvest problems experienced by growers producing similar crops in developed countries. In order for developing countries to break into the international market it would be advantageous to produce fruit that have enhanced characteristics such as higher yield, longer storage life, improved taste, fruit colour and seedlessness. To increase the impact of these crops on local consumption, increased nutritional value is highly important. Furthermore, the rapid expansion of these industries will be dependent on the development of more appropriate propagation techniques that will ensure the rapid release of disease and virus-free selections or cultivars.

The need for a FAO/IAEA CRP to address these problems:

a) Foster relationship between technology-rich research groups and germplasm-rich groups for mutual benefits.
b) Generate desirable mutants on a large scale and high frequency.
c) Lack of international organizations dealing specifically with research on tropical and subtropical fruit trees.
d) Ensure distribution and dissemination of information and plant material.
e) Identification, characterization and dissemination of genes and promoters.

This CRP will meet the need to close the gap between the limited mutant resource and the full range of phenotypes that is essential to fully exploit tropical and subtropical tree crops. It will also increase food and economic security by ensuring sustainable crop production and increase in yield and quality.

Criteria used for selection of crops:

Crops should be tropical or subtropical fruit or nut trees.
Evidence of breeding activities in particular species.
Potential for improvement of food and/or economic stability.

Crop list:

Mango, Datepalm, Coconut, Cashew, Avocado, Papaya, Passionfruit, Jujube, Guava, Anona, Lychee

Citrus spp can be used as the model tropical fruit tree crop because there are already several defined mutants in several breeding programmes located in developing and developed countries. The available expertise will be invaluable for training scientists of collaborating groups of the CRP.

Overall Objective:

To generate and characterize radiation induced and natural genetic diversity in tropical and subtropical fruit trees for improving nutrition balance, food security, and enhancing economic status of growers in Member States.

Specific Research Objectives:

- To overcome major constraints in plant regeneration by tissue culture for large-scale multiplication of desirable induced mutants in order to sustain natural and induced fruit tree biodiversity leading to improved economic viability of growers and nutrition component of their diets
- To assess the impact of induced mutants on fruit yield and quality components, depending on the fruit tree life cycle, under the field conditions.
- To assess root stocks of induced mutants, especially tolerant to abiotic and biotic stresses, for grafting and their impact on yield.

Research agreement and contract proposals have been received and are under evaluation. The first Research Co-ordination Meeting will be held from 25-29 September 2000 in Vienna, Austria.

F. TECHNICAL CO-OPERATION PROJECTS

Development of new ornamental plant and mungbean mutant varieties in Thailand (THA/5/039, THA/5/045)

In Thailand, ornamental plants have economic importance for the local as well as foreign markets. In order to meet the demand for attractive ornamentals, there is a need to produce new varieties with different traits *e.g.* flower colour, flower morphology, flower size, long shelf-life. In addition to ornamentals, food legumes are economically important crops that play a major role in sustaining agricultural productivity. The Kasetsart University has been supported to establish a gamma greenhouse, a gamma irradiation service to plant breeders in the country and to initiate new breeding programmes under the projects THA/5/039 and THA/5/045. A close collaboration with three other institutions involved in breeding research and extension has been established to strengthen the development and evaluation of new varieties. Mutated traits have been obtained such as flower colour and flower shapes by gamma irradiation of single node cuttings of chrysanthemum. The testing and evaluation of these mutants are done in Chiang Mai, in the Northern part of the country, especially for flower colour in the milder climate. Four stable flower colour mutants of *Canna hybrida* ornamental plant induced by gamma treatment of rhizomes have been registered as new varieties in 2000 and being multiplied for distribution to growers.

In mungbean, three promising mutants have been isolated, which are ready to go to the farmer's field trials aiming to develop mutant cultivars. These mutants have high yield and tolerance to *Cercospora* leaf spot disease. Two other mutants M5-16 and M5-29 show resistance to cowpea weevil. Within 2-3 years, mutant cultivars will be released. They will offer farmers new options to control disease and pest problems of mungbean as successfully already done at the Field Crops Research Institute, Department of Agriculture with the development of the mutant variety Chai Nat 72 which is resistant to bean fly.

Current Operational Projects are:

BGD/5/019	Extension services to farmers on promising mutant varieties
COL/5/017	Mutation breeding of plantain and rice
COS/5/021	Radioactive probes for plant disease diagnosis
COS/5/023	Improved mutant varieties of rice and banana
CPR/5/010	Induced mutations for improvement of rice

CPR/5/011	Improvement of cotton and rapeseed through induced mutations
CPR/5/013	Induced mutations to improve rice quality
ECU/5/020	Resistance to disease in cacao and babaco
ELS/5/008	Improvement of potato through *in vitro* mutation breeding
ETH/5/011	Improvement of tef through mutation breeding
GHA/5/026	Improvement of cassava through mutation breeding
GUA/5/012	Mutations and biotechnology for crop improvement
INS/5/026	Mutation breeding of bananas
INS/5/027	Mutation breeding of ornamental plants
IRA/5/007	Mutation techniques for crop improvement
IRQ/5/011	Nuclear techniques in cereal production
IRQ/5/015	Induction of mutations in crops through *in vitro* culture
KEN/5/021	Improved drought resistance of crops by induced mutations
MAG/5/008	Mutation techniques and biotechnology for rice and cassava
MAK/5/004	Mutation and doubled haploid techniques to improve wheat
MAL/5/021	Mutation breeding and biotechnology for plant improvement
MLI/5/014	Field performance of selected mutants of sorghum and rice
MON/5/009	Nuclear techniques to improve production of wheat and legumes
MYA/5/008	Mutation breeding in grain legumes
PAK/5/033	Development of leaf curl tolerant varieties of cotton
PAK/5/035	Development of salt tolerant varieties of basmati rice
PAK/5/039	Pest resistant chickpea through induced mutation
PER/5/024	Introduction of barley and other native crop mutant cultivars
PHI/5/027	Mutation breeding of priority agricultural crops
RAF/5/029	Nuclear techniques in plant breeding and biotechnology (AFRA X)
RAF/5/035	Control of bayoud disease in date palm
RAF/5/042	Development of improved crop varieties (AFRA III-18)
RAS/5/037	Mutational enhancement for genetic diversity in rice (RCA)
RLA/5/035	Evaluation of cereal crop mutants (ARCAL XXIA)
SRL/5/030	Mutation breeding in bananas and plantains
SUD/5/023	Improving cotton and sugar cane crops (phase II)
THA/5/045	Radiation induced mutations for bean and chrysanthemum
URT/5/020	Improving productivity of basic food crops in Tanzania
VEN/5/018	Genetic improvement of fruits and pepper
VIE/5/013	Improvement of basic food crops through induced mutations
VIE/5/014	Rice mutant varieties for saline land
ZAM/5/020	Improvement of beans through mutation breeding

G. ACTIVITIES AT THE PLANT BREEDING UNIT, SEIBERSDORF

Increasing anther culture efficiency in rice (*Oryza sativa* L.) using anthers from ratooned plants

Anther culture response for the variety Taipei-309 was compared using anthers of ratooned and non-ratooned plants. Anthers were plated onto N6 liquid media with 0, 2, 5 and 10 mg l^{-1} ABA, respectively. After 5 days in culture, the media were removed and N6 medium without ABA was added. A higher number of pre-mitotic P-pollen grains were observed in anthers from ratooned plants. A significant synergistic effect was found between anthers from ratooned plants/ABA concentrations and the increase in embryogenic-like structures (ELS) induction and green plant regeneration efficiency. The highest frequency of anthers producing ELS was found in N6 medium (6% sucrose and 2 mg l^{-1} 2,4-D) with 5 and 10 mg l^{-1} ABA for anthers derived from ratooned plants. Mean green plant regeneration efficiencies were raised from 24.2 to 42 % (non-ratooned) and from 30 to 70 %, (ratooned donor plants) using a combination of 10 mg $l0^{-1}$ABA in induction media, and MS with 2 mg l^{-1} BAP, 1 mg l^{-1} NAA, 2 mg l^{-1} kinetin and 0.45 % agarose. The highest number of green shoots per ELS were obtained from ratooned-derived anthers (up to 82). The use of ratooned plants in anther culture, its importance and relative advantages are also discussed. **Plant Science, 151 (2000) 107-114.**

Effect of spikelet position on rice anther culture efficiency

The potential of anthers from different parts of the panicle to induce callus was investigated with the japonica rice variety Taipei 309. The results showed that the callusing abilities of anthers from different spikelet positions were significantly different. After planting 4483, 4496, 4348 anthers from the basal, middle and top parts, the percentage of anthers forming calli was 20% in the basal part, 12% in the middle part and 8% in the top part. The anthers of basal parts containing pollen at all uninucleate stages, including early, middle and late, showed higher callus induction frequency than those from middle and top parts. The green plantlet regeneration frequencies of top, middle and basal spikelets were around 18% in all three cases. From the results it would appear that anthers from the basal part of the panicle should be used in anther culture of rice in order to obtain higher efficiencies, and thereby optimise the usefulness of this technique in rice breeding programmes. **Plant Science, 153 (2000) 155-159**

Modification of a rapid screening method of rice mutants for NaCl tolerance using liquid nutrient culture

The isolation or identification of mutants requires an efficient screening method. The screening technique must be reliable and able to evaluate large amounts of mutated material. Salinity screening under field conditions is often inaccurate and difficult due to strong environmental effects. **Mutation Breeding Newsletter No. 44, (pg. 25).**

Bottlenecks in the generation and maintenance of morphogenic banana cell suspensions and plant regeneration via somatic embryogenesis therefrom.

During the last decade, *Musa* embryogenic cell suspensions were successfully initiated from scalps and (fe)male flowers of many genotypes and landraces. The initiation of a banana suspension takes 8 to 26 months depending on the explant type and landrace. Low embryogenic responses hamper the optimisation of the induction and early initiation steps. Only 1 out of 2 to 1 out of 5 good embryogenic calluses result thus far in a "good" e.g. highly regenerable and transformation-competent suspension. Clearly, their initiation is still far from routine. The main problem related to the use of scalps is the need for a prolonged culture in the presence of very high BA concentration and its possible effect on the ploidy level. Flow cytometry analysis provides a very powerful tool to quickly determine the ploidy level of starting material and suspensions. This is important, as the initiation of suspensions is so time and labour consuming. Also of prime importance is the cryopreservation of cell suspensions at an early stage as they can get contaminated very quickly, loose their morphogenetic potential with time and are prone to somaclonal variation. The first data on somaclonal variation among suspension-derived plants are now available. **INFOMUSA Vol. 8 No. 2 pp 3-7, Dec. 1999**

Chimerism in *Musa* spp.

The genetic improvement of bananas and plantains (*Musa* spp. L.) using biotechnological approaches, such as *in vitro* mutagenesis (Novak, 1992) and genetic transformation (May et al. 1995, Sagi et al. 1995) of multicellular meristems leads to a high degree of chimerism. Usually, repeated vegetative propagation must be carried out to dissociate chimeras but detailed studies as to the number of cycles required (e.g. three) (Van Harten et al., 1998) have yet to be verified. In general, mutated cell are difficult to monitor, however mutations that result in a change in genome number may be an exception in this respect since they can easily be induced by colchicine treatment. Monitoring cytochimera dissociation requires a rapid and precise method for ploidy screening at an early stage of plant development. Various phenotypic traits including stomata size, stomata density and pollen size are unsuitable for large-scale selection because screening for cytochimeras and polyploids by these indicators is slow and unreliable (Adniya & Ardian 1994, Van Duren et al., 1996, van den Hout et al, 1995). While ploidy estimation can be done by chromosome counting, this is difficult in *Musa* due to the small size of its chromosomes (Osuji et al., 1996, Dolezel et al., 1998), flow cytometric analysis of nuclear DNA content is being increasingly used for large-scale ploidy screening (Dolezel, 1998), and this has already been established in *Musa* spp. (Dolezel et al., 1994, Dolezel et al., 1997).

To monitor chimerism dissociation assessed by three propagation systems (shoot-tip culture, multi-apexing culture and corm slide culture), ploidy chimerism dissociation was monitored by flow cytometry.

The results showed that when using shoot-tip culture, during three subcultures just after colchicine treatment, the average percentage of cytochimeras was reduced from 100% to 36% and from 100% to 24% when propagating by the corm slide culture technique; whereas the multi-apexing technique allowed a reduction of the average percentage of cytochimeras from 100% to 7% after the same number of subcultures. Nevertheless, none of the systems led to complete elimination of chimerism. The general concept of chimerism was reviewed and factors that may influence chimera dissociation *in vitro* were discussed in the presentation at the third FAO/IAEA Research Co-ordination Meeting on cellular biology and biotechnology

including mutation techniques for creation of new useful banana genotypes. **INFOMUSA Vol. 8 No. 2 (PROMUSA X), Dec. 1999.**

Participation in Meetings
At the third RCM "Cellular biology and biotechnology including mutation techniques for the creation of new useful banana genotypes", Sri Lanka, 2-9 October 1999.
Lecture: Chimerism in *Musa* spp.

Ploidy Determination Service

The Unit is now offering a service for ploidy determination using flow cytometric analysis. Our main expertise is in *Oryza sativa* spp. and *Musa* spp., nevertheless any other crop can be analysed as long as ploidy controls are supplied together with the samples. Any part of the plant can be provided, although, best results are obtained from young tissues and i*n vitro* rooted plantlets.

Radiation Service Statistics

During the first half of the year the Unit received 10 requests for radiation treatment with ^{60}Co from 9 Member States. 13 different species were treated, compromising 12 seed samples and one *in vitro* material.

H. PUBLICATIONS

Bhatia, C. R., K. Nichterlein, and M. Maluszynski**,** 1999. Oilseed cultivars developed from induced mutations and mutations altering fatty acid composition. Mut.Breed.Rev. **11**: 1-36

Khan, I., M. D. Gaj, and M. Maluszynski**,** 1999. *In vitro* mutagenesis in sugarcane callus culture. MBNL. **44**: 19-20

Kodym, A., S. Hollenthoner and F.J. Zapata-Arias. Tubular skylights used for the natural lighting of *in vitro* culture growth rooms, Plant Science (in press)

Nielen, S., M. Guzman and F. J. Zapata-Arias. Studies on Tos 17 retrotransposons in rice plants derived from irradiated seeds and gemetoclonal variants, 7th International Congress of Plant Molecular Biology, Quebec, Canada.

Jain, S.M. 2000. Mechanisms of spontaneous and induced mutations in plants. Radiation Res. Vol. 2: 255-258.

Maluszynski, M. and B.S. Ahloowalia, 2000. Mutation techniques in plant breeding. Radiation Research. Vol. 2:251-254.

Maluszynski, M., 1999. Crop germplasm enhancement through mutation techniques. In: Proceedings of the International Symposium on Rice Germplasm Evaluation and Enhancement.

Rutger,J.N., J.F.Robinson and R.H.Dilday (Eds.) Arkansas Agricultural Experiment Station, Fayetteville. pp.74-82

Maluszynski, M., B. Ahloowalia, A. Ashri, K. Nichterlein, and L. van Zanten, 1999. Induced mutations in rice breeding and germplasm enhancement. In: Proceedings of the 19th Session of the International Rice Commission. FAO, Rome. pp.194-204

Maluszynski, M. and B.S. Ahloowalia, 2000. Mutation techniques in plant breeding. In: Radiation Research Vol. 2: Proceedings. Moriarty, M., C. Mothersill, C. Seymour, M. Edington, J.F. Ward and R.J.M. Fry (Eds.) Int. Assoc. Rad. Res., Lawrence. pp.251-254

Minocha, R. and S.M. Jain, 2000. Tissue culture of woody plants and its relevance to molecular biology. In: Molecular biology of woody plants, Vol. 2, S.M. Jain and S.C. Minocha (eds). Kluwer. Pp 315-340.

Szarejko, I. and M. Maluszynski**,** 1999. High frequency of mutations after mutagenic treatment of barley seeds with Na N_3 and MNH with application of interincubation germination period. MBNL. **44**: 28-30

Publication of abstract of presentations of FAO/IAEA 3rd RCM on "Cellular biology and biotechnology, including mutation techniques for creation of new useful banana genotypes" in PROMUSA Journal (1999), vol. 8, No. 2.

PLEASE COMPLETE THIS REGISTRATION FORM AND SEND IT TO THE PLANT BREEDING AND GENETICS SECTION AT THE FOLLOWING ADDRESS:

WAGRAMERSTRASSE 5, P.O. BOX 100, A-1400 VIENNA, AUSTRIA
TELEFAX: (+43-1) 26007, TELEPHONE: (+43-1) 2600

NEW CROP VARIETY DEVELOPED THROUGH MUTATION INDUCTION OR BY CROSSING WITH INDUCED MUTANTS

A. Latin name of species:

____

English name: ____________________

B. Name of new variety (cultivar):

____

C. Year of release from breeder: _____

D. Place and Date of official approval: ____

E. Parent variety(ies) - if new variety results from a cross with mutant, indicate which is the mutant:

		mutant
1.	________________________	**yes / no**
2.	________________________	**yes / no**
3.	________________________	**yes / no**

F. Main improved characters of variety (indicate if character is derived from mutation or not):

		mutation derived
1.	________________________	**yes / no**
2.	________________________	**yes / no**
3.	________________________	**yes / no**

G. Kind(s) of mutagenic treatment: ________________________

H. Doses(s) and/or concentration(s): _____________

I. Year of mutagenic treatment: _____

J. How was the variety bred: ____

K. Name(s) of breeder(s) and institute(s):

____

____

address: ________________________

____

____

L. Extent of acceptance by growers:

- **Commercial value:** _____
- **Hectares of cultivation:** _____
- **Other:** _____

M. References (published articles, official documents, etc.):

Name of person contributing this information: _____

THANK YOU FOR YOUR KIND COLLABORATION !