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
Vienna

No. 11 July 2003

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ISSN 1564-2569

TO THE READER

Implementation of a new CRP on "Physical mapping technologies for the identification and characterization of mutated genes contributing to crop quality", organization of mutant germplasm database and repository, implementation of new TC projects and activation of work on molecular characterization of *Musa* putative germplasm as well as sequencing of BAC clones were the major activities of our sub-Programme on Plant Breeding and Genetics during the last six months. A lot of work has been concentrated on organizing a mutant germplasm repository. The first collections of rice and linseed mutants have already arrived and their descriptions have been introduced into the mutated germplasm database. We found this activity especially important to stimulate exchange of crop germplasm among plant breeders. Similarly there is an urgent need to collect mutants of various crops as necessary material for functional genomics and germplasm enhancement. Nevertheless, many crop research institutes are initiating large-scale mutation programmes with the use of their own plant material. To help them in selecting the mutagen, doses and mutation treatment procedure, we published the third issue of Mutation Breeding Newsletter Index of No. 21-44. The Index is also available through our website *http://www.iaea.org/programmes/nafa/d2/index.html*. The numerous requests for issues of the Mutation Breeding Newsletter already received from various countries indicate the value of this 80-page index for plant breeders and research institutes.

We were invited to present the activities, achievements and trends of our sub-Programme at two very important, international meetings: The International Conference on the Status of Plant and Animal Genome Research, known as the Plant and Animal Genome (PAG XI), and The International Congress on "In the Wake of the Double Helix – From the Green Revolution to the Gene Revolution". At this last meeting, an initiative was taken to organize the Crop Root Research Consortium to stimulate and coordinate investigations on root system in crop plants.

The implementation of Technical Co-operation projects this year has been more complicated than usual, due to the political situation in Iraq and the SARS disease in some countries of Asia. Numerous activities had to be postponed or organized in other countries. This situation mainly affects implementation of two large-scale regional projects: Enhancement of Genetic Diversity in Food, Pulses, and Oil Crops and Establishment of Mutant Germplasm Network in Asia (RAS5040) and Monitoring of Food Fortification Programmes Using Nuclear Techniques – Induced Mutations for Rice with Low Phytic Acid Content (RAS7014). Nevertheless, we expect to meet all major objectives foreseen in our work plans for this year.

There are several important changes within the team of the sub-Programme: Dr. Chikelu Mba (Nigeria) joined the Plant Breeding Unit at Seibersdorf in March 2003, while Dr. Qing Yao Shu (China) and Dr. Pierre Lagoda (Luxemburg) will join the Plant Breeding and Genetics Section in July 2003. Unfortunately, due to retirement, Dr. F. Javier Zapata-Arias, the Head of Plant Breeding Unit will leave the Agency at the end of June 2003. We thank Dr. Zapata-Arias for his work with us and wish our new colleagues much success in initiating work in our sub-Programme.

Miroslaw Maluszynski

A. STAFF

Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna International Center, Wagramer Strasse 5, P.O. Box 100, A-1400 Vienna, Austria (Phone: +431 2600 + extension)

		E-mail	**Phone (431) 2600 +**
James D. Dargie	Director	j.dargie@iaea.org	21610

Plant Breeding and Genetics Section

Miroslaw Maluszynski	Head of Section	m.maluszynski@iaea.org	21619
Mohan Jain	Technical Officer	s.m.jain@iaea.org	21623
Stephan Nielen	Technical Officer	s.nielen@iaea.org	21618
Pierre Lagoda	Technical Officer	p.lagoda@iaea.org	21624
Qing Yao Shu	Technical Officer	q.y.shu@iaea.org	21617
Kathleen Weindl	Secretary	k.weindl@iaea.org	21620
Katayon Allaf	Secretary	k.allaf@iaea.org	21621

FAO/IAEA Agriculture and Biotechnology Laboratory, Plant Breeding Unit of the Agency's Seibersdorf Laboratory, A-2444 Seibersdorf, Austria

Christopher Rigney	Head, Agriculture and Biotechnology Laboratory	c.rigney@iaea.org	28267
F. Javier Zapata-Arias	Head of Unit	f.zapata-arias@iaea.org	28279
Chikelu Mba	Plant Biotechnologist	c.mba@iaea.org	28268
Rownak Afza	Senior Technician	r.afza@iaea.org	28208
*Andrea Kodym	Technician	a.kodym@iaea.org	28213
Mirta Matijevic	Technician	m.matijevic@iaea.org	28317
Guenter Berthold	Technician	g.berthold@iaea.org	28414
Andreas Draganitsch	Technician	a.draganitsch@iaea.org	28414
Franz Zwiletisch	Technician	f.zwiletisch@iaea.org	28414
Arsenio Toloza	Technician	a.toloza@iaea.org	28275
Mohammed Elagamawy	Technician	m.elagamawy@iaea.org	28213
Elisabeth Swoboda	Secretary	e.swoboda@iaea.org	28281

* presently on maternity leave

B. FORTHCOMING EVENTS

New CRP on the "Effects of Mutagenic Agents on the DNA Sequence in Plants" – Expected to be Approved in 2003

Technical Officer: P. Lagoda

A new Co-ordinated Research Project on the effects of mutagenic agents on the DNA sequence in plants is planned for 2003 and research may be supported by the Agency.

Modern breeders and farmers can tap into a widely broadened diversity through mutation induction techniques to improve their crops. The impact of induced mutations on crop improvement programs is reflected in more than 2200 entries in the IAEA's database on officially registered mutant varieties (MVD), of which about 75% are direct mutant varieties mainly derived from treatment with gamma rays. In contrast to data on the potential of mutation techniques very little is known about the effects of the different mutagenic agents on the DNA sequence in plants. In particular, detailed knowledge is missing on the category of changes a certain mutagen is causing (point mutations, size of deletions, translocations, inversions) as well as on the frequency of these changes. Data on the effect of mutagens, however, will substantially facilitate mutational analysis of plant traits, which is regarded as one of the most efficient approaches for identification and isolation of agronomically important genes. Additionally this information will be extremely helpful to the plant breeder to identify a successful dose for treatment of his/her material. This CRP will utilize mutation technique principles, genetic and cytogenetic methods of mutation frequency evaluation and high-throughput genomic techniques to address these questions of paramount importance to mutation oriented breeding programs.

Consultants Meeting on "Effect of Mutagenic Agents on DNA Sequence in Plants", Vienna, Austria, 7-9 July 2003

Technical Officer: P. Lagoda

Three consultants will meet with the Joint FAO/IAEA Division Plant Breeding and Genetics Section and Plant Breeding Unit staff. Dr. G. Caetano-Anollés (USA) is a worldwide-accepted crop expert on evolution of macromolecular structure, evolution of transcript networks and evolutionary role of spontaneous mutations among others. Dr. M. Dizdaroglu (USA) is a major scientist in DNA damage and repair in human molecular biology and oncology, which are the most advanced fields in this respect. Both experts are outstanding scientists representing complementary aspects of the molecular effects of induced mutations, essentially needed to get advice on the topics and tasks to be addressed within the CRP. Together with the third expert, Dr. M. Gale (UK), who will contribute his outstanding expertise in co-linearity and microsynteny in crops, the consultant team will have the challenging task of guiding the planning of this CRP on the effects of mutagenic agents on the DNA sequence in plants.

3rd Interregional Training Course on “Mutant Germplasm Characterization using Molecular Markers”, Seibersdorf, Austria, 6-31 October 2003

Technical Officer: S. Nielen

This will be the third training course of a new programme that has been successfully implemented during the last two years at the Agency’s Laboratories in Seibersdorf, Austria. The purpose of the course is 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 programmes.

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. It will include substantial hands-on training and the topics covered will include molecular biology theory, DNA extraction, purification and restriction, the polymerase chain reaction (PCR), nucleic acid electrophoresis, radioactive labelling and detection, commonly-used DNA markers (AFLP, SSR, ISSR, and retrotransposon based marker techniques), chromosomal analysis using fluorescence *in situ* hybridisation, applications of DNA markers (marker-assisted selection, DNA fingerprinting, linkage analysis and genetic mapping principles) to enhance the utilisation of plant mutants.

The course is open to 20 participants from developing Member States of FAO and IAEA. They should have basic experience in molecular techniques and be actively involved in crop breeding programmes. All applications have to be submitted through official channels (FAO Country Representative, National Atomic Energy Authority or Office of the UNDP).

Regional (AFRA) Workshop on “Standardisation of Crop Breeding Methods for the Improvement of Drought Tolerance” (RAF/5/050-002), Lusaka, Zambia, 10-14 November 2003

Technical Officer: S. Nielen

This Workshop is a follow-up activity of the regional (AFRA) training workshop on “Adoption of appropriate selection techniques for the development of drought tolerant germplasm” held at IITA, KANO Station, Nigeria from 9-13 October, 2000. By the time of the training Workshop, national research teams participating in the AFRA plant breeding project will have used new screening techniques to develop advanced mutant generations of major African crops with tolerance to drought. The participants will report on their research achievements and experience in adopting/developing appropriate drought screening techniques. The purpose of this Workshop is to share experience with other collaborating scientists from the region and prepare guidelines for drought screening in mutant populations as a base for the preparation of a drought screening manual. Presentations by participants, international experts and Agency staff, a visit to a research station, group discussions and working sessions will address this area.

The Workshop is open to 15 principal investigators participating in the project on “Increasing Production of Nutritious Food through Mutation Breeding and Biotechnology” (AFRA III-3).

C. PAST EVENTS

International Triticea Mapping Initiative Workshop, San Diego, California, USA, 9-14 January 2003

Invited Speaker: M. Maluszynski

The International Conference on the Status of Plant and Animal Genome Research, known as the Plant and Animal Genome (PAG), has been organized on an annual basis for the last ten years, always in the Town & Country Hotel, San Diego, California and always in January. More than 1800 scientists registered for PAG XI. In addition to a few plenary lectures (six during PAG XI) numerous workshops were organized, which covered various aspects of plant and animal genomics. There were 72 workshops implemented during the five days of PAG XI. Among them the following were related directly to plant genome research: Abiotic Stress, Allele Mining, Apomixis, Apple, Arabidopsis, Barley, Brassicas, Citrus, Comparative Genomics of Plants, Compositae, Cool Season Legumes, Cotton, Forage and Turf Plants, Forest Trees, Fruit and Nut Crops, Functional Genomics, Grape Genome Project, Host Pathogen Interactions, International Grass Genome Initiative, ITMI, Legumes, Lolium Genome Initiative, Maize, Maize Expression, Molecular Markers, Plant Alien Introgression, Plant Cytogenetics, Plant Interactions, Plant Transgene Genetics, Polyploidy, Rice, Rice Blast, Root Genomics, Solanaceae, and Sugar Beet.

The use of induced mutations in functional genomics was presented in many papers in various workshops. The International Rice Research Institute developed a mutation project on rice. The organized Rice Deletion Mutant Collection has almost 40,000 mutants of M_3/M_4 families. Preliminary data indicated that in 4 analysed mutants more than 920 genes were detected as deleted. Mutations as well dominated the workshop on Root Genomics. However, 4 out of 5 papers in this workshop were presented by agreement or contract holders of the FAO/IAEA CRP on Mutational Analysis of Root Characters in Annual Food Plants Related to Plant Performance.

I attended the Conference and presented a paper on Mutational analysis of gene expression in cereals during the International Triticeae Mapping Initiative (ITMI). More than eight hundred scientists from various countries participated in this session of ITMI. In our presentation we indicated that the era of genomics brought systemic access to the transcriptome of studied organisms and the post-genomics processing of this new paradigm through high-throughput technologies brought a wealth of anonymous differentially expressed genes. In order to put meaning to these data, as well on the fundamental side as applying the newfound knowledge to the "grass roots", it is essential to assign a biological function to them. Mutational analysis of plant traits, in breadth and depth, appears to be one of the simplest current approaches to generate polymorphism and tag the function with the sequence of differentially expressed genes. Gene discovery by forward genetics and map based cloning is being optimised through reverse genetics approaches which are based on the systemic paradigm of genomics and lead to the construction of gene machines in which every mutation is molecularly tagged and retrievably deposited in individual organisms (e.g. transposon induced mutation lines in maize, rice, arabidopsis). Insertion mutagenesis is very labour and cost intensive, whereas radiation and chemical mutagenesis are more efficient but the mutations are anonymous. Fortunately, microarrays and other high-throughput methods - TILLING (Targeting Induced Local Lesions IN Genomes), DArT (Diversity Array Technology), GMS (Genomic Mismatch Scanning), RDA (Representational Difference Analysis of cDNA), HAPPY mapping (*in vitro* linkage mapping technique) - will compensate for this identification deficit and make it possible to "clone a hole". Mutation technique principles - including retrotransposon activation, genetic and cytogenetic methods (comet and TUNEL assay) of mutation frequency evaluation, dissection of traits through mutational analysis as well as economic impact of induced mutations in crop plants have also been presented.

On the basis of presented papers dealing with functional genomics and numerous discussions with other scientists it became clear that the lack of clear data on the frequency of point mutations and various size deletions generated by radiation and chemical mutagens is one of the major factors limiting wide application of radiation in functional genomics. The establishment of a CRP dealing with this problem is very desired and requested by scientists from many Member States. There is also an urgent need to speed up organization of a mutant germplasm depository and collection of data for a mutant database.

Asian Development Bank Meeting on "Breeding for Iron Dense Rice", (TC Project RAS/5/014), Dhaka, Bangladesh, 27-30 January 2003

Technical Officer: P. Lagoda

In 2001, a project "Breeding for Iron-Dense Rice: A Low-Cost, Sustainable Approach to Reducing Iron deficiency Anemia in Asia" has been initiated by the Asian Development Bank, the International Food Policy Research Institute and the International Rice Research Institute (ADB-IFPRI-IRRI) in co-operation with NAR(E)S in Asia to develop iron dense rice. This research project, coordinated by IFPRI, has two components: Plant breeding, and Nutrition. The plant breeding component is coordinated by IRRI. The current participants include Bangladesh, Indonesia, Philippines and Vietnam, IRRI, and advanced country laboratories from Australia, Denmark and the USA. Two new members were added in 2003: China and Thailand.

Iron-deficiency is the world's most widespread nutritional disorder and affects about 5 billion people in developed and developing countries. Iron-deficiency leads to anemia, which, in the severe form affects nearly 2 billion people worldwide. Overall, 39% of preschool children and 52% of pregnant women are anemic, more than 90% of them live in developing countries. Iron deficiency anemia also has profound negative effects on human health and development. In infants and young children, it impairs physical growth, cognitive development and immunity; at school age it affects school performance and reduces activity levels; in adults it reduces work capacity and decreases resistance to fatigue. In pregnant women, iron deficiency anemia is associated with an increased risk of maternal mortality and illness, as well as an increased risk of pre-term delivery, retarded growth of the fetus, low birth weight and increased risk that the newborn baby dies soon after birth. Food fortification and supplementation with iron, zinc and vitamin A has been the choice to overcome malnutrition.

Breeding for improved nutritional quality is a relatively new, but under-appreciated activity with great potential. Much of the plant breeding effort so far has been focussed on improving yield and resistance to biotic and abiotic stress. Very little attention has been directed to improve staple foods for increased iron, zinc and vitamins. Rice that is high in iron content can contribute to solve the above-mentioned deficiency problems in populations that use rice as a staple food.

It is under discussion that iron-dense rice is only part of the solution. This iron must also be available to the human organism. This bioavailability depends on different factors, such as phytic acid content. In this respect, RAS/7/014 "Monitoring of Food Fortification Programmes Using Nuclear Techniques" is an ideal complement. The objectives of the RAS/7/014 project are twofold: i) to evaluate and monitor the food fortification intervention programmes in five participating Member States (China, Indonesia, Pakistan, Thailand, Vietnam), and ii) to develop rice mutants with low phytic acid from the country's high-yield rice varieties. Breeding for more nutritious foods can be accomplished using conventional breeding enhanced by biotechnology techniques. The plant breeding and mutation induction part is coordinated by the Plant Breeding and Genetics Section of the Joint FAO/IAEA Division.

Project Review and Coordination Meeting on “Increasing Production of Nutritious Food through Mutation Breeding and Biotechnology (RAF/5/050), Pretoria, South Africa, 10-14 March 2003

Technical Officer: S. Nielen

This was the first Project Review and Coordination Meeting under the Regional AFRA project RAF/5/050, which is a follow up of the previous project RAF/5/042. The meeting was hosted by the ARC-Roodeplaat Vegetable and Ornamental Plant Institute, South Africa and was attended by Project Coordinators (PCs) from Algeria, Cameroon, Egypt, Ethiopia, Kenya, Madagascar, Mauritius, Morocco, Sierra Leone, South Africa, Sudan, Tunisia, United Republic of Tanzania, Zambia and Zimbabwe, as well as by the Technical Officer (S. Nielen) and the Project Officer (P. Bhakta).

The objective of the project is to assist AFRA Member States in the development and field evaluation of improved crops for higher agricultural productivity, better nutrition and greater tolerance to stress. The crops are divided into three components: 1) Development of drought tolerant mutant lines/clones; 2) Development of improved mutant lines of neglected African crops; 3) Multi-location evaluation of advanced mutant lines/clones. The meeting started with the country presentations by all participating PCs describing the progress and results obtained so far under the three components, the country work plans and arrangements for future project activities. Significant progress was noted in the implementation of the projects in most countries. A new banana mutant variety (‘Elbeili’) with improved yield and quality characters has been recently released in Sudan (February 2003) and spineless safflower mutants exhibiting high seed yield and improved oil quality are being submitted to national variety trials in Egypt. Selection of advanced mutant lines were reported for finger millet (Zambia), sesame, lupine and wheat (Egypt), banana and groundnut (Sudan), amaranthus (South Africa), durum wheat (Morocco), rice (Tanzania), and doubled haploid lines of bread wheat (Kenya).

Individual meetings were held with each PC to discus in detail the status of their projects, specific issues and outstanding problems as well as the input needed from the Agency for further implementation. On a field trip to the facilities of the ARC Roodeplaat the participants had the opportunity to visit a fully functioning and to some extent commercially operated tissue culture laboratory, rainout shelters for screening for drought tolerance, as well as the local field experiments on cassava and sweet potato.

The Meeting enabled face-to-face contact between the IAEA officers and the PCs so as to improve future dialogue and co-operation. The status of the project in the respective countries has been assessed and national and regional work plans have peen formulated.

First Research Coordination Meeting on "Physical Mapping Technologies for the Identification and Characterization of Mutated Genes Contributing to Crop Quality", Vienna, Austria, 31 March – 4 April 2003

Technical Officer: S. Nielen

The first RCM in this new CRP was attended by 13 participants from Argentina, Bulgaria, China, Czech Republic, Germany, Iceland, Pakistan, Poland, Ukraine, United Kingdom, and Viet Nam. The main aim of the meeting was to discuss the individual project activities, to adapt the work plans, and to facilitate possible collaboration between the research teams. The CRP is directed towards 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. Among the traits that have been reported to be assessed for improved quality within the CRP are bread making (wheat/ *Leymus*), fruit colour and carotinoide (tomato and pepper), aroma and waxes (rice), fibres (cotton), oils and fatty acids (*Brassica*), and secondary metabolites, in particular medicinal alkaloids (poppy). A wide range of material and resources, such as genetic aneuploid stocks, chromosomal translocations and deletions, wild germplasm, mutant lines, genetic maps and BAC libraries are available within the CRP. The CRP is based on the development and use of cutting-edge technologies, including DNA microarrays, preparation and screening high density colony arrays and fluorescence *in situ* hybridization on mitotic and meiotic chromosomes and DNA fibres. Their application relies on sophisticated instrumentation and experience with advanced methods of plant molecular biology and genomics. In order to facilitate the access to these technologies it was recommended, during the meeting, to strengthen existing links and establish new links between participants. In particular, participants working on related species and objectives are going to share and complement techniques. The participants agreed to share DNA clones, probes and vectors where appropriate. The participants recommended that within the framework of the subprogramme of the Joint FAO/IAEA Division, the Plant Breeding Unit at Seibersdorf Laboratories should become a technology platform for the CRP (mutation induction, flow cytometry, fingerprinting, BAC end sequencing).

A detailed report on the RCM is in preparation and copies will be available upon request from the Plant Breeding & Genetics Section.

Workshop on “Physical Mapping Technologies for the Identification and Characterization of Mutated Genes Contributing to Crop Quality”, Seibersdorf, Austria, 7–9 April 2003

Technical Officer: S. Nielen

This three-day Workshop, which was held directly following the first Research Coordination Meeting of the CRP (see above), was intended to ensure that a platform of key technologies is available to all participants at the time of project initiation. Eleven participants attended the Workshop. Because of its major importance for physical mapping the Workshop programme focused on fluorescence *in situ* hybridisation (FISH). Dr. Pat Heslop-Harrison, University of Leicester, UK, and Dr. Kesara Anamthawat-Jonsson, University of Reykjavik, Iceland, two outstanding experts in the field of molecular cytogenetics participated as lecturers/advisors in the Workshop, which had a strong emphasis on the practical aspects of FISH. The following techniques were covered in the exercises: chromosome preparations from root tips of various species relevant to the CRP using the squashing techniques; labelling of rDNA and genomic probes using Alexa fluorochromes; hybridization of labelled probes to metaphase preparations; screening of slides using fluorescence microscopy, and analysis and evaluation of results. Since some of the participants already had good experience with FISH techniques the Workshop also functioned as a place for exchange of practical tips, which usually cannot be found in published papers. The usefulness of the Workshop was highly acknowledged by the participants and it was recommended to organize another Workshop for a selected group of participants after the second RCM, which is planned for 2005. This second workshop should be focussed on answering high-technology physical mapping, adding to the data obtained by the participants, and centred on the newly developed material. For example, extra low-copy probes where technology was not available in the home laboratory, or fibre DNA *in situ* hybridisation to increase the resolution of physical mapping, or microarray construction to examine deletions in mutated material, might be course modules.

Meeting on “Field Evaluation of Bayoud-resistant Date Palm Mutants”, (Project RAF/5/049), Sfax, Tozeur, Tunisia, 5-10 May 2003

Technical Officer: S.M. Jain

The first date palm project RAF/5/035 began in 1995 with the main objective to isolate Bayoud disease resistant date palm mutants. Initially, date palm tissue culture technology was developed via somatic embryogenesis and organogenesis for plant regeneration. At Sfax, the major focus has been to regenerate date palms via somatic embryogenesis. They have made substantial progress and have published the first paper on date palm somatic embryogenesis: Fki, L., R. Masmoudi, N. Drira & A. Rival (2003). An optimised protocol for plant regeneration from embryogenic suspension cultures of date palm, *Phoenix dactylifera* L. cv. Deglet Nour. Plant Cell Reports 21: 517-524.

With this technique, thousands of somatic embryos can be produced, germinated into plantlets and transferred to the field. They have induced somatic embryogenesis in 15 date palm varieties. Similarly, somatic embryogenic cell suspension cultures are used for irradiation purposes in order to isolate mutants. The major benefit of this technology is the considerable time saved in dissociation of chimerism as it takes several years to dissociate it. Recently, the Agency has provided cryopreservation equipment for long-term storage (few months to 10-20 years) of somatic embryos and that would lead to establishment of a germplasm bank of date palm mutant varieties. Tunisia, Sfax is the only place that has cryopreservation facilities and these will be further expanded when successful results start coming in.

In 2001-2002, the Moroccans supplied 100 mg toxin, which has resulted in the selection of *Fusarium oxysporum* sp. *albedinis* (FOA) toxin tolerance in 4 out of 45 regenerated plants. These plants are maintained under *in vitro* conditions and will be micropropagated before transfer to the field. More Bayoud disease tolerant mutant plants will be selected as soon as FOA toxin is received from Morocco.

A 2-day international workshop on "Brittle leaf disease of date palms" was held in Tozeur. The serious threat to date palm, caused by maladie des feuilles cassantes (MFC) or brittle leaf disease, was discussed and strategies were formed to overcome it. This disease is known in Southern Tunisia since the 1960s, and has become more prominent since 1986. According to a survey in 2002, over 36,000 date palm trees (up from 4,700 in 1991) are infected with MFC in Tunisia; MFC symptoms are also seen in Libya, Algeria and Egypt. The symptoms are associated with manganese deficiency and the presence of a small double stranded RNA of host origin. The causal pathogen of this disease is still unknown and needs further extensive research.

Third Research Coordination Meeting on "Application of Biotechnology and Mutation Techniques for the Improvement of Local Food Crops in LIFDCs", Pretoria, South Africa, 19-23 May 2003

Technical Officer: S.M. Jain

Twelve participants attended this meeting from Bolivia, Costa Rica, France, Ghana, Indonesia, Slovak Republic, South Africa, Syria and Thailand. Each participant gave a 45-minute oral presentation, followed by extensive discussion. Important results have been achieved in this CRP and will be published either as a TECDOC or a book:

- A reliable strategy for plant regeneration from *in vitro* cultured nodal explants was developed in *Dioscorea* spp. Plants were multiplied to the M_1V_6 generation. Dwarf plants were selected and currently are being micropropagated for large-scale plant multiplication.
- A genetic diversity bank of bitter potato (S*olanum juzepczukii)* was established A total of 14 potentially useful mutants were kept for further field experiments.
- 17 putative root rot disease tolerant mutants of cocoyam were obtained after irradiation and screening. The genetic relationships amongst 70 accessions has also been established by DNA fingerprinting.
- A novel strategy for shortening generation cycles *in vitro* gave 7 and > 4 generation cycles/year for pea and grass pea, respectively. Gene transfer with *Agrobacterium tumefaciens* + GUS gene was successful as regenerated transgenic plants expressed GUS activity inherited in the progeny in a Mendelian fashion.

- Three selected mutants of taro (B43, B63 and B133) had the desired characters: high tolerance to leaf blight disease, early maturing, heavy corms and good taste.
- Bambara groundnut mutants resistant to *Cercospora* leaf spot with desirable agronomic traits were identified. A newly developed technique shortened its generation cycle using *in vitro* and *in vivo* methods.
- 22 mutant lines of okra were selected showing tolerance to yellow vein mosaic virus. 12 of them gave satisfactory yields but fruits had an undesirable shape, and the remaining 10 mutant lines produced fruits of a desirable shape but the yield is still to be determined
- 15 drought tolerant leafy mutants of *Amaranthus tricolor* were selected in the field for their comparatively higher performance in artificially created drought conditions
- 48 *Amaranthus cruentus* selected mutants and 18 mutants of K-433 hybrid were obtained, that bore various useful traits, i.e. determinate growth, uniformity in flowering and seed maturity, leaf-less inflorescences and an increased seed size.
- 15 research papers have been published in both national and international refereed journals.

This RCM was successfully concluded with a final document on the Conclusions and Recommendations, which included the following general recommendations:

- It is recommended that IAEA should facilitate the continuation of the research projects, which have generated very useful results.
- IAEA should support research on the evaluation of the mutants generated from this CRP for nutritional quality and acceptability by consumers.
- It is strongly recommended that IAEA supports the improvement of facilities in participating countries and the training of personnel in the use of mutagenesis, tissue culture and other biotechnologies to improve neglected and underutilized crops.
- Human resources development activities are highly recommended to allow the dissemination of know-how from this CRP, to benefit researchers from various LIFDCs.

Project on "Virus Screening of Improved Banana Mutants for Large-scale Dissemination" (TC Project SRL/5/036), Colombo, Sri Lanka, 26-30 May 2003

Technical Officer: S. Nielen

The aim of this project is to establish mass screening tests for various banana attacking viruses such as Banana Streak Virus (BSV), Banana Bract Mosaic Virus (BBrMV), Banana Bunchy Top Virus (BBTV), and Cucumber Mosaic Virus (CMV). ELISA and PCR techniques suitable to detect the local virus strains are being developed and will be used to screen micropropagated banana plants before distributing them to the farmers. In particular through this project the large-scale dissemination of an Embul banana mutant with improved quality characters, which has been developed using induced mutagenesis (TC project SRL/5/030), will be facilitated. Banana is playing a more and more important role in the dry zones of southern Sri Lanka, in particular because it requires less water compared to paddy. The Technical Officer assessed the status of the project during his mission to Sri Lanka. It was noted that in collaboration with Prof. Lockhart, University of Minnesota, USA, who went on expert mission to Sri Lanka the counterpart has successfully developed an ELISA kit for BSV detection through local antibody production using the hen-egg technique. Furthermore the tissue culture laboratory at the Magampura Agro-Technology and Community Services Centre (MATCSC) of the University of Colombo at Welligata, Hambantota, has made substantial progress in micropropagation of banana. Currently the production amounts to 4000 plants per month and more than 150 farmers acquired the material for a reasonable price and planted it on their fields. Due to the healthy and improved planting material the income of the farmers is significantly increased. The centre in Weligatta is going to be extended by a new laboratory building, which is currently under construction and financed by the NGO World Vision. The new facilities and equipment provided by the Agency will enable the envisaged production of 10,000 plants per month. It is planned that in the long run the centre, which also has activities on banana processed food and flour, will be run in a self-sufficient manner using the income gained from sold plantlets and other products.

D. STATUS OF EXISTING CO-ORDINATED RESEARCH PROJECTS

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

Technical Officer: S.M. Jain

Today, the world's economy and food security are dependent on a few crop species. Also, continuous loss of biodiversity due to an increase in human population, global warming, and gaseous pollution etc., are making the situation more risky for future food supply and rural economies. Agrobiodiversity in many countries is being destroyed, especially in developing countries. It is a source of wealth that has potential to contribute to improved incomes, food security and nutrition. This CRP was started to overcome major constraints to increase productivity of neglected and underutilized, both vegetatively and seed propagated crops, by genetic improvement with induced mutations and biotechnology for enhancing economic viability and sustain crop species diversity; benefiting small farmers in terms of food security, nutrition, enhanced socio-economic benefits. The final RCM was held in Pretoria, South Africa, 19-24 May, 2003. The publication of results achieved through this CRP is under preparation.

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.
(For more information see below)

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 joint RCM was organized by the Department of Genetics, University of Silesia, Katowice and held in Krakow, Poland, 10-14 June 2002. 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 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 in consequences on drought tolerance of crop plant (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. That crop plant agriculture has benefited hugely from mutagenesis programmes is beyond any dispute whatsoever. In modern times, however, with the advent of crop surpluses in developed nations, there is a partial shift away from yield per se, towards food quality and resistance to biotic and abiotic stresses, in order to reduce usage of pesticides and other agrochemicals. It is becoming particularly evident that existing genetic variation for resistance to abiotic stresses is insufficient to achieve 'quantum' shifts in crop plant breeding required for these key traits. Further advances will rely heavily on increased exploitation of germplasm resources, as well as mutated genes arising from mutagenesis programmes or gene engineering endeavours, as significant genetic gains will require extensive novel genetical variation. It is also likely that future advances will rely heavily on genomic resources, such as the EST databases, used to identify candidate genes and large insert DNA libraries for map-based isolation of ‘root-trait’ genes, and the application of molecular markers to crop plant improvement.

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 *Arabidopisis 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. Such tools will undoubtedly soon be available for legumes and *Solanaceous* crops, as there are plans to sequence *Medicago* and tomato, and conserved synteny has been clearly demonstrated between tomato, potato and pepper.

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.

Markers, as well as their application in linkage and diversity analysis, can also be used in the analysis of mutated plant material. For example, AFLPs, which essentially assay SNP variation in/near restriction sites, offer the potential for performing genome ‘scans’ of mutated lines to measure directly the single nucleotide changes due to mutagenic treatment. However, large genomic lesions, such as deletions will also cause differences in AFLP profiles, but for different reasons. At the present time, SNP-based markers offer the best potential for quantifying single-base changes in DNA, caused by mutagenic treatment.

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.

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

Technical Officer: S.M. Jain

Tropical and subtropical fruits are mainly grown in developing countries, in which they take care of nutrition, food security aspects depending on the fruit type and the employment situation. Mostly, there is a lack of initiative on the part of Federal Governments to pay attention to the genetic improvement of fruit crops, their economic potential as export items and development of agro-industries. Neglect of improving horticultural crops, human population growth factors such as deforestation are also leading to the loss of valuable germplasm for genetic improvement of fruits. The longer these problems are unattended, that would lead to an increase in import of fruits and destroy the local economy. Most of the developing countries have a serious nutrition problem, which could readily be solved by improving: fruit production, post harvest storage facilities, better transportation to easy access to market and also cut down the price of fruits. Generally fruit is too expensive for the common man to include in his regular diet. Because of poor storage facilities and access to market, there is a tremendous loss of fruits and farmers are unable to make a profit. There is a great potential of tropical fruits in the international markets, *e.g.* mango, banana, citrus, litchi, papaya etc., both are consumed as a fresh or canned fruit or as a pulp or puree. The European market has a huge potential for fruit imports due to long winter. This market can be captured only when a high quality of fruits is maintained, e.g. disease free. Moreover, genetically modified (GM) fruits are unacceptable to consumers in Europe and other parts of the world. Thereby, plant breeders are under pressure to develop new fruit cultivars, which are cheap, disease free, high quality, better nutrition, and excellent raw material for the industry. Moreover, the development of fruit industry would create more jobs in the rural sector.

Genetic improvement of tropical and subtropical fruits is essential for increasing the productivity of fruits. One of the major problems with fruit breeding work is long life cycle of many fruit crops, which has hampered fruit breeding work. However, genetic variation is necessary for crop breeding, which can either be induced by physical or chemical mutagens besides using natural genetic diversity. Induced mutations are highly effective to enhance natural genetic resources, and have assisted in developing improved cultivars of fruits and other crops. FAO/IAEA funded Co-ordinated Research Project (CRP) entitled "Improvement of tropical and subtropical fruit trees through induced mutations and biotechnology" was initiated in year 2000 to address major constraints in tropical and subtropical fruits such as mango, citrus, guava, cashew, avocado, papaya, litchi, Annona, jujube, carambola, pitanga and jaboticaba (for more see Working Material, IAEA-312.D2.RC.823; Vienna 2001)." The first RCM was held in Vienna, 25-29 September 2000, with an overall objective to generate and characterize radiation-induced and natural genetic diversity in tropical and subtropical fruit trees for improving nutrition balance, food security, and enhancing economic status of the growers in Member States.

At the 2nd RCM, held in Vienna, 2-8 September 2002, a total of 13 participants came from China, India, Indonesia, Iran, Malaysia, Pakistan, Philippines, South Africa, Thailand, Israel, UK, and USA. The highlights of the major achievements during year 2000-2002 are as follows:

- Radiosensitive curve was determined to calculate LD 50 dose of gamma radiation in all experimental fruit crops of this CRP. Almost all fruit crops had different radiosensitive curve. Different explants as well as seeds were irradiated to induce mutations.
- In order to dissociate chimerism, shoots were multiplied up to M_1V_4 generation for the selection of stable mutants.
- Plant tissue culture protocols for plant regeneration were standardized, especially somatic embryogenesis and organogenesis. Somatic embryogenic cell suspension cultures were also developed for irradiation purposes. Low germination rate of somatic embryos is still a major problem.
- Some of the important traits, depending on the fruit crop, will be selected during this CRP. They are- seedlessness/less seeded (citrus, guava, pitanga, jaboticaba), disease resistance (avocado, papaya, mango, cashew, citrus, jujube), high yield (mango, guava), Quality including flavor (mango, jujube), drought tolerance (guava, cashew), early flowering (guava), improved shelf-life (papaya, annona), seed size (litchi, pitanga, jaboticaba), plant height (annona, mango)
- Seed irradiated papaya plants are already in the field for the evaluation and identification of mutants.

The third and final RCM will be held in South Africa from 13-17 September 2004. We will publish the results of this CRP in a book.

Physical Mapping Technologies for the Identification and Characterization of Mutated Genes Contributing to Crop Quality

Technical Officer: S. Nielen

This CRP started in October 2002 with 14 participants from Argentina, Bulgaria, China, Czech Republic, Germany, Iceland, Pakistan, Poland, Ukraine, United Kingdom, USA, and Viet Nam. The main objective of this project is to facilitate the improvement of crop quality through the application of physical mapping and mutation techniques. The work plan applies the technology involved in accessing the genetic and physical position of quality genes in various crop genomes and building physical map information for crop improvement.

The first RCM was held in Vienna from 31 March to 4 April 2003, followed by a three day Workshop on fluorescence *in situ* hybridization (FISH) at the Plant Breeding Unit, Seibersdorf.

E. TECHNICAL CO-OPERATION PROJECTS

Current Operational Projects

Project Number	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/5/013	Induced mutations to improve rice quality	M. Maluszynski
GHA/5/030	Improved cocoa productivity through control of cocoa swollen shoot virus disease	S. Nielen
GHA/5/031	Enhancing cassava production through supplementary nutrient application	S.M. Jain
INS/5/027	Mutation breeding of ornamental plants	S.M. Jain
INS/5/030	Sustainable agriculture development in Yogyakarta	S.M. Jain
INS/5/031	Mutation breeding of horticultural crops	S.M. Jain
INT/5/147	Developing salt-tolerant crops for sustainable food and feed production in saline lands	S.M. Jain
IRQ/5/015	Induction of mutations in crops through *in vitro* culture	S.M. Jain
JOR/5/008	Establishment of *in vitro* mutagenesis laboratory	M. Maluszynski
KEN/5/024	Crop improvement and management through application of nuclear and biotechnology techniques	Q.Y. Shu
MAG/5/008	Mutation techniques and biotechnology for rice and cassava	Q.Y. Shu
MAK/5/004	Mutation and doubled haploid techniques to improve wheat	P. Lagoda
MAL/5/024	*In vitro* mutagenesis for horticultural crop plants	S.M. Jain
MYA/5/010	Development of improved rice with tolerance to drought	Q.Y. Shu
NIR/5/031	Radiation-induced mutations for the development of cowpea varieties	P. Lagoda
PAK/5/038	Development of drought and heat tolerant canola mutants	S. Nielen
PAK/5/039	Pest resistant chickpea through induced mutation	S. Nielen
PAK/5/040	Improvement of heat-tolerant semi-dwarf bread wheat	S. Nielen
PAK/5/042	Induced mutation to improve salt-tolerance in non-aromatic rice varieties	S. Nielen
PER/5/024	Introduction of barley and other native crop mutant cultivars	M. Maluszynski
PHI/5/029	Enhancing agricultural productivity through radiation technology in Mindanao	S.M. Jain
RAF/5/035	Control of bayoud disease in date palm	S.M. Jain
RAF/5/042	Development of improved crop varieties (AFRA III-18)	S. Nielen
RAF/5/049	Field evaluation of bayoud-resistant date palm mutants	S.M. Jain

Project Number	Title	Technical Officer
RAF/5/050	Increasing production of nutritious food through mutation breeding and biotechnology	S. Nielen
RAS/5/037	Mutational enhancement for genetic diversity in rice	S.Y. Shu
RAS/5/040	Enhancement of genetic diversity in food, pulses and oil crops and establishment of mutant germplasm	S.Y. Shu
RAS/7 /014	Monitoring of food fortification programmes using nuclear techniques	P. Lagoda
RLA/5/035	Evaluation of cereal crop mutants (ARCAL XXIa)	M. Maluszynski
ROK/5033	Quality improvement of major crops and integrated plant nutrition management in the low-input agricultural system	P. Lagoda
SRL/5/034	Radiation-induced mutations for black pepper improvement	S.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	Q.Y. Shu
VIE/5/014	Rice mutant varieties for saline land	Q.Y. Shu
YEM/5/003	Applying nuclear techniques for improvement of crop yield	S.M. Jain
ZAI/6/009	Mutation techniques for improving medicinal plants with a curative effect on human diseases	S.M. Jain
ZAM/5/022	Crop improvement through *in vitro* mutation techniques	S. Nielen

F. ACTIVITIES AT THE PLANT BREEDING UNIT, SEIBERSDORF

SSR Characterization of *Musa* Putative Mutant Germplasm

The genus *Musa* (of the family Musaceae) contains two important world food crops, banana and plantain. Combined, they are a major staple for millions of people in the tropical and sub-tropical regions of Africa, Asia Pacific and South America where they are produced in over 100 countries. These robust giant herbs are usually grown all year by small-scale farmers, mostly for local consumption. Because of their adaptation to a wide range of environments, amenability to low input agriculture, adaptation to intercropping and mixed farming systems, they constitute a major component of food security in these hard-pressed regions of the world. The small percentage of the total produce that is exported to the more temperate regions of the world also earn the much needed foreign exchange for local economies.

One of the major production constraints to these crops is the banana leaf spot, or black sigatoka, caused by *Mycosphaerella fijiensis* Morelet. This disease reduces yield by up to 50%. Though chemical control for this disease does exist, the environment implications are enormous. Also, the sterility of the edible banana and plantains make conventional breeding programmes tedious, expensive and in addition hardly ever give rise to new superior varieties.

It is for this reason that non-conventional breeding methods such as mutation induction hold a lot of promise for the improvement of the crop.

We have developed about 500 banana mutants through the irradiation of shoot tips using a Cobalt-60 source. Eight of these have, under green house conditions, shown a high degree of tolerance to juglone (SIGMA), a synthetic form of one of the toxins associated with black sigatoka disease. We are taking the next logical step of having them evaluated in a sigatoka endemic region in Uganda in collaboration with the National Agricultural Research Organization (NARO), Uganda.

Parallel to field evaluations, these mutants are now undergoing preliminary molecular assays. We are using a panel of highly polymorphic banana simple sequence repeat (SSR) markers to assay the mutants and the parent. It is hoped we will be able to find SSR loci that discriminate between the parent and the mutants. Such a locus that produces polymorphic alleles could then be used as a molecular tag and applied in early screening of banana mutants resistant to the black sigatoka disease. This is the first step in developing a body of reliable PCR-based molecular markers that could be used in early screening of Musa germplasm for resistance to this disease.

End Sequencing of *Musa* BAC Clones

Our Unit is actively participating in the Musa Genomics Consortium through the following activities:

- Fingerprinting of 100 BAC clones
- 200 BAC-end sequencing (2 end-sequences per clone)
- Making of 100 TOPO sub-libraries (1 library/BAC clone)

We have commenced the pre-sequencing activities for the initial BAC clones that have been received from the Genomics Resource Centre for the Consortium at the Institute of Experimental Botany of the Academy of Sciences of the Czech Republic, Olomouc, Czech Republic.

Molecular Characterization of Rice Mutant Lines Tolerant to Salinity

This is on-going collaborative work with the International Rice Research Institute, Philippines aimed at the development of rice lines that are tolerant to saline soils. Putative salt tolerant mutants have been identified. In addition to fine tuning the early screening techniques, a batch of rice molecular markers, SSR, AFLP and ISSR, are being used to characterize these mutant lines along with their wild type parent as well as a traditionally grown salt tolerant variety. We hope to identify molecular markers that can be used to screen for this trait at an early stage as well as gain some insight into the influence of irradiation on the genetics of salt tolerance in rice.

Improvement of Ethiopian Tef through Mutation Induction

Tef, a major staple in Ethiopia, has lodging as one of the main production constraints. Breeding for this trait has hitherto been met with little success on account of the very low genetic base of the germplasm available to the breeder and also because of the difficulties in artificial hybridisation programs. The PBU has over the years therefore been involved in the development of alternative breeding methods for this crop.

This current project being undertaken in collaboration with the Ethiopian Agricultural Research Organization, Debre Zeit Agricultural Research Centre is aimed at the development of homozygous tef lines for mutation induction through anther culture and doubled haploid techniques. Such homozygous lines would then be irradiated in order to generate variability for this trait that could be exploited in breeding programs in Member States.

We have been testing different media for the induction of callus from mature embryos of Tef. Also on-going is the irradiation of such calli and the regeneration of putative mutants from the irradiated calli. We are also developing the protocols for haploid induction via anther culture.

Improvement of Malagasy Rice Lines through Mutation Induction

A major production constraint for rice in Madagascar is the susceptibility of the locally cultivated rice varieties to cold. There are no cold tolerant rice lines available to the rice breeders in Madagascar. The PBU has therefore been working in collaboration with the Université d`Antananarivo Faculté des Sciences Lab. Physiology Végétale Biologie et Ecologie Végétale Antananarivo, Madagascar, to develop breeding materials from which this trait could be introgressed into the local lines.

The adopted strategy is aimed at ultimately contributing to the broadening of the genetic base of rice germplasm in Madagascar using 5 Malagasy rice varieties. The series of techniques being employed include the application of callus induction, cell suspension and anther cultures techniques coupled with irradiation.

Irradiation Services

Irradiation services provided from January to May 2003:

Number of Requests	5
Number of Species	5
Number of Varieties	29
Number of Treatments	67
Number of Countries	5

Ploidy Determination

The following flow cytometry assays were carried out in order to determine the ploidy levels of the plants during the period under review:

Crop	*Institution & Country*	*No. of Samples*	*Objective*
Banana	Katholic University, Lueven, Belgium	250	Confirmation of plants from cell suspension cultures
Banana	Internal, PBU	150	Aneuploid Detection in Calcutta-4

Mutant Germplasm Repository

Mutant germplasm accessions deposited in the repository from January – May 2003:

Crop	*No. of accessions*	*Remarks*
Rice	107	
Lentil	3	
Flax	2894	M_3 EMS

With these, we now have a total of 3004 accessions distributed over 3 crops from 3 countries.

Fellowship Training and Scientific Visits

Name	*Country*	*Subject Area*	*Period*
Ms. Andrianaivo, H.V.S	Madagascar	Anther culture and mutation induction in rice	06.01 – 05.08. 2003
Mr. Tadesse, A	Ethiopia	Anther culture, doubled haploid and mutation induction in tef	08.01 – 07.07. 2003
Mr. Ndiaye, El H.S.	Senegal	Maintenance of laboratory equipment	01-21.04. 2003

Consultants/Visiting Scientists

Name	*Affiliation*	*Project*	*Period*
Dr. Gregorio, G.	International Rice Research Institute (IRRI), Philippines	Salinity tolerance in rice	13-21.03. 2003

G. PUBLICATIONS

Bhalla-Sarin, N., U.S. Prasad,. A.S. Kantharajah and S.M. Jain, 2003**.** Micropropagation of litchi (*Litchi chinensis* Sonn.). In: Micropropagation of woody trees and fruits. S.M.Jain and K. Ishii (Eds.). Kluwer. pp 721-731.

Dris, R., R. Nisikainen and S.M. Jain (eds.). 2003. Post harvest storage and plant nutrition in horticultural crops., Volume 2, Science Publishers, New Hampshire, USA.

Jain, S.M. and K. Ishii (eds.), 2003. Micropropagation of woody plants and fruits. Kluwer Academic Publishers, The Netherlands.

Siobhan, M.C., A. Cassells, and S.M. Jain, 2003. Stress and aberrant phenotypes *in vitro* culture. Plant Cell Tissue and Organ Culture 74: 103-121.

Watt, M.P., F. Blakeway, M.E.O. Mokotedi and S.M. Jain, 2003. Micropropagagtion of Eucalyptus. In: Micropropagation of woody trees and fruits. S.M. Jain and K. Ishii (Eds.). Kluwer. pp 217-244.

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:

<u>mutant</u>

1. ____________________ **yes / no**

2. ____________________ **yes / no**

3. ____________________ **yes / no**

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

<u>mutation derived</u>

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 COLLABORATION!

Plant Breeding and Genetics Newsletter No. 11

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

International Atomic Energy Agency
Wagramer Strasse 5, P.O. Box 5,
A-1400 Vienna, Austria

Printed by the IAEA in Austria,
July 2003

03-01261