

PLANT BREEDING AND GENETICS



NEWS LETTER

Joint FAO/IAEA Division
of Nuclear Techniques
in Food and Agriculture
and FAO/IAEA Agriculture and
Biotechnology Laboratory, Seibersdorf
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Vienna



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TO THE READER

There have been a number of important events related to the activity of the Plant Breeding and Genetics sub-programme in the past six months. The joint FAO/IAEA RCMs on “Molecular characterization of mutated genes controlling important traits for seed crop improvement” and “Mutational analysis of root characters in annual food plants related to plant performance” were held in June, in Krakow, Poland. It was the third RCM of the CRP on crop plant genomics and the second in the CRP on root systems. The joint RCM was hosted by the Department of Genetics, University of Silesia, Katowice. More than 40 scientists from twenty countries (Argentina, Australia, Belgium, Brazil, Bulgaria, Canada, China, Cuba, Germany, India, Israel, Korea, Mexico, The Philippines, Poland, Portugal, Turkey, South Africa, United Kingdom, USA and the International Centre for Genetic Engineering and Biotechnology – New Delhi) participated in the meeting. Significant progress was achieved in presented projects of diverse areas of both CRPs. Although genomics and root genetics are methodologically among the most rapidly developing disciplines, the participants successfully tried to follow the latest developments.

The Consultants Meeting on “Physical mapping technologies for the identification and characterization of mutated genes contributing to crop quality” was also held in June, in Vienna. Physical mapping technologies provide new tools for the rapid advancement of breeding programs and are highly applicable to neglected crops in developing countries. Furthermore, they open new opportunities for developing modern approaches to plant improvement research. Consultants recommended the organization of a Co-ordinated Research Project dealing with application of these new technologies to breeding programmes with the use of induced mutations for crop improvement. It is expected that the new CRP will be initiated this year.

In close collaboration with EU COST 851 ‘Gametic cells and molecular breeding for crop improvement’ project we started with preparation and editing of a book on "Doubled haploid production in crop plants. A manual." More than 40 manuscripts were collected, reviewed by a team of EU COST 851 experts and are now in the final editing phase. Similarly, we finished editorial work on publishing the training material from the FAO/IAEA Training Course “Mutant germplasm characterization using molecular markers. A Manual.” The manual is now in print and will be published and distributed in August 2002.

The Technical Co-operation activities concentrate on evaluation of new project proposals for the implementation cycle 2004-2005 and on organization of two new regional projects in Asia: “Enhancement of genetic diversity in food pulses and oil crops and establishment of mutant germplasm network” and “Elimination of micronutrient malnutrition in East Asia and the Pacific.” Under the Regional AFRA project a workshop was held in Cameroon to review mutation methodology and results of experiments on genetic improvement of neglected African crops.

The activities of the Plant Breeding Unit, Seibersdorf concentrated on the use of cell suspensions for the induction and selection of mutants in banana and on establishing of DNA fingerprinting service. Also, work on isolation and characterisation of rice mutants with salinity tolerance was continued.

We are very glad to inform you that the 2nd FAO/IAEA Interregional Training Course on “Mutant germplasm characterization using molecular markers” will be held at Seibersdorf, 4-29 November 2002.

Mirosław 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

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 “Improvement of Tropical and Subtropical Fruit Trees through Induced Mutations and Biotechnology”, Vienna, Austria, 2-6 September 2002

Technical Officer: M. Jain

The second RCM will be held in Vienna, 2-6 September 2002. Fourteen participants from Australia, China, India, Indonesia, Philippines, Iran, Israel, Malaysia, Pakistan, South Africa, Thailand, United Kingdom and USA are expected to attend. They will present the results of their last 2-years-work, mainly on radiation induced mutations in tropical and subtropical fruit trees such as mango, cashew, avocado, citrus, litchi, jujube, papaya, annona, carambola, pitanga, and jaboticaba. We expect that all participants have followed the work plan proposed during the first RCM, held in Vienna, 25-29 September 2000. Accordingly, radiosensitive curves of different fruit trees are supposed to be established and hopefully identified as an optimal radiation dose as well as multiplication of mutated material up to MV_1 - M_1V_3 generations before moving to the next step for true mutant isolation. During this RCM, we will discuss the ideal approach for mutant isolation, multiplication, and characterisation. Irradiation of differentiated or multicellular structures will cause chimerism in mutated plants and will require multiplication phases, M_1V_1 to M_1V_4 , for its dissociation, which could take 5-6 years, even more, depending on the fruit crop.

Consultants Meeting on “Low Cost Technology in Plant 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 layout 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.

2nd Interregional Training Course on “Mutant Germplasm Characterisation Using Molecular Markers”, FAO/IAEA Agriculture and Biotechnology Laboratory, A-2444 Seibersdorf, Austria, 4-29 November 2002

Technical Officer: M. Maluszynski & S. Nielsen

Background: Molecular marker technology enhances precision in characterizing induced mutants in plant breeding. These markers offer new opportunities in genetic research and plant breeding particularly in the assessment of biodiversity, for fingerprinting of germplasm/mutants, genome mapping, marker assisted selection (MAS), and in gene linkage analysis. Training in applying these methods is essential for plant breeders and geneticists interested in their integration into breeding programs.

Purpose of course: To enhance knowledge and provide practical training on current molecular marker techniques and their use in evaluation and characterization of crop biodiversity, focusing on mutants to facilitate breeding programs.

Nature of course: The course will entail lectures and practical laboratory exercises covering the theory and use of DNA markers with particular emphasis on their applications in plant breeding and in utilisation of crop plant mutants. The course will include substantial hands-on training and the topics covered will include molecular biology theory, DNA extraction,

purification and restriction, polymerase chain reaction (PCR), nucleic acid electrophoresis, commonly used DNA markers (AFLP, SSR, ISSR, STMS, IRAP, REMAP), TILLING, applications of DNA markers (marker-assisted selection, DNA fingerprinting, linkage analysis and genetic mapping principles) to enhance the utilisation of plant mutants.

C. PAST EVENTS

3rd Research Co-ordination Meeting of FAO/IAEA Co-ordinated Research Project on “Molecular Characterization of Mutated Genes Controlling Important Traits for Seed Crop Improvement”
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and

2nd Research Coordination Meeting of FAO/IAEA Co-ordinated Research Project on “Mutational Analysis of Root Characters in Annual Food Plants Related to Plant Performance”, Krakow, Poland, 10-14 June 2002

The joint FAO/IAEA RCMs on “Molecular characterization of mutated genes controlling important traits for seed crop improvement” and “Mutational analysis of root characters in annual food plants related to plant performance” were held in Krakow and Katowice, Poland, 10-14 June 2002. It was the third RCM of the CRP on crop plant genomics and the second CRP on root systems. The joint RCM was hosted by the Department of Genetics, University of Silesia, Katowice. More than 40 scientists from twenty countries (Argentina, Australia, Belgium, Brazil, Bulgaria, Canada, China, Cuba, Germany, India, Israel, Korea, Mexico, The Philippines, Poland, Portugal, Turkey, South Africa, United Kingdom, USA and the International Centre for Genetic Engineering and Biotechnology – New Delhi) participated in the meeting. It was possible to implement all planned activities and meet all objectives of the meeting thanks to its excellent and timely organization.

Significant progress was achieved in the implementation of research programs of almost all contract and agreement holders. It was pointed out, that the improvement of screening techniques, development of molecular markers as a way to alleviate the need for direct root assessment, root architecture models as a tool to address the space-time dynamics of the soil/plant system, and genetic analysis of these traits will ultimately contribute to breeding crops with root systems suitable for high or low-input agriculture (USA - lettuce, Italy and Germany – maize). In this context, mutational analysis of root characters is devoting major efforts to clarify root-specific characteristics (Poland – barley, Australia – lupines, India –tomato). The understanding of root form and function is inherently important for creating root ideotypes that are well adapted to specific environments (UK – barley, Cuba and Brazil –wheat). Root systems are highly dynamic. Formation of fine roots, branching and forming clusters of fine roots, differentiation of root hairs, rooting depth and associations with rhizosphere organisms are among those root characteristics which affect nutrient and water acquisition from the soil and have consequences on drought tolerance of crop plants (Belgium – banana, Israel – tomato, Brazil – wheat, South Africa – cowpea and bambara groundnut, China – soybean, Turkey – barley and cowpea). In addition, other characteristics such as nutrient absorption kinetics, root exudates and microbial activity in the rhizosphere affect nutrient availability and therefore plant uptake of nutrients from the soil. Mutational analysis makes possible the genetic study of gene interactions and the recognition of regulatory pathways enhanced by various types of mutants

(Poland and UK – barley). Specific EST libraries and protein/metabolite profiles from mutants are increasingly available for exploitation in root research. Cultivar comparison also provides information for traits that can be useful in improving adaptation of crops, especially to low-input environments.

Comparative genomics, especially in grasses and Brassicas, has provided major insights into the evolution of plant genomes, and has allowed researchers to postulate positions of genes based on map homology across the species divide. With the complete sequencing of the genomes of *Arabidopsis thaliana* and rice, the utility of comparative maps will be enhanced greatly, as they will allow the identification of candidate genes in crops from information from models, based on shared map location of putative orthologous genes. Presented reports indicated that molecular markers have become essential tools in plant genetics (USA –rice, Brazil – maize, Bulgaria – wheat, Canada - flax), for linkage map construction (USA - maize), trait mapping (Korea – rice, Turkey - wheat), and gene isolation (Poland – barley, Korea – soybean). Generally, markers have been increasing in sophistication since the advent of RFLPs, moving towards almost universal use of PCR-based marker systems. Nonetheless, given the importance of comparative genomics, use of strictly ‘orthologous’ markers such as RFLPs and STS markers derived from them, still have a major role to play (UK –pearl millet, rice and wheat, Brazil - rice). Highly multiplex markers, such as AFLPs, are seen largely as an efficient way of rapidly filling out linkage maps and targeting markers to trait/QTL locations (India – rice, China – maize and foxtail millet, Portugal - peas). Such markers can be converted to single locus PCR markers but the costs/effort involved render it suitable only for small numbers of markers (i.e. those mapping to a particular genetic interval or locus).

There is currently a move towards markers based on the assay of single nucleotide polymorphisms (SNPs), as these are amenable to non-gel based assays (and therefore potentially higher throughput), and furthermore, represent the most frequent and universal type of polymorphism found in plant genomes (Korea – soybean). However SNPs are, at present, relatively expensive to deploy, and are thought to be more useful in situations where markers are being targeted to a particular candidate gene or genomic region. Global SNP mapping strategies are prohibitively expensive in crop plants at the present time, but doubtless, this will change as SNP application technology becomes less expensive.

Given the amount of success in utilizing mutated genes in plant genome research, it is clear that there is a strong requirement for further mutagenesis programmes in plants. For many crops this will increase the number of mutations available to plant breeders (Poland - barley, The Philippines, China and Brazil - rice), whereas for others it will make large numbers of mutants available for the very first time (UK - potato). Given the increasing focus on quality and stress traits, it is becoming essential to develop high throughput ‘smart’ phenotypic screening procedures. It is also possible that reverse genetic approaches, methods aimed at detecting lesions in a specific target gene identified from a DNA sequence database (e.g. TILLING) will become increasingly important. Such methods require a detailed knowledge of the spectrum of mutational effects caused by particular mutagens (e.g. comparisons between different chemical and radiation treatment), as the methodologies employed for mutation detection are dictated by the nature and spectrum of mutational events caused by the specific mutagen.

An important event of the joint RCM was the visit to the Faculty of Biology and Environmental Protection, University of Silesia in Katowice. The participants had an opportunity to visit Departments of Genetics and Plant Anatomy and Cytology. Scientists from these departments

described their work and demonstrated various methods dealing with root system analysis, molecular markers application for isolation of mutated genes, gene sequencing, *in situ* hybridisation methods, flow cytometry and fluorescence microscopy with image analysis.

Consultants Meeting on “Physical Mapping of Genes Conferring Quality Traits”, Vienna, Austria, 17-21 June 2002

Technical Officer: S. Nielen

A consultants meeting was organized to consider needs and possibility for organization of a new CRP dealing with physical mapping of genes responsible for quality characters. The following experts were invited to participate in the meeting: Perry Gustafson (USA), J.S. (Pat) Heslop Harrison (UK) and C.F. Quiros (USA). Physical mapping technologies provide new tools for the rapid advancement of breeding programs and are highly applicable to neglected crops in developing countries. In the past twenty years, major investments in several model species have been made, advancing our understanding of plant genomics. Utilizing classical breeding, mutation, markers and molecular cytogenetic techniques, considerable progress has been made in ability to manipulate genes and gene complexes. However, plant breeders are still some distance from being able to utilize all the genomic information, and a better understanding of genome structures and variation (natural and induced) to carry out the sort of directed gene manipulation required. It has been well established that the application of mutagens can be a very important approach for manipulating many crop characters including quality. Future advances in our ability to improve production and quality will become more dependent on the utilization of many technologies. Physical mapping can provide an effective approach facilitating manipulation of various quality characters, including the transfer of genes between varieties and even species. The technology is applicable to seeded as well as non-seed propagated crops. A particular advantage is that genetic polymorphisms and large segregating populations are not required for physical mapping.

Recent technological developments are changing the way breeders approach the manipulation of genes and gene complexes for crop improvement. Clear among these have been the emergence of current model systems *Oryza sativa* and *Arabidopsis thaliana* and an increased knowledge of conservation of gene sequences and synteny among species. Previously, various aneuploid, genetic, and mutation stocks (spontaneous and induced by radiation and other means) have advanced breeding approaches for the improvement of many crops. Building on genetic maps of phenotypic characters, molecular markers have provided an almost unlimited number of polymorphisms so that detailed genetic linkage maps have been made for all the world's major crop species. At the same time, vast amounts of DNA sequence information has become available – complete sequences for at least two plant species, and tens of thousands of complete and partial (EST) sequences for genes in several more crops.

Techniques for physical mapping of the plant genome start with chromosome analysis by microscopy. Development of fluorescent staining methods now enable rapid analysis of the chromosomes in all plant species, regardless of the genome size (from little more than 100 Mbp, million base pairs, in *Arabidopsis*, rose, some tropical trees, to well over 15,000 Mbp in wheat, pine, onion). These powerful techniques enable significant understanding of genome structure to be gained in all species. It is critical to know the ploidy level, chromosome composition, and breeding potential of any line being introduced into a breeding programme: mutation processes

lead to gain or loss of chromosomes or chromosome segments, or even entire genomes, and knowledge of this variation in chromosome number can lead directly to identification of plant breeding options. In many cases this knowledge has prevented adoption of inappropriate strategies such as crossing of different ploidy levels. Recently, fluorescence *in situ* hybridization methods added a powerful new dimension to chromosomal analysis. In virtually all species, individual chromosomes can be identified, enabling the genetic linkage group to be joined with the physical chromosome, and allowing chromosomes to be followed through breeding programmes, particularly when single chromosomes carrying useful genes, and even more gene combinations/complexes, can be tracked. Alternate technologies (for example PCR-based) can also be used to answer some/many of the same questions, but might require more marker development in the specific crop and might require detailed evaluation.

The improvement of quality traits in food and industrial crops is one of the most important goals in plant breeding and is gaining more and more attention. Improved crop quality is considered to be of great economical value for both developed and developing countries and in the case of nutritional quality it will have a significant positive effect on human health. This applies in particular to regions suffering from malnutrition. A constraint to improving quality in agricultural crops is a lack of understanding of the basis of trait gene manipulation.

This CRP will address the problems associated with physical placement of a gene or gene complex in a chromosome. The work plan will apply the technology involved in accessing the genetic and physical position of quality genes in various crop genomes and build towards using physical map information for crop improvement. This CRP will address the following problems:

- 1) There is a need to accelerate breeding programs in developing countries to improve quality traits in crops – especially neglected crops, grown by smallholder farmers, that contribute to food security, health, and agricultural sustainability;
- 2) There is a lack of basic genetic and biological information for most quality traits;
- 3) Fine physical maps do not exist in most crops so the location of genes to be transferred is unknown;
- 4) Resources (i.e. characterized markers, genetic and cytogenetic stocks) for conducting comprehensive genetic research are limited;
- 5) There is a lack of availability of mutant phenotypes and alleles for key quality traits in many crops;
- 6) There is an availability gap in technology and information required for developing countries;
- 7) Poor linkages among research communities (between developing and developed countries and among developing countries) exist;
- 8) Inadequate training and awareness of the opportunities for transferring genomic information and material from model species to crop species exists.

This CRP will provide a platform for crops that are diploids and polyploids, seed and vegetatively propagated, dicot and monocot, annual and perennial, hence demonstration and dissemination of methods, their application and knowledge of their capability, seems to be more important than a focus on either particular crop species or particular quality traits. The technologies are particularly relevant to crops and especially neglected crops with limited research programs and background information.

The consultants recommended that the Agency implement a new CRP with the general objective to assist Member States in accelerating crop breeding programmes through the application of physical mapping and complementary genomic approaches, and the characterization and utilization of induced mutants for improvement of crop quality with the objective of increasing agricultural sustainability, food security, economic stability, and alleviating local quality-related food problems. The deadline for sending Research Contract Proposals expires at the end of September 2002.

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: S. Nielen

The Workshop was a follow-up activity of the regional 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. Thirteen participants from ten African countries attended the workshop and presented the results and work plans with respect to their new project proposals. International experts and Agency staff gave lectures and presentations on the use of induced mutations in improvement of seed and vegetatively propagated crops. Selection of advanced mutant lines were reported for lupine (earliness, increased yield, reduction in alkaloid content), tef (lodging resistance), bambara groundnut (increased yield), tropical pumpkin (tolerance to powdery mildew disease), amaranth (drought tolerance) and cowpea (drought tolerance). In Zambia improved finger millet mutant lines are under national variety trials. During the workshop each participant discussed in detail with other collaborating scientists from the region and individually with the experts the status of her/his project, possible drawbacks and improvements of future work plans. On a field excursion to the Institute de Recherche Agronomique pour le Development in Buea the local cocoyam *in vitro* mutagenesis project was demonstrated. A field trial with putative mutants, now under selection for root rot resistance, was visited. The workshop was completed by a discussion session on possibilities for promotion and popularization of neglected African crops. It was emphasized that one important factor for later acceptance of the new variety is the participation of local farmers in the breeding programme. Furthermore, the breeder should seek a market for improved neglected crops as well as try to establish contacts with the food industry.

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 being applied for the improvement of various vegetatively and seed propagated crops: cocoyams (*Colosasia esculenta*, *Xanthosoma* spp.), yams (*Dioscorea* spp.), grain and vegetable amaranths (*Amaranthus* spp.), Bambara groundnut (*Vigna subterranea*), grasspea (*Lathyrus sativa*), okra (*Abelmoshus esculentus*), bitter potatoes (*Solanum jucepszukii*, *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 mutations, *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 M₁V₄ and M₁V₅ 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.

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. The second RCM was organized by the Department of Genetics, University of Silesia and held in Krakow, Poland, 10-14 June 2002.

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. The third RCM was organized by the Department of Genetics, University of Silesia and held in Krakow, Poland, 10-14 June 2002.

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

Technical Officer: M. Jain

The first RCM was held in Vienna, Austria, 25-29 September 2000 with an overall objective to generate and characterise 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. The participating institutes are from Australia, China, India, Indonesia, Philippines, Iran, Israel, Malaysia, Pakistan, South Africa, Thailand, United Kingdom and USA. The specific objectives of this CRP are: a) to establish research groups focussing on a specific crop or a specific technology, b) to identify individual mutant trees with the potential of beneficial performance, and c) to characterise mutated genes in both field tests and by molecular tools. The selected crops were mango, cashew, avocado, citrus, litchi, jujube, papaya, annona, carambola, pitanga, and jaboticaba. The selection criterion used was that the crops should be tropical or subtropical fruit or nut trees. In addition, there is evidence of local breeding activities and potential for improvement of food and /or economic stability. Citrus is more like a model plant because it has been widely used for biotechnology and mutations. In most of the selected fruit trees, somatic embryogenic cultures can be induced, which are highly desirable for induced mutations. Since embryogenic cultures originate from a single cell in fruit trees chosen for this CRP, the chances of chimerism are very much diminished. Moreover, handling large numbers of embryogenic cell population for irradiation is rather simple and consequently chances of getting mutations are high. The major disadvantage of this approach is that plant regeneration from somatic embryos is very poor and can't be used for large-scale multiplication of mutant lines. This area requires more basic research before it can be commercially exploited. Therefore, a combination of somatic embryogenesis and *in vitro* micropropagation would be an ideal approach for mutation induction and large-scale multiplication of mutant populations. Moreover, embryogenic cultures of mutants can easily be used for flow cytometry to study cytological variation, and can also be cryo-stored for a long time for future use and help in establishing germplasm banks of mutant lines. In the forthcoming RCM, we expect some progress in radiation-induced mutations in selected fruit trees.

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 <i>in vitro</i> culture	M. Jain
JOR/5/008	Establishment of <i>in vitro</i> 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	<i>In vitro</i> mutagenesis for horticultural crop plants	M. Jain
MYA/5/010	Development of improved rice with tolerance to drought	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
RAS/5/040	Enhancement of genetic diversity in food, pulses and oil crops and establishment of mutant germplasm network (RCA)	M. Maluszynski
RLA/5/035	Evaluation of cereal crop mutants (ARCAL XXIa)	M. Maluszynski

Project No.	Title	Technical Officer
ROK/5/033	Quality improvement of major crops and integrated plant nutrition management in the low-input agricultural system.	M. Maluszynski
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	M. Maluszynski
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 <i>in vitro</i> mutation techniques	K. Nichterlein

TC project on “Development of Improved Crop Varieties” (RAF/5/042)

Over the past decades the Agency has assisted African Member States in applying radiation technology to increase agricultural production. Improved breeding lines of various crops have been produced but often remained on the breeding stations. The regional project was started in 1997, aiming to assist in the further multi-location and on-farm evaluation of selected mutant lines for their official release, or in large-scale dissemination of already released improved mutant varieties.

Project assistance to field trials through smaller equipment, consumables and expert services supported the release and dissemination of mutant varieties in the Africa region. Sorghum, an important cereal and rotation crop in cotton-based production systems of Mali and other countries of the region was improved through application of mutation techniques in local germplasm. Eight mutant varieties with improved grain yield, reduced plant height, earliness and lodging resistance were released in Mali in 1998 under a national TC project. Three of the eight registered mutant varieties namely ‘Fambe’, ‘Tiedjan’ and ‘Gnome’ have been multiplied under the regional AFRA project for extension. The distribution of seed to farmers and demonstrations ‘on-farm’ in cotton-based production systems has been done in cooperation with the Compagnie Malienne de Développement Textile (CMDT), an organization for cotton production and marketing. These varieties were grown in 1998-99 by 507 farmers, in 11 villages, on almost 1,500 ha in CMDT Fana region and since then the area has been increasing continuously. Seed has also been distributed to other regions in Mali and exchanged in the SSA region. Currently sorghum mutants from Mali are in evaluation trials in Ghana and in other countries of the West and Central African Network on Sorghum (ROCARS). Nine new mutant varieties were registered in early 2002; either late maturing - adapted to southern Mali (Guinean area), or medium maturing - adapted to the Sudanian/Sahelian area, or with drought tolerance. The new varieties (‘Soble’, ‘Djakele’, ‘Kolobakari’, ‘N’gno-deni’, ‘Kolossina’, ‘Tassouma’, ‘Kolodjan’, ‘Ansona’, ‘Souroumani’) have improved lodging resistance, grain quality and grain yield components. In African rice (*Oryza glaberrima*), the support through research and a national technical cooperation project to IER Sikasso, in Mali led to the release of six mutant varieties of the parent Gorbali in 1998 and of one mutant variety of the parent Haira in 2001. The improved

varieties have white seed as the parents, but induced resistance to seed shattering. Seed multiplication, further evaluation and dissemination to farmers has been supported through the regional project.

Other promising mutant varieties released under the regional project are three varieties of sesame ('Taka 1', 'Taka 2' and 'Taka 3') officially released in Egypt in 2000 with high yield, disease and insect resistance. These varieties were cultivated in 2001 on 20 ha for further multiplication in farmer fields to raise certified seed (about 50 growers). In wheat the mutant variety Njoro-BW1 was released in Kenya only seven years following mutagenic treatment of the late maturing and tall variety 'Pasa' three years after testing the National Dryland Wheat Performance Trials. It has a good seed yield and higher protein content than the check variety Duma with good milling and baking quality and moderate resistance to rust under experimental conditions in marginal areas. It has short stature and early maturity of only 80 days under lowland conditions, and is suitable for low-altitude, marginal rainfall areas. Currently, Njoro-BW1 is multiplied in off-season trials for production of 900 kg pre-basic seed. In 2002, it will be further multiplied for the production of 36 t of basic or certified seed for distribution to seed companies and farmers.

Mutant varieties in the pipeline are two mutant clones of desert banana with higher yield and good quality submitted for official release in Sudan in 2002, three mutants of lentil with high yields, improved biological nitrogen fixation and moderate tolerance to fusarium expected for official release in Morocco in 2004, two spineless mutants of safflower with high yields, high oil content and high oil quality for testing in National Variety Trials in the 2002/2003 season in Egypt.

Drought is the most widespread crop production constraint in North Africa, northern parts of West Africa, East and South Africa. Due to the climate and anthropogenic factors (deforestation, overpopulation and overgrazing) drought will continue to be a constraint and will become more serious in the future. Plant breeding is probably the most viable approach to stabilizing food production under normal (non-catastrophic) environmental drought problems. The project assisted through regional training, expert services and provision of specialized equipment the transfer of mutation and improved drought screening techniques to AFRA Member States. Thereby plant breeders were encouraged to develop drought tolerant breeding lines of major cereal and legume crops. From 1997 to 2001, one planning workshop, three training courses and workshops, individual expert services to participating countries and the establishment of improved drought screening facilities were organized by the project in collaboration with host institutes of Member States and International Agricultural Research Institutes (e.g. ICRISAT and IITA). This has increased the capacity in the region to breed and screen for drought tolerance. The use of radiation-induced mutations in combination with improved screening techniques increased the genetic variation for drought tolerance of important food crops. The first drought tolerant crop mutants were released in Mali (sorghum) and Kenya (wheat). Other countries have to do further mutant evaluations prior to submission to the National Variety Release Committees.

There are many traditional African food crops, which are adapted to the agro-climatic and biotic stresses in the region and used by local communities as a primary source of carbohydrates, proteins, minerals, vitamins and other micronutrients. Some of these crops are grown only in specific regions to supplement basic dietary needs and are not known to the outside world, while other food crops are completely neglected since little or no attention has been given to

developing improved lines compatible with modern farming. It is therefore essential that neglected traditional crops be developed and improved in yield, quality, disease resistance and stress tolerance through conventional and mutation techniques. One planning and one training workshop were organized and expert services provided to encourage the genetic improvement of neglected crops. Early mutant generations have been produced in cocoyam (Cameroon), white lupin (Egypt), noug and tef (Ethiopia), African yam bean (Ghana), bambara groundnut (Madagascar, Zimbabwe), African rice (Mali), amaranths (South Africa), lablab (Tanzania). The new regional project RAF/5/050, will support the breeding work for drought tolerance and the improvement of neglected crops in the region initiated under the project RAF/5/042, which was completed at the end of 2001.

F. ACTIVITIES AT THE PLANT BREEDING UNIT, SEIBERSDORF

Usefulness of Embryogenic Cell Suspensions for the Induction and Selection of Mutants in *Musa* spp.

Induced mutation techniques are particularly important for bananas and plantains (*Musa* species) where there is limited sexual reproduction that could generate genetic variation, the basis for selection. Even though spontaneous mutations have contributed to the genetic diversity of *Musa* and significantly increased the variation used to breed *Musa* spp. their occurrence is too low. The use of *in vitro* cultures for induced mutations in *Musa* spp. could be a method of choice if several steps of the mutation induction process could be optimized. The following aspects were investigated: the possibility to detect genetic instability in DNA content, the determination of an optimal mutagenic dose, the elimination of chimerism and the application of an early mass screening for the selection of useful mutants.

With the increased use of 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, chromosome instability itself 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 with 5 missing chromosomes was reported.

To irradiate embryogenic cell suspensions (ECS), several preliminary studies were performed. The first radiosensitivity tests of *Musa* ECS were performed and it has been found that cell suspensions from *Musa* can tolerate up to 200 Gy. At 100 Gy the growth curve is 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. Colchicine treatment induced polyploidy and mixoploidy (chimerism) if

embryos are not of single cell origin. To date no mixoploid regenerated plants from colchicine treated ECS were detected.

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 black Sigatoka disease. The test was applied when the acclimatized plants reached the 6 leaf stage. The dose of 25 ppm permitted to differentiate between the tolerant variety Fougamou and the susceptible variety Grande Naine. To date, from around 4000 irradiated Grande Naine plants screened, 8 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.

Contact: N. Roux (e-mail: N.Roux@iaea.org)

Services

Molecular markers are valuable tools for plant breeding and genetics in both practical aspects and basic research. The most frequently used markers today are all based on PCR because they require only limited amounts of DNA, are fast and relatively inexpensive. The most sensitive method currently in use 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. The DNA is restricted 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 one fluorescently labelled selective primer and separate the fragments on a 3100 genetic Analyzer from Applied biosystems. This machine is able to automatically calculate the size of the separated fragments using fluorescently labelled DNA standards, which are run in the same capilleries together with the probes.

We have recently compared three different lentil mutants with their parent variety. The restriction enzymes used were EcoRI and MseI. The selective primers for MseI had 3 selective bases. For EcoRI we first used primers with 2 selective bases. However, these primer combinations did not lead to the amplification of clearly separated fragments. We therefore tried EcoRI primers with 3 selective bases. This resulted in defined fragment patterns. The reason for this different behavior is the large genome size of lentil (4×10^9 base pairs).

Contact: M. Matijvic (e-mail: M.Matijvic@iaea.org)

Services

Cobalt 60 Irradiation

Total number of:

requests	13
treatments	112
Seeds	107
<i>in vitro</i>	5
Plant species	13
Varieties	44
Member States	9

Training

The following scientists visited and/or received training:

Fellows:

1. Mr. Alexander Centhew	Moldovia	July 2001 - June 2002
2. Mr. Ibrahim Mohammed Rawashdeh	(NCARTT) Jordan	February - May 2002
3. Ms. Ana Maria S. Veluz	(PNRI) Philippines	February - May 2002
4. Mr. J. M. Seneviratne	Sri Lanka	February - June 2002
5. Mr. Paul Mbogo Kusolwa	Morogoro, Tanzania	January - September 2002

Visiting Scientists:

1. Dr. Vuong Dinh Tuan
Cuulong Delta Rice Research Institute, Omon, Cantho, Vietnam, 20-24 May 2002
2. Dr. Mr. Abdel Hafeez Elhassan
Univer. of Gezira, Fac. of Agriculture, Wad Medani, Sudan. 21-24 May 2002

G. PUBLICATIONS

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

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- Newton, R.J., J.C. Bloom, D.H. Bivans and S.M. Jain, 2001. Stable genetic transformation of conifers. Phytomorphology (Golder Jubilee Issue) pp 421-434.

H. VACANCIES

Food and Agriculture Organization of the United Nations

Vacancy Announcement No. 1016-AGE

Deadline for Application: 19 August 2002, Post Number: 0054941

Position and Grade: PLANT MOLECULAR BIOLOGIST (P-4)
Duty Station: Vienna, Austria
Type/Duration of Appointment: Fixed term, 3 years

Duties and responsibilities:

Under the general supervision of the Director, Joint FAO/IAEA Division and the immediate supervision of the Senior Officer (Plant Breeding & Genetics), will provide scientific and technical support to programmatic activities dealing with applications of molecular methods for aiding selection, improvement and characterization of plant genetic resources in food and agriculture. Specifically will:

- Plan and implement coordinated research projects dealing with applications of molecular and mutation techniques in crop improvement
- Plan and implement Technical Cooperation projects and related training courses and workshops dealing with molecular and mutation techniques in crop improvement
- Keep under continuous review the applications of molecular techniques, particularly molecular markers for selecting and improving traits of agronomic importance in food and industrial crop plants and for characterizing plant genetic resources in food and agriculture
- Design, organize and contribute to the conduct of studies dealing with policy and technical issues related to the above
- Prepare documentation from technical and policy meetings for publication
- Manage the coordination and exchange of information relating to the above within and outside the organization through information systems, databases
- Perform other related duties as required

Qualifications and experience – Essential:

- Advanced university degree (PhD or equivalent) in Molecular Biology.
- Seven years of responsible professional experience in planning and implementing projects using molecular markers in plant breeding and genetics.
- Working knowledge (level C) of English, French or Spanish and limited knowledge (level B) of one of the other two.
- Ability to analyze technical and scientific information. Ability to write clearly and concisely, edit reports and make effective oral presentations. Initiative and a high sense of responsibility. Ability to organize and coordinate meetings and training activities. Computer literacy and ability to use word processing and other standard software. Courtesy, tact and ability to establish and maintain effective working relationships with people of different national and cultural backgrounds.

Qualifications and experience – Desirable:

Working experience in developing countries. Willingness to travel and participate in meetings and field missions outside the duty station.

* Applications should be sent to the Division of Personnel
IAEA, Wagramerstrasse 5, P.O. Box 100, A-1400 Vienna, Austria

International Atomic Energy Agency
Vacancy Announcement No. 2002/063
Deadline for Application: 16 September 2002

Position and Grade: PLANT BREEDER AND GENETICIST (P-4)
Duty Station: Vienna, Austria
Type/Duration of Appointment: Fixed term, 3 years

Duties and responsibilities:

Under the general supervision of the Director, Joint FAO/IAEA Division and the immediate supervision of the Senior Officer (Plant Breeding & Genetics):

- To evaluate technical co-operation requests and advise on equipment needed, assist in finding qualified experts for technical co-operation programmes, evaluate the reports of experts in the field, and assist in the technical evaluation of fellowship applicants and in guiding their programme of study.
- To evaluate research contract and agreement proposals, to monitor progress in research projects and to organize periodic meetings with participants in co-ordinated research programmes.
- To support the Section Head in guiding a relevant laboratory programme, especially in the field of genetics, molecular markers and doubled haploids in seed propagated crops, and to provide scientific information on the use of induced mutations, especially in the staff member's field of specialization to other divisions of the organization.
- To prepare the scientific programme of technical meetings related to the specialized field, to serve as a scientific secretary for such meetings, and to edit reports and proceedings.
- To support the Section Head in planning and implementing training courses.
- To serve on scientific-technical missions and represent the FAO and the IAEA at relevant meetings as required.

Education and Experience:

- PhD or equivalent in plant breeding and genetics, with a sound background in genetics, plant biotechnology and mutation techniques.
- 10 years of experience at the national level is required and a minimum of 1 year at the international level.

Knowledge, Skills and Abilities:

- A sound knowledge of research related to the genetic improvement of crop plants through the application of mutation techniques and other advanced genetic biotechnology methods, especially as it relates to the genetic improvement of seed propagated crops.
- Proficiency in English. Knowledge of French, Spanish or Russian desirable.

* Applications should be sent to the Division of Personnel
IAEA, Wagramerstrasse 5, P.O. Box 100, A-1400 Vienna, Austria

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

Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture
and FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf

International Atomic Energy Agency
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