PLANT BREEDING AND GENETICS NEWSLETTER

Joint FAO/IAEA Division
of Nuclear Techniques
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TO THE READER

The FAO/IAEA Interregional Training Course on Mutant Germplasm Characterisation Using Molecular Markers was definitely the most important event of the Plant Breeding and Genetics sub-Programmes activity in 2001. The course was held at the FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria, 1-25 October. The programme covered various DNA marker techniques such as genomic DNA isolation, restriction analysis of genomic and plasmid DNA, gel electrophoresis, southern transfer of genomic DNA, DNA hybridization, autoradiography, RFLP, AFLP, SSR, ISSR and RAPD analysis, and inverse PCR. The course was very successful mainly due to three major components: outstanding lecturers, very enthusiastic and motivated trainees, and last but not least, very efficient organization and excellent preparation of the course. The lectures and exercises were covered by Günter Kahl (Germany), Uri Lavi (Israel), Katrien Devos (UK), Jeff Bennetzen (USA), Madan Mohan (ICGEB) and Stephan Nielen, (FAO/IAEA). The staff of the Plant Breeding Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf helped in conducting all exercises and practical demonstrations. In total, 21 participants from the following countries attended the course: Algeria, Bulgaria, Brazil, China, Croatia, Cuba, Ethiopia, Ghana, Guatemala, India, Indonesia, Iran, Kenya, Malaysia, Moldavia, Pakistan, Republic of Slovakia, Romania, Syria, Thailand and Turkey. In the last week of the course, the participants were able to successfully apply the above-mentioned techniques to their DNA samples of wheat, avocado, cassava, garlic, amaranthus and tomato.

We decided to prepare a laboratory manual on the basis of the reading materials and laboratory protocols, that were provided to the participants by the lecturers. Perry Gustafson (USA) and Brian Forster (UK), with the help of the lecturers, compiled and edited all protocols. It is expected that the manual will be printed before March 2002 and freely distributed to requesting scientists. The manual will also be available on CD-Rom and through our Homepage.

2001 was the last year of the Co-ordinated Research Project on "Cellular biology and biotechnology including mutation techniques for creation of new useful banana genotypes." The fourth and final Research Co-ordination Meeting was held in Leuven, Belgium, 24-28 September. This location was selected as the Belgium Government co-sponsored the CRP over the last five years. The CRP yielded many interesting results, which stimulated participants to apply to the Common Funds for Commodities for continuation of the work on banana improvement.

This year will bring a lot of interesting events in the sub-Programme. We are expecting to organize three RCMs related to molecular markers, root systems and induced mutations in fruit trees. Preparations have also been initiated for the FAO/IAEA Consultants Meeting on "The future of molecular marker technology in crop plant improvement", which should be held in Vienna in July 2002.

Miroslaw Maluszynski

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B. FORTHCOMING EVENTS

International FAO/IAEA Symposium on the "Use of mutated genes in crop improvement and functional genomics" – POSTPONED TO 2004

Technical Officer: M. Maluszynski

This is to inform you that the sponsoring Organizations have decided to reschedule the above Symposium to the year 2004. Rapid progress has recently been observed in methodology dealing with functional genomics where induced mutations, especially fast neutron mutagenesis, have wide applications. The new approach combining microarrays technologies, DNA markers and quantitative trait loci statistical analysis has brought a real revolution in research on gene functions. Many laboratories have already initiated research in this area but the first, more comprehensive results should be expected next year.

We certainly take note of your interest in meetings related to the use of mutated genes in crop improvement and functional genomic and will keep you informed of Agency activities relevant to these issues. We hope that this postponement has not caused too much of an inconvenience.

2nd Research Co-ordination Meeting on "Mutational analysis of root characters in annual food plants related to plant performance", Krakow, Poland, 10-14 June 2002

Technical Officer: M. Maluszynski

This meeting under will be held in Krakow, Poland, 10-14 June 2002. Twenty participants will present the results of their work on mutational dissection of root characters in crop plants. The results of genetic analysis of developed root system mutants and mutated genes tagging will be the major subject of the discussion.

3rd and final Research Co-ordination Meeting on "Molecular characterization of mutated genes controlling important traits for seed crop improvement", Krakow, Poland, 10-14 June 2002

Technical Officer: M. Maluszynski

This meeting will be held in Krakow, Poland, 10-14 June 2002. Twenty-one participants will present results of their work on application of DNA marker techniques in plant breeding and basic research. Current approaches in characterization of mutated germplasm will be the major topic for the discussion.

Regional (AFRA) Training Workshop to "Review mutation methodology and results of experiments on genetic improvement of neglected African crops" RAF/5/050-001, Douala, Cameroon, 24-28 June 2002

Technical Officer: K. Nichterlein

This AFRA Training Workshop is the first regional activity of the new project of AFRA (RAF/5/050; 2002-2006) on "Increasing Production of Nutritious Food Through Mutation Technique Breeding and Biotechnology". The Workshop will be jointly organized by the International Atomic Energy Agency in collaboration with the Government of Cameroon. It will be held in Douala, Cameroon and hosted by the Institut de Recherches Agricoles pour le Développement (IRAD), Centre Régional d'Ekona, from 24-28 June 2002 for Principal Investigators participating in the AFRA project with the improvement of neglected African Crops. This Workshop is a follow-up activity of the regional planning workshop on "Planning of regional activities in improvement and rehabilitation of traditional and neglected food crops through mutation techniques", held at ARC Roodeplaat, in Pretoria, South Africa from 24 to 28 November 1997 and various subsequent regional training courses on mutation techniques for crop improvement. By the time of the training Workshop, national research teams will have extended germplasm collections evaluated and initiated breeding programmes on various neglected/underutilized crops. The participants will report on their experience in producing segregating mutant populations of those crops, identification of useful mutants and their evaluation by presenting their research results and plans. The purpose of this Workshop is to share experiences with other collaborating scientists from the region and to discuss and agree on suitable breeding techniques for regional crop improvement programmes. Presentations by participants, international experts and FAO/IAEA staff, a field visit and a practical demonstration of cocoyam *in vitro* mutagenesis and group discussions will address these areas. Deadline for submission of nominations from AFRA participating countries is 31 March 2002.

2nd Research Co-ordination Meeting on "Improvement of tropical and subtropical fruit trees through induced mutations and biotechnology", Florida, USA, 1-6 September 2002

Technical Officer: M. Jain

The 2nd Research Co-ordination Meeting under the FAO/IAEA Co-ordinated Research Project will be held in Florida, USA, 2-6 September 2002. The RCM Group will present various strategies in order to recover mutants with such traits as resistance to abiotic and biotic stresses, fruit quality, tree architecture etc., for enhanced food security and sustainability.

Consultants Meeting on "Low cost tissue culture", Vienna, Austria, 8-12 July 2002

Technical Officers: M. Maluszynski & B.S. Ahloowalia

It is planned to produce a manual on "Low Cost Technology in Plant Tissue Culture", which is intended for end-users in developing countries.

Examples of topics are:

- Physical components of the technology. Low cost lay-out of the laboratories, preparation of room (kitchen), transfer room, culture or growth room, hardening and weaning area, soil growing, greenhouse facility, packaging and shipping. Related facilities – office, storage for chemicals, containers and supplies; Low cost media and culture containers; Reducing electricity/lighting/gas/water; Increasing labour efficiency; Overhead costs, costs of the various components and which components will save costs.
- Incorporation of short cuts and low cost in various stages of technology: explant initiation – surface sterilization, establishment of mother explants; subculture for multiplication/proliferation of explants; shoot and root production; weaning and hardening; transfer to soil and growth in glasshouse; delivery to the marker/growers.
- Maintenance of high efficiency and increasing efficiency; How to save costs without reducing efficiency and sacrificing quality. Standard practices; reducing contamination rate; reducing losses during weaning/hardening/soil growing/packaging; quality assurance of the end product to the market, supplier/growers.
- Use of micropropagated plants by growers, farmers, on-farm conventional multiplication of the clonally propagated material for cost reduction.

C. PAST EVENTS

Research Co-ordination Meeting on "Cellular biology and biotechnology including mutation techniques for creation of new useful banana genotypes", Leuven, Belgium, 24-28 September 2001

Technical Officer: M. Jain

The fourth and final RCM of the banana CRP on "Cellular biology and biotechnology including mutation techniques for creation of new useful banana genotypes" was held at the Katholieke Universiteit Leuven (KUL), Leuven, Belgium, 24-28 September 2001. A total of ten participants attended this RCM, from Austria (FAO/IAEA), Belgium, Cuba, Czech Republic, Germany, Israel, Mexico, Philippines, and Sri Lanka. All participants were in favour of publishing the results of CRP in a book entitled "Banana Improvement: Cellular and Molecular Biology, and Induced Mutations". This book will be published with an international publisher.

Overall achievements

Tools were developed for germplasm characterization and improvement through induced mutations, cryopreservation, somatic embryogenesis, somaclonal variation and genetic engineering. Some of the existing cultivars have been improved for disease tolerance and important agronomic traits. Collaborations among participating laboratories were established, including exchange of staff, training and technology transfer.

Practical achievements

Research contract holders J. Lopez Torres (Cuba), Mak Chai (Malaysia), A. James (Mexico), and J. Dolezel (Czech Republic) did exceedingly well in this CRP. Nicolas Roux

(FAO/IAEA) has been instrumental in dissociation of chimerism and developing flow cytometry, and has already published results in international refereed journals. From this CRP, several young students benefited in completing their master degree as well as in completing/in the process of completing their Ph.D. programs in Israel, Czech Republic, and Belgium Some of the participants presented their results in international conferences. From this CRP, several research papers (51) have been published in conference proceedings and international refereed journals.

Many international trainees received training on several aspects of banana tissue culture, molecular cytogenetics and molecular markers at KUL and Faculte universitaire des sciences agroniques, Gembloux (FUSAGx), Belgium; Institute of Experimental Botany (IEB); and University of Frankfurt, Germany. The trainees came from developing countries such as China, Cuba, Egypt, Mexico, and Rwanda.

- Laboratories for flow cytometry were established in the International Institute for Tropical Agriculture (IITA, Nigeria) and in Malaysian Institute for Nuclear Technology MINT, Malaysia). The transfer involved staff training in the Institute of Experimental Botany (IEB,Czech Republic) and expert visit.
- The outcome of this training was very successful. For example, the Cuban trainee was successful in establishing new somatic embryogenic banana cell suspensions from Cuban plant material. In addition, he successfully irradiated plant material in Cuba.
- In Sri Lanka, 20 persons from the countryside were given training in tissue culture technology for mass production of banana. Post-graduate training on indexing of banana viruses was organized.

Specific achievements

1. Detection of DNA methylation polymorphism in banana micropropagated plants with amplified fragment length polymorphism (AFLP).
2. Embryogenic cell suspension cultures were developed for several banana cultivars including plantains (AAB), and also their cryopreservation protocol.
3. Three cryopreservation techniques were developed for long-term conservation of meristems. An INIBAP technical guideline for cryopreservation of banana was published.
4. Induced mutations generated a series of improved clones that were screened for different traits such as early flowering, reduced height, large fruit size, and tolerance to Fusarium, in CRP participating countries Cuba, Malaysia, Philippines, and Sri Lanka.
5. Both *Agrobacterium*-mediated transformation and particle bombardment methods were used for banana transformation, and transformation rate was cultivar dependent.
6. Virus indexing procedures were transferred to Sri Lanka for indexing local banana virus strains.
7. In Malaysia, an early screening technique was developed for Fusarium wilt using tissue culture-derived plants in a double-tray system.
8. A selection system was developed against Black sigatoka disease by using *Mycosphaerella fijiensis* crude extract, the semi-purified, and one purified fraction (juglone)
9. Screening techniques for nematode resistance in *Musa* under shade-house and field conditions. Aseptic cultures of *Radopholus similes* and *Pratylenchus coffeae* were established using alfalfa calli., and their pathogenicity was confirmed after greenhouse tests.
10. DNA flow cytometry was used for detection of polyploidy, monitoring of cytochimera

dissociation, and analysis of karyological stability of ECS.

11. Transposon mutagenesis was explored for gene tagging, using maize Ac element, in banana genome. A substantial number of distinct mutants were generated and characterized
12. Fluoresence *in situ* hybridization (FISH) protocol was developed for *Musa* for detailed studying of karyotypes, providing distinct chromosome landmarks, gene localization, analysis of long-range chromosome structure, and linking to physical and genetic maps.
13. A total of 28 allele-specific simple sequence repeat (SSR) markers were generated for *Musa* and used to detect: polypmophisms between the A and B genomes, identify hybrids, and trace back the B genome in hybrids. These markers are now used within the CRP and worldwide. A total of 24 locus-specific, highly polymorphic SSR markers were also produced for *Mycosphaerella fijiensis* to discriminate them from other species.

Group Meeting on "Novel approaches on improving crop tolerance to salinity and drought" INT/5/141, Vienna, Austria, 12-16 November 2001

Technical Officers: K. Nichterlein & G. Keerthisinghe

A Group Meeting on "Novel Approaches on Improving Crop Tolerance to Salinity and Drought" (INT/5/141) was held in Vienna, Austria, 12-16 November 2001. This meeting was conducted in conjunction with a Consultants' Meeting on "Identification of Crop Species/Cultivars for Drought and Salinity Tolerance for Sustained Crop Yields by Using Nuclear Techniques, in Particular the Carbon Isotope Discrimination". Nine consultants from Australia, Canada, China, Germany, Israel, India (ICRISAT), Pakistan, South Africa and USA attended the Group Meeting. Five participants from Australia, Mexico (CIMMYT), Pakistan, UK, the USA and a representative from FAO attended the Consultants Meeting. Ms. K. Nichterlein and Mr. G. Keerthisinghe (Soil, Water & Crop Nutrition Section) served as Scientific Secretaries of the Group Meeting and Consultants Meeting respectively. Mr. J. Quian, Deputy Director General, Department of Technical Cooperation and Mr. J. Dargie, Director, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, welcomed the participants and emphasized constraints in increasing crop and food production and sustaining soil fertility under saline and drought conditions. Five technical sessions were held during the first two days, in which the participants presented papers on various approaches for improving crop tolerance to drought and salinity and the role of nuclear techniques in the identification of crop tolerance. After the presentations two working groups were formed: one consisting of the participants of the Group Meeting and the other consisting of the participants of the Consultants Meeting.

Participants of Group Meeting:
Dr. Pichu Rengasamy, Adelaide University, AUSTRALIA
Dr. Herald Steppuhn, Semi-arid Prairie Agricultural Research Centre, CANADA
Prof. Dr. Debao Li, Zhejiang University, PR CHINA
Dr. Arnd Heyer, MPI Molecular Plant Physiology, Potsdam, GERMANY
Dr. Nirit Bernstein, Volcani Research Center, ISRAEL
Dr. Rachid Serraj, ICRISAT, INDIA
Dr. Mujtaba Naqvi, Nuclear Institute for Agriculture and Biology, PAKISTAN
Dr. Kobie de Ronde, Agricultural Research Council, SOUTH AFRICA

Prof. Dr. Majid Foolad, Pennsylvannia State University, USA

Participants of Consultants Meeting:
Prof. Dr. Anthony Hall, University of California, USA
Dr. Anthony Condon, CSIRO Division of Plant Industry, AUSTRALIA
Dr. Philippe Monneveux, CIMMYT, MEXICO
Dr. John Gorham, University of Wales, UK
Dr. Shafqat Farooq, Nuclear Institute for Agriculture and Biology, PAKISTAN
Dr. Helena Gomez Macpherson, FAO, ITALY

The Group Meeting participants reviewed conventional and molecular approaches for the improvement of crop tolerance to salinity and drought and identified research priorities for future work on the productivity improvement under such abiotic stresses. They concluded that the complexity of those stress syndromes should be resolved with a holistic approach integrating physiological dissection of the resistance traits and molecular genetic tools, together with conventional breeding and agronomical practices that lead to better conservation and utilization of soil moisture as well as matching crop genotypes with the environment. A systematic characterization of the drought environments should be done where the crops are grown to enable adequate targeting of drought resistance traits, using historical climatic series, GIS tools, water balance and crop simulation models. It was recommended that preliminary surveys be conducted among the target farmers to obtain their views, preferences and cropping ambitions with respect to crop improvements and new stress tolerant crops.

It was recommended to evaluate the available germplasm (natural and induced variations/mutations) for salt and drought tolerance. Specific emphasis must be placed on important food and forage crops. A high priority objective in the improvement of crop productivity and system sustainability under drought and salinity should focus on the genetic improvement of symbiotic and associative nitrogen fixation under stress conditions, for both agricultural and natural systems. Important traits for drought tolerance improvement should include plant phenology (drought escape) and root characteristics (drought avoidance). For salt tolerance, it is recommended to improve the nutrition status of the shoot for K and Ca under salt stress, using physiological and molecular techniques, as well as transgenic plants over-expressing ion transporters. It is recommended to identify and characterize genes/QTLs conferring stress tolerance using appropriate genetic populations (e.g. recombinant inbred lines or inbred backcross lines) and target environments. Such QTLs can be transferred to target genetic background using marker-assisted selection and breeding. It is recommended to develop plant ideotypes incorporating saline/drought resistance on the basis of soil and hydrological processes.

Overall, it is recommended to initiate and support crop improvement programmes for stress conditions, following an integrated strategy. For agricultural cropping systems of arid and semi-arid zones stress, main emphasis should be on the improvement of stress tolerance of crops with high yield potential (durum wheat, barley, triticale, pearl millet, sorghum, upland rice, chickpea, cowpea, groundnut etc.). The approach for extreme drought and salt affected zones should be on the identification and improvement of plant species with tolerance to extreme stress conditions. The role of nuclear techniques in such crop improvement programmes will be their use in characterisation of soil properties (neutron probes, isotope exchange studies), in biological nitrogen fixation studies of legumes (^{15}N), feeding experiments of forage crops,

molecular marker development (^{32}P, ^{33}P), and in genetic enhancement through induced mutations (irradiation with ^{60}Co).
The Consultants Meeting proposed various strategies for using carbon isotope discrimination technique as a selection tool for identifying higher yielding crop genotypes in wheat and rice cropping systems under drought and salt stresses. A proposal was formulated to address the above issues in the framework of a Co-ordinated Research Project.

Regional AFRA Training Course on "Improved mutation, *in vitro* culture and drought screening techniques for the improvement of African crops" (RAF/5/042-008), Agricultural Research Council (ARC) – Roodeplaat, Pretoria, South Africa, 15-26 October 2001

Technical Officer: K. Nichterlein

Background
The purpose of this Training Course was to train skilled plant breeders/technicians belonging to the national research team of project RAF/5/042 on "Development of improved crop varieties". Thirteen participants from the region (Algeria, Cameroon, Ghana, Kenya, Libya, Madagascar, Mali, Morocco, Nigeria, Sierra Leone, Sudan, Tanzania, Zimbabwe and South Africa) have been trained in the use of induced mutations for the improvement of African crops, for screening of large mutant populations for traits related to tolerance to drought stress and use of *in vitro* culture techniques in crop improvement. The two participants from Egypt and Mali did not attend the Training Course.

The AFRA designated Regional Center for Induced Mutations and Related Biotechnology includes the ARC Roodeplaat, its sister institutes ARC-ITSC Nelspruit and ARC-GCI Potechefstroom. ARC Roodeplaat is the major institute for AFRA group training activities. In the current training activity, 10 local and 4 international experts including the Technical Officer were involved in lectures, practicals and group discussions on the various subjects and methods covered during the two-week period. Field and greenhouse trials on screening of mutant populations of Bambara groundnut (for drought under a rainout shelter), vegetable amaranth and cowpea (in wooden boxes in the greenhouse) were visited.

Interregional Training Course on "Mutant germplasm characterization using molecular markers", FAO/IAEA Agriculture and Biotechnology Laboratory, A-2444 Seibersdorf, Austria, 1-25 October 2001

Technical Officer: M. Maluszynski

Background
The objective of this training was to train scientists from developing countries on different aspects of molecular markers and to teach the applications of molecular markers in characterizing mutations and their role in plant breeding. Recent developments in DNA marker technology together with the concept of marker-assisted selection (MAS) provide new solutions for selecting and maintaining desirable phenotypes. Molecular marker technology is

now integrated into existing plant breeding programs all over the world in order to allow researchers to access, transfer and combine genes at a rate and with a precision not previously possible. Molecular markers offer great scope for improving the efficiency of conventional plant breeding by carrying out selection not directly on the trait of interest but on molecular markers linked to that trait.

Progress has been made in recent years in mapping, tagging and isolating many agriculturally important genes utilizing molecular markers. Over the last years, many molecular techniques have been developed which help us in finding molecular markers of interests. A few techniques, which are particularly promising in assisting selection for desirable characters are: RFLPs (Restriction Fragment Length Polymorphism), AFLPs (Amplified Fragment length Polymorphism), RAPDs (Random Amplified Polymorphic DNA), Microsatellites and PCR based DNA markers such as SCARs (Sequence Characterized Amplified Regions), STS (Sequence Tagged Sites) and ALPs (Amplicon Length Polymorphism). These techniques help in direct selection of many desired characters simultaneously using F_2 and back-cross populations, near isogenic lines, double haploids and recombinant inbred lines.

List of Topics

Many techniques were taught in the laboratory. Participants gained experience in Genomic DNA isolation, Restriction analysis of genomic and plasmid DNA, Gel electrophoresis, Southern transfer of Genomic DNA, Random priming of plasmid DNA, DNA hybridization, Autoradiography, RFLP analysis, RAPD analysis, AFLP analysis, SSR and ISSR analysis, and Inverse PCR. In addition to hands on experience on many techniques, participants were also given demonstrations on the DNA sequencer.

Theory lectures covered many chapters related to molecular markers and their application in detecting DNA polymorphism. Many of the latest molecular techniques and their application in genomics studies were also discussed.

Participation

Twenty participants from different developing countries were invited to this four-week training course. Participants carried out their own projects and some were able to repeat, on their own, some of the techniques learned here.

Faculty

The following scientists participated in teaching different aspects of molecular markers:

Dr. Madan Mohan, ICGEB, New Delhi, INDIA
Dr. Gunter Kahl, Frankfurt University, GERMANY
Dr. Uri Lavi, ARO, Volcani Centre, ISRAEL
Dr. Jeff Bennetzen, Purdue University, USA
Dr. Katrien Devos, John Innes Center, UK
Dr. Stephan Nielen, Joint FAO/IAEA Division,Vienna, AUSTRIA

The Interregional Training Course was successful as participants were able to learn many techniques in molecular biology with special reference to application of molecular markers in characterizing mutations and their role in plant breeding.

Consultants Meeting on "Development of low cost tissue culture techniques", Seibersdorf, Austria, 8-12 October 2001

Technical Officer: J. Zapata-Arias

The meeting was organised at the end of a two-year technical contract between the FAO/IAEA Seibersdorf Laboratory and collaborators in Brazil and Cuba. The research was discussed and evaluated by the following scientists:

Dr. Maria Caridad Gonzalez Cepero, Departamento de Genetica y Mejoramiento, CUBA
Prof. Dr. Pierre Debergh, University of Gent, BELGIUM
Dr. Richard Litz, University of Florida, Tropical Research and Education Center, USA
Dr. Benedita Ines Franco Possignolo Rodrigues, CENA, Sao Paulo, BRAZIL
Dr. H.M. Sankararamasubramanian, M.S. Swaminathan Research Foundation, INDIA

Recommendations can be summarised as follows:

This project has addressed some of the high cost parameters of micropropagated tropical crops: sucrose quality, gelling agents, growth environment and the use of natural light. The results indicate that there can be significant cost savings if local products are used for standard procedures. Most important, however, is the module concept for a plant tissue culture facility, involving the use of a standard container-sized growth room of 18 m^2 that could facilitate the growth of approximately 100,000 plants/year and natural lighting provided by tubular skylights. This system could vitalise commercial micropropagation in developing countries and could serve as the basis for provisioning FAO/IAEA TC projects in these countries. Additional studies e.g. on water quality are necessary in order to optimise a truly low cost system for commercial use.

Training Course on "Application of molecular marker technology in the characterization and utilization of induced mutants in rice breeding", Manila/Los Baños, the Philippines, 7-21 July 2001

Technical Officer: M. Maluszynski

The purpose of the Training Course was to enhance knowledge and provide practical training on current molecular marker techniques and their use in evaluation and characterization of rice mutants for breeding programmes and functional genomics. A total of 14 participants from different countries in the region were invited to this training. The course was composed of lectures and exercises on various molecular marker techniques and especially: new horizons in rice breeding; hybridisation- and amplification-based DNA markers with hands-on training on SSRs and SNPs markers; construction of molecular maps; DNA fingerprinting of germplasm/mutants; general strategies of genome mapping; molecular tagging of genes governing agronomic traits; PCR based markers and their utilization in rice breeding; marker

assisted selection in rice breeding; application of molecular markers in cloning of agronomically important genes and in functional genomics.

D. STATUS OF EXISTING CO-ORDINATED RESEARCH PROJECTS

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

Technical Officer: K. Nichterlein

This CRP was initiated in 1998 with the objective of overcoming major constraints to increase productivity of neglected 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 various vegetatively and seed propagated crops: 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 present there are 16 participating institutes from Bolivia, Costa Rica, Ecuador, France, Germany, Ghana, India, Indonesia, Slovakia, South Africa, Syria and Thailand including an agreement holder from IPGRI based at ICARDA. This CRP made good progress. In most participating countries, significant progress was achieved in collecting germplasm, establishing germplasm banks and developing mutation, *in vitro*, and molecular techniques for the improvement of so far scientifically neglected crops with importance to household food security of LIFDCs. Radiosensitivities have been established after mutagenic treatment of seed and/or *in vitro* cultures for a number of crops for which no or only little information was available before initiating this CRP. A combined *in vitro*/*in vivo* system to shorten the breeding cycle of pea was successfully adapted to bambara groundnut. Methodology for stress screening of mutated populations has been developed or improved, such as screening for root rot and leaf blight in cocoyam and for drought in amaranth. The protocols have been used to develop early generations of mutant populations in okra, naranjillo, grain and vegetable amaranths, Bambara groundnut, grass pea, quinoa, cocoyam, yam and bitter potato. Putative mutants with desired traits have been identified in the seed propagated species, whereas in the vegetatively propagated species mutated material was first multiplied *in vitro* to dissociate chimera, and M_1V_4 and M_1V_5 plants have been transferred to soil for greenhouse and/or field screening. It is planned to hold the third Research Co-ordination Meeting in 2003.

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

Technical Officer: M. Jain

The 4th and final RCM was held in Leuven, Belgium, 24-28 September 2001.

This CRP was initiated in 1994 with the general aim of integrating 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 organogenesis for large-scale plant production. Somatic embryogenesis cell suspension cultures are ideal for *in vitro* mutagenesis and the selection of mutants with desirable agronomic traits. Since 1996, Belgium has been an important financial contributor to this CRP.

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

Technical Officer: M. Maluszynski

This CRP was initiated in 2000, with the overall objective of assisting Member States to apply 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. Reports were obtained from all and evaluated.

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

Technical Officer: M. Maluszynski

This CRP was initiated in 1999 with the aim of assisting Member States to apply molecular genetics of mutated genes for improving production in both major cereals and related under-utilised crops. More specifically to collectively develop, characterise and data-base mutant collections of key crops for application in breeding programmes and to molecularly characterize 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.

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

Technical Officer: M. Jain

This CRP was initiated in 2000 and the first RCM was held in October 2000, with 12 participants. This RCM was reported on in detail in Newsletter No. 6, December 2000. The overall objective of this project is 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.

E. TECHNICAL CO-OPERATION PROJECTS

Current Operational Projects

Project No.	Title	Technical Officer
COS/5/021	Radioactive probes for plant disease diagnosis	S. Nielen
COS/5/023	Improved mutant varieties of rice and banana	M. Maluszynski
CPR/5/011	Improvement of cotton and rapeseed through induced mutations	M. Maluszynski
CPR/5/013	Induced mutations to improve rice quality	M. Maluszynski
GHA/5/026	Improvement of cassava through mutation breeding	M. Jain
GHA/5/030	Improved cocoa productivity through control of cocoa swollen shoot virus disease	S. Nielen
INS/5/027	Mutation breeding of ornamental plants	M. Jain
INS/5/030	Sustainable agriculture development in Yogyakarta	M. Jain
INS/5/031	Mutation breeding of horticultural crops	M. Jain
IRQ/5/015	Induction of mutations in crops through *in vitro* culture	M. Jain
JOR/5/008	Establishment of *in vitro* mutagenesis laboratory	M. Maluszynski
KEN/5/021	Improved drought resistance of crops by induced mutations	M. Maluszynski
MAG/5/008	Mutation techniques and biotechnology for rice and cassava	M. Maluszynski
MAK/5/004	Mutation and doubled haploid techniques to improve wheat	K. Nichterlein
MAL/5/024	*In vitro* mutagenesis for horticultural crop plants	M. Jain
MYA/5/010	Development of improved rice with tolerance to drought	K. Nichterlein
PAK/5/035	Development of salt-tolerant varieties of basmati rice	K. Nichterlein
PAK/5/038	Development of drought and heat tolerant canola mutants	K. Nichterlein
PAK/5/039	Pest resistant chickpea through induced mutation	K. Nichterlein
PAK/5/040	Improvement of heat-tolerant semi-dwarf bread wheat	K. Nichterlein
PER/5/024	Introduction of barley and other native crop mutant cultivars	M. Maluszynski
PHI/5/027	Mutation breeding of priority agricultural crops	S. Nielen
RAF/5/035	Control of bayoud disease in date palm	M. Jain
RAF/5/042	Development of improved crop varieties (AFRA III-18)	K. Nichterlein
RAF/5/049	Field evaluation of bayoud-resistant date palm mutants	M. Jain
RAF/5/050	Increasing production of nutritious food through mutation breeding and biotechnology	K. Nichterlein
RAS/5/037	Mutational enhancement for genetic diversity in rice	M. Maluszynski
RLA/5/035	Evaluation of cereal crop mutants (ARCAL XXIa)	M. Maluszynski
ROK/5/033	Quality improvement of major crops and integrated plant nutrition management in the low-input agri-cultural system.	M. Maluszynski
SRL/5/034	Radiation-induced mutations for black pepper improvement	M. Jain

SRL/5/034	Radiation-induced mutations for black pepper improvement	M. Jain
SRL/5/036	Virus screening of improved banana mutants for large-scale dissemination	S. Nielen
SUD/5/026	Improvement of the productivity and sustainability of industrial crops	S. Nielen
URT/5/020	Improving productivity of basic food crops in Tanzania	M. Maluszynski
VEN/5/018	Genetic improvement of fruits and pepper	M. Jain
VIE/5/014	Rice mutant varieties for saline land	K. Nichterlein
YEM/5/003	Applying nuclear techniques for improvement of crop yield	K. Nichterlein
ZAM/5/022	Crop improvement through *in vitro* mutation techniques	K. Nichterlein

TC project "*In vitro* mutagenesis for horticultural crop plants" (MAL/5/024)

The worldwide floriculture industry's estimated worth is over € 50 billion and is increasing annually by 8-10 percent, especially in the developing countries. The Malaysian Government has targeted the increase in land area for floriculture products and the expected floriculture industry will contribute substantial income as national exports. Small farmers will get economic benefits, and consequently, floriculture would become a 'cash crop'. Under this project, one new radiation induced mutant variety of orchid, namely Dendrobium 'Sonia KeenaAhmadSobri' was released to the growers. This mutant has diamond shaped petals, flowers are of parental types and shelf life is 15 days. Dendrobium is one of the largest orchid genera, consisting of more than 1500 species, with many hybrids. Another radiation-induced mutant variety *Tradescantia spathcea* var. Sobril, was released, which is variegated with creamy stripes and best used as a potted specimen. *T. spathcea* is a fast growing tropical succulent normally used as a ground cover to edge boarders/pathways, or as a potted specimen.

F. ACTIVITIES AT THE PLANT BREEDING UNIT, SEIBERSDORF

Improvement of *Musa* ssp. through *in vitro* mutagenesis

When using embryogenic cultures in micropropagation of banana, somaclonal variation occurs among regenerated plantlets. This variation may interfere with mutations, which could be obtained through mutation techniques. Although the causes of this chromosome instability are poorly understood, it is believed to be one of the most common causes of tissue culture-induced variation. Using flow cytometry, variation in chromosome number could be detected among plants regenerated via somatic embryogenesis from tissue culture. The results obtained by flow cytometry were verified by chromosome counting in meristem root-tip cells. After standardization of the method, the results indicated that flow cytometry was sensitive enough to detect aneuploidy in *Musa* with ± 1 chromosome accuracy. Abnormalities in DNA content could be detected at an early stage, during *in vitro* culture. For the first time, a banana embryogenic cell suspension (ECS) with 5 chromosomes missing was reported.

The first radiosensitivity tests of *Musa* ECS were performed and it has been found that cell suspensions from *Musa* could tolerate up to 200 Gy. At 100 Gy the growth curve was only affected at 50% compared to the control.

When irradiating cell suspensions, large populations can be handled under controlled conditions and if embryos are of single cell origin, they overcome the problem of chimerism. We simulated this by treating ECS with colchicine and determined the ploidy of the regenerated plants by flow cytometric analysis. To date no mixoploid regenerated plants from colchicine treated ECS were detected. These results suggest that embryos are of single cell origin, thus by using ECS mixoploidy (chimerism) could be overcome.

An early mass screening method based on the use of the toxin Juglone (5-Hydroxy-1,4-naphthoquinone), the main toxin to be responsible of the global effect of the fungus *Mycosphaerella fijiensis,* was used to screen for resistance to the black Sigatoka disease. The test was applied when the acclimatized plants reached the 6 leaf stage. The dose of 25 ppm permitted differentation between the tolerant variety Fougamou and the susceptible variety Grande Naine. Presently, from around 4000 irradiated Grande Naine plants screened, 19 putative mutants were selected for their tolerance to 25 ppm of Juglone. These plants are now being evaluated for their tolerance to the inoculation of the fungus at the Plant Pathology Unit of the University of Gembloux, Belgium.

Gamma radiation is being used to enhance genetic variation in *Musa*. About 2000 irradiated banana plants have now been tested with juglone (the main toxin of the fungus *Mycosphaerella fijiensis* which is causing black Sigatoka disease) and 20 plants showed different levels of tolerance. Resistance to *Mycosphaerella fijiensis* in these plants still has to be confirmed through inoculation with the fungus, which is a very slow process. This is being done in cooperation with the University of Gembloux, Belgium.

Instead of just discarding all plants that show no resistance to juglone, we have now started to screen these for aneuploid mutants. Aneuploid mutants can be detected through chromosome counts but this is a rather time-consuming screen. We have therefore evaluated the use of flow cytometry to detect these mutants. Results obtained by flow cytometry were compared to chromosome counting in meristem root-tip cells. It could be shown that flow cytometry is sensitive enough to detect aneuploidy in *Musa*. With such a sensitive and fast technique at hand we are now screening routinely for aneuploid mutants in addition to screening for juglone resistance. Such aneuploid mutants will probably not have a practical value but would be important tools for basic reasearch in *Musa*.

The Plant Breeding Unit is also offering ploidy determination by flow cytometry as a service to Member States. For further information please contact Nicolas Roux.

DNA Fingerprinting Service

The DNA fingerprinting laboratory of the Plant Breeding Unit is equipped with a MultiPROBE II robotic workstation from Packard Bioscience and a 3100 Genetic Analyser from Applied Biosystems. The Genetic Analyser is automatically performing capillary electrophoresis and can be used for sequencing or DNA fragment analysis. We routinely separate DNA fragments

which leaves the possible use of 4 different dyes. In principle, all molecular marker techniques that produce DNA fragments in the detectable size range can be used as long as the fragments are labelled. We are currently using primers that have incorporated a fluorescent label but it would also be possible to use fluorescent nucleotides to be incorporated into the final product.

The preferred marker technique in our laboratory is AFLP (Amplified Fragment Length Polymorphism) which has been developed by Vos *et al.* (Nucl. Acids Res. 23, 1995, 4407-4414). This method can be applied to any species without prior sequence information. DNA is restricted (usually with 2 different restriction enzymes) and synthetic adapters are ligated to the ends, which serve as starting points for two rounds of PCR amplification. The second amplification round also called selective amplification, uses primers which extend for a few bases into the unknown template DNA thus resulting in amplification of only a subset of fragments which can then be separated by electrophoresis. Using various combinations of primers with specific "selective" bases different patterns (fingerprints) of DNA fragments can be obtained. We are using EcoRI primers, which are fluorescently labelled in the selective amplification for separation of the products on the 3100 Genetic Analyser.

Another method that we have adapted for use on the 3100 Genetic Analyser is ISSR (Inter Simple Sequence Repeats). SSRs or microsatellites are tandem repeats of 2, 3, or 4 bp, which are frequent in plant genomes. The number of repeats is highly variable and can be used as a molecular marker if sequence information adjacent to the repeats is available to derive primers for PCR. This means that microsatellites containing sequences have to be cloned and sequenced to derive the markers, which is very costly and labour intensive. However, instead of amplifying the microsatellites, it is also possible to amplify the region between two microsatellites using primers that bind at the microsatellites. Since these ISSR primers contain the repeated microsatellite sequence it is necessary to anchor them either at the 3´or the 5´end. We are using fluorescently labelled ISSR primers for separation of the products on the 3100 Genetic Analyser. We have also shown that the diversity of the ISSR markers can be further increased by digesting the amplified fragments with different 4cutter restriction enzymes.

The Plant Breeding Unit will start its DNA Fingerprinting Service in 2002. We will consider requests for the characterization of mutant varieties developed in Member States that are received for storage in the FAO/IAEA Mutant Germplasm Repository.

Contact: Holger Bohlmann (h.bohlmann@iaea.org)

Services

Radiation treatment with ^{60}Co

10 individual requests from 8 different countries

treatments: 164
- 5 *in vitro*
- 30 *in vivo*
- 129 seed samples

plant species: 9
- 1 *in vitro*
- 1 *in vivo*
- 7 seed samples

Ploidy determination using flow cytometry

Mexico	*Agave* (75 samples)
India	*Musa* (45 samples)

Training

The following scientists visited and/or received training:

Mr. M. Chai	Malaysia	June
Mr. A. Centthew	Moldovia	July-December
Mr. F. Arguello Delgado	Costa Rica	July-August
Ms. A. Sienkiewicz	Poland	July-August
Ms. B. Ruiz Aranda	Spain	July-September
Mr. C. Osei	Ghana	August
Mr. M. Robert	Mexico	August

G. PUBLICATIONS

- Bhatia, C.R., Nichterlein, K., and Maluszynski, M., 2001: Mutations affecting nodulation in grain legumes and their potential in sustainable cropping systems. Euphytica. 120(3): 415-432.

- Chen, Q.F., Wang, C.L., Lu, Y.M., Shen, M., Afza, R., Duren, M.V., and Brunner, H., 2001: Anther culture in connection with induced mutations for rice improvement. Euphytica. 120(3): 401-408.

- Kodym A., Hollenthoner S. & Zapata-Arias F.J. (2001) Cost reduction in the micropropagation of banana by using tubular skylights as source for natural lighting. *In Vitro* Cellev. Biol.-Plant 37:237-242.

- Kodym A. & Zapata-Arias F.J. (2001) Low-cost Alternatives for the Micropropagation of Banana. Plant Cell, Tissue and Organ Culture 66:67-71.

- Maluszynski, M., Ahloowalia, B.S., Nichterlein, K., Jain, S.M., Lee, S., Nielen, S., Zapata-Arias, J., Bohlman, H., Roux*, N., Afza, R., and Kodym, A (2001). Mutating genes to meet the challenge for crop improvement and food security ABN 071 AgBiotechNet.

- Maluszynski, M., Szarejko, I., Barriga, P., and Balcerzyk, A., (2001): Heterosis in crop mutant crosses and production of high yielding lines using doubled haploid systems. Euphytica. 120(3): 387-398.

- Nawrot, M., Szarejko, I., and Maluszynski, M., (2001): Barley mutants with increased tolerance to aluminium toxicity. Euphytica. 120(3): 345-356.

- N.S. Roux, A. Toloza, J. Dolezel and F.J. Zapata-Arias, (2001). Induction, detection and use of aneuploids for genetic studies in Musa spp. 2nd International Symposium on Molecular and Cellular Biology of *Musa* spp. Byron Bay, Australia October 29 – November 3 2000. Infomusa Vol. 10 No1 Pp II-III (Abstract).

- N.S. Roux, J. Dolezel, R. Swennen and F.J. Zapata-Arias, (2001). Effectiveness of three micropropagation techniques to dissociate cytochimeras in *Musa* spp. Plant Tissue and Organ Culture 66: 189-197

- N.S. Roux 2001. Use of Mutants in *Musa* Improvement and in Genomics. Global *Musa* Genomic Consortium, Arlington, USA, 17-20 July, 2001 (Abstract)

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!

Plant Breeding and Genetics Newsletter

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