

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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CONTENTS

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TO THE READER	2
A. STAFF	3
B. FORTHCOMING EVENTS.....	4
C. PAST EVENTS.....	5
D. STATUS OF EXISTING CO-ORDINATED RESEARCH PROJECTS	13
E. TECHNICAL CO-OPERATION PROJECTS	16
F. ACTIVITIES AT THE PLANT BREEDING UNIT, SEIBERSDORF	18
G. PUBLICATIONS	22

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TO THE READER

The most important event related to the activity of the Plant Breeding and Genetics sub-programme in the past six months was the 2nd FAO/IAEA Interregional Training Course on “Mutant Germplasm Characterization using Molecular Markers” which was held at Seibersdorf, 4-29 November 2002. Drs S. Nielen (Course Director) and P. Lagoda organized and implemented this course with the help of the staff of Plant Breeding Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf. It provided training for 20 participants from Bangladesh, Belarus, Cote d’Ivoire, Egypt, Ghana, Guatemala, India, Indonesia, Malaysia, Peru, Philippines, Poland, Slovakia, Sri Lanka, Sudan, Syria, Thailand, Turkey, Ukraine and Yugoslavia. This time the lectures and exercises were covered by Uri Lavi (Israel), Petra Wolters (USA), Suk-Ha Lee (Republic of Korea), J.S. (Pat) Heslop-Harrison (UK), Pierre J.L. Lagoda (FAO/IAEA), Stephan Nielen (FAO/IAEA). In addition to basic molecular and marker techniques, which were also a subject of the first training course last year, fluorescence *in situ* hybridisation methods were included in the teaching and demonstration programme. As we informed you in the last edition of this Newsletter, a laboratory manual was published with detailed protocols on molecular markers techniques entitled “Mutant germplasm characterization using molecular markers. A Manual”. (IAEA Training Course Series No. 19). (available for free distribution under conditions provided on page 11 of this Newsletter).

We have also finished editing a book on “Doubled haploid production in crop plants. A Manual.” This book was prepared in close collaboration with EU COST 851 activities. Ken Kasha (Canada), Brian Forster (UK) and Iwona Szarejko (Poland) helped to edit more than 40 protocols for doubled haploid production in at least 23 crop species. The preparation of this manual reflects our interest in the development and application of this technology for crop improvement. Two CRPs and numerous Technical Co-operation projects greatly contributed to the development of doubled haploid methods and also to implementation of this technology in crop improvement programmes of many countries.

Numerous other important activities have been undertaken by the Plant Breeding and Genetics sub-programme during the last 6 months. A consultants meeting on “Low cost technology in plant tissue culture” was held in Vienna and its results will be summarized in the form of an IAEA-TECDOC which is now in the final stage of preparation. In addition to the implementation of five Co-ordinated Research Projects, nine workshops and national or regional training courses were held in Algeria, Kenya, Malaysia, Macedonia, Sri Lanka, Vietnam, Yemen and Zambia. In the Plant Breeding Unit, Seibersdorf activities focused on identifying putative rice mutants with tolerance to salinity and of polymorphism in salt tolerant rice mutants and on collection of the first mutants in an organized Mutants Repository.

After seven years of excellent service, Dr. Karin Nichterlein left the Plant Breeding and Genetics Section for a position in FAO. Similarly, Dr. Nicolas Roux, the leader of our banana team, left the Plant Breeding Unit after nine years of service. We will miss them and wish them all the best in their new positions. Dr. Pierre Lagoda- molecular biologist – joined the Plant Breeding and Genetics Section on a temporary assignment in June 2002.

On behalf of the staff of the Plant Breeding and Genetics Section and the Plant Breeding Unit, I would like to take this opportunity to wish you all a very happy and successful New Year.

Mirosław 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

James D. Dargie Director

Plant Breeding and Genetics Section
--

Mirosław Maluszynski	Head of Section	M.Maluszynski@iaea.org
Mohan Jain	Technical Officer	S.M.Jain@iaea.org
Karin Nichterlein	Technical Officer	K.Nichterlein@iaea.org
Stephan Nielen	Technical Officer	S.Nielen@iaea.org
Pierre Lagoda	Technical Officer	P.Lagoda@iaea.org

Secretaries:

Kathleen Weindl	K.Weindl@iaea.org
Katayon Allaf	K.Allaf@iaea.org

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

Javier Zapata Arias	Head of Unit	F.Zapata-Arias@iaea.org
Nicholas Roux	Research Scientist	N.Roux@iaea.org
Rownak Afza	Senior Technician	R.Afza@iaea.org
Andrea Kodym	Technician	A.Kodym@iaea.org
Mirta Matijevic	Technician	M.Matijevic@iaea.org
Günther Berthold	Technician	F.Zwiletisch@iaea.org
Andreas Draganitsch	Technician	F.Zwiletisch@iaea.org
Franz Zwiletisch	Technician	F.Zwiletisch@iaea.org
Arsenio Toloza	Technician	A.Toloza@iaea.org

Secretaries:

Muriel Weinreich	M.Weinreich@iaea.org
Elisabeth Swoboda	E.Swoboda@iaea.org

B. FORTHCOMING EVENTS

First Research Coordination Meeting on “Physical Mapping Technologies For The Identification And Characterization Of Mutated Genes Contributing To Crop Quality”, Vienna, Austria, 31 March – 9 April 2003

Technical Officer: Stephan Nielen

This first meeting under the new Co-ordinated Research Project will be held followed by a technical workshop at the Agency’s Laboratories at Seibersdorf from 7–9 April. The RCM Group will discuss and develop strategies for accessing the genetic and physical position of quality genes in various crop genomes, with the aim of using physical map information for crop improvement. The hands-on workshop will ensure that a platform of key technologies is available to all participants at the first phase of the project.

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: Mirosław Maluszynski

Fifteen participants from Bolivia, Costa Rica, Ecuador, France, Germany, Ghana, India, Indonesia, Slovakia, South Africa, Syria and Thailand are expected to attend this RCM, including an agreement holder from IPGRI based at ICARDA. They will present the main results of their research under this CRP, mainly on the use of mutations, *in vitro* and molecular techniques to increase productivity of neglected and underutilized crops such as 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*). The results will be reviewed, reports improved, guidelines for mutagenic treatment, breeding techniques and characterisation of germplasm of neglected crops formulated and the first draft of the Technical Document of the CRP will be prepared.

New CRP on the “Effects Of Mutagenic Agents On The DNA Sequence In Plants” – expected to be approved in 2003

Technical Officer: Stephan Nielen

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.

C. PAST EVENTS

Consultants Meeting on “Low Cost Technology In Plant Tissue Culture”, Vienna, Austria, 24-30 August 2002

Technical Officer: Mohan Jain

Tissue culture is mainly used for large-scale plant multiplication especially with micropropagation. This approach is being used mostly in vegetatively propagated crop plants such as fruits, ornamental plants and forestry. In commercial tissue culture companies it is used routinely for plant multiplication, and can provide thousands of *in vitro* propagated plants in a short period of time all year round. Micropropagation is a simple and labour intensive technology. However, the cost of *in vitro* plant production is high due to various factors such as the cost of electricity, water, chemicals etc., even though labour is cheap in developing countries. Therefore, it is necessary to cut down the costs of *in vitro* plant production for wider use of this technology. This program was started with the intention of developing low cost technology for developing countries for year round multiplication and supply of *in vitro* plants of elite germplasm including induced mutants.

Six consultants from Austria, Bangladesh, India, Israel, and USA participated in this meeting to discuss low cost propagation and prepare a manual for end-users in developing countries. This manual is now ready for publication. The chapters include: plant tissue culture, low cost options in tissue culture, physical components of tissue culture technology, culture media and containers, low cost option for energy and labour, bioreactors, disease detection and elimination, quality assurance and priming tissue cultured propagules. At the end, an addendum provides important information on equipment, common tissue culture terms, common abbreviations, composition of commonly used culture media, layout and design of tissue culture laboratory, figures and related publications and website. This document may be able to inculcate ideas in cutting the cost for *in vitro* plant production.

FAO/IAEA National Training Course on “Mutation Techniques For Improvement Of Field Crops” (YEM/5/003), Agricultural Research and Extension Authority (AREA), Dhamar, Yemen, 27 July – 3 August 2002

Technical Officer: Karin Nichterlein

The Agricultural Research and Extension Authority hosted this training course on mutation techniques for crop improvement. The programme covered mutagenic treatment including various radiosensitivity tests, handling of mutant populations of seed propagated crops, identification and selection of mutants, screening for disease resistance and tolerance to drought and salinity, improvement of wheat and oilseeds through induced mutations, economic impact of mutant varieties of wheat, cotton and legumes in Pakistan. Seventeen researchers and research technicians from AREA Headquarters and various research stations (central, northern, Tihama, El-Koud) were trained. Two international lecturers, Dr. M.A. Awan, Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan and Dr. A.I. Ragab, Nuclear Research Center, Cairo, Egypt taught at this course.

FAO/IAEA Regional Training Workshop on “Application Of Molecular Markers In Disease Diagnostic And Mutant Characterisation In Date Palm”, (RAF/5/049), Algiers, Algeria, 24-28 August 2002

Technical Officer: Mohan Jain

This Workshop was held for the first time at the Institut National de la Recherche Agronomique d' Algerie, Algiers. Eight participants attended; five from Algeria and three from Morocco. They had different academic backgrounds with similar goals of date palm improvement and protection against Bayoud disease. The basic purpose of this Workshop was to introduce molecular marker technology for early detection of Bayoud pathogen in date palm and also to induce genetic variability for the identification of mutants. At the end of the Workshop all participants suggested that more practical demonstrations be included, theory lectures be reduced and that such Workshops be organized on a yearly basis.

Second 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: Mohan Jain

This meeting was attended by 13 participants from China, India, Indonesia, Iran, Malaysia, Pakistan, Philippines, South Africa, Thailand, Israel, UK, and USA. The main aim was to review the overall progress made by the participating countries during the last years and propose a work plan for the subsequent two years. Considerable progress has been made according to the work plan in tissue culture and induced mutations in many crops.

Conclusions and Recommendations

The RCM Group has made good progress in utilising various strategies in order to recover mutants that address the breeding goals such as resistance to abiotic and biotic stresses, fruit quality, tree architecture etc., for enhanced food security and sustainability. Irradiation assays have been established for all crops and methods of propagation have been tested and refined (see achievements and Table 1). Emphasis should now be given to selecting and identifying mutants. Several methods are available for assessment (Table 1).

1. Exchange of genetic materials

Exchange of material and information among partners has been accomplished and should be encouraged once mutants are identified. It is recognised that there are serious constraints to international exchange of germplasm and DNA. It is recommended that FAO/IAEA explore ways in which barriers can be overcome, for example through the development of bilateral material transfer agreements, and by agreements that clearly distinguish between research and commercial application.

2. Collaborative linkages

The application of induced mutations and biotechnology to fruit crop improvement depends on a multidisciplinary approach. This CRP has served as a valuable medium for fostering close collaboration and co-operation among scientists working within/between these species, and also with those having technological expertise in other crops or model systems. We recommend that the new linkages that have been built are further strengthened, and full use made of options for training courses and visits, and of fellowship programs building on the framework of the CRP.

3. DNA markers

Applications of DNA markers and generic molecular tools have been shown to be possible in tropical and sub-tropical crops in a limited number of species such as banana, citrus, and papaya. It is highly recommended that consideration is given to expand their use, particularly for characterizing generic and induced variation coming from this CRP. In this rapidly changing genomics research area, new high throughput technologies are becoming available which will be efficient and low cost for tropical and sub-tropical fruit crop improvement; options to use these must be seized when possible. Single Nucleotide Polymorphisms (SNPs) are new single locus markers that are very abundant and highly suitable for automation and high throughput. Micro-array hybridisation makes it possible to screen large amount of markers and clones and their distribution and expression in a semi-quantitative manner. Since their application requires sophisticated equipment, this research avenue offers an opportunity for collaboration between laboratories in developing and developed countries.

4. Mutagenesis and selection of mutants

In vitro and *in situ* selection of desired variants through mutagenesis is recommended where there is an appropriate screening agent. It is also recommended as an alternative means for increasing genetic variation, without the intervention of any other genetic material. This technology has been utilised with citrus, guava, and mango and good progress has been reported with avocado. Somatic embryogenesis of several species in this CRP has now been well defined. The technique has a number of advantages over the conventional use of irradiated budwood. Embryogenic cultures can be utilized effectively for mutation induction followed by selection (assuming that an appropriate selection agent is available). The use of these

techniques should be particularly encouraged if *in vitro* selection techniques can be applied. If no selection pressure can be applied to cultures the value of obtaining solid mutants derived from single cell cultures must be weighed against the convenience and speed of utilising grafting technologies. Embryogenic systems are also the preferred method for genetic transformation of trees.

5. Sustainability and food security

It is recognised that preservation and utilisation of biodiversity is of utmost importance. Development of improved varieties by *in vitro* or *in situ* mutagenesis is recommended for increasing genetic diversity, both for qualitatively and quantitatively inherited characters (such as disease resistance, stress tolerance, post-harvest disorders, fruit quality and yield). These approaches are environmentally sustainable and can make an important contribution to food security. The efficiency of the recovery of useful mutants of vegetatively propagated crop species can potentially be greatly enhanced if embryogenic cultures are utilized. Mutation induction followed by selection for resistance to an appropriate agent, e.g., phytotoxin for disease resistance, etc., enables the targeting of a specific trait for improvement. This approach to plant improvement should be viewed as a viable alternative to genetic transformation, particularly for this group of tropical and subtropical fruit crop species, which have significant markets in countries that have rejected GMO technology.

6. Dissemination

The research coordination meeting has proven to be a very effective method for exchange of information and ideas regarding the planning and execution of projects. Some of the scientific aspects of the project have been published. Establishment of a mailing list devoted to induced mutation for plant breeding would be a very effective way of communication between RCM participants and other interested parties interactively and instantly.

7. Appropriate selection of technology

A wide variety of research tools have been utilized in the CRP and are summarised in the below table. A number of the projects would benefit if they began to use embryogenic cultures to accelerate the recovery of useful mutants. Thought needs to be given to screening mutants for the breeding goals identified. Initially, plants can be grown in high density for pre-screening before planting at commercial spacing.

Research tools utilized or recommended for use in the CRP

Method	Crop/methodology currently used in this CRP	Crop/methodology in this CRP potentially to be used
Propagation		
Somatic embryogenesis	Avocado, Citrus, Mango, Papaya, Cashew	Guava, Jaboticaba.
Bud wood (grafting)	Citrus, Guava, Cherimola, Liches	Mango, Avocado
Seeds	Papaya, Jujube, Guava, Citrus	Carambola
Shoot culture	Citrus, Jujube, Guava, Avocado	Cherimola, Papaya
Adventitious shoots		Carambola
Irradiation		
Type	Acute	Chronic
Tissue	Embryogenic cultures, seeds,	Nucellus, pollen

	budwood,	
Dose	LD25-50	
Chemical mutagenesis	no	Consider
<i>In vitro</i> Selection	Avocado, Mango	Citrus, Guava, Papaya, others if selection agent identified
Assessment		
Morphology (fruit characteristic, seedlessness, etc.)	All	All
Disease resistance	Avocado, Mango, Papaya	Guava, Jujube
Physiological traits (ethylene biosynthesis)	Papaya	Mango, Avocado, Guayava
DNA markers	AFLPs, SSRs	SNPs, retro-elements
Molecular and analytical cytology	Chromosome number	<i>In situ</i> hybridization, flow cytometry, microarray

FAO/IAEA Training Course on “Use Of Neutron Probe For Drought Tolerance Screening” (RAF/5/050), Kenyan Agricultural Research Institute (KARI) Njoro, Kenya, 1-3 October 2002

Technical Officer: Karin Nichterlein

This training course was organised under the regional project RAF/5/050, and focused on the use of neutron probes to study soil-water-plant relationships in order to screen crops for drought tolerance under controlled and reproducible conditions with rain shelters and/or controlled irrigation in the off-season. This activity contributes to standardisation and improvement of screening for drought tolerance in the African region. The course consisted of theoretical lessons and practical demonstrations and exercises on drought screening, soil water relationships, neutron probes, soil water content (SWC) measurement with the neutron probe, neutron probe calibration, conversion of raw data counts to SWC and health and safety aspects of neutron probes. Fifteen staff members of both KARI Njoro and the neighboring Egerton University completed the course. Dr. J.M. Steyn from the Department of Plant Production and Soil Science of the University of Pretoria, South Africa served as an expert to this course.

FAO/IAEA National Workshop on “Applications Of Induced Mutation And Molecular Tools In Horticultural Crops Including Ornamental Plants” (MAL/5/024), MINT, Malaysia, 8-12 October 2002

Technical Officer: Mohan Jain

This was the second Workshop under MAL/5/024 project, and mainly focused on tissue culture, genetic variability, and molecular cytogenetics and markers. This Workshop was attended by 19 participants from MINT, local universities, research organizations and a small company. Most of the participants had varied background and research interests. However, the common interest of the participants was induced mutations in plants for genetic improvement. The Workshop involved both lectures (each one hour duration) and practical demonstrations.

The lecturers of this course were Dr. Saxena, Canada; Dr. Schwarzacher, UK; Dr. Jain, IAEA; and Dr. Rusli, MINT.

FAO/IAEA/University of Skopje National Seminar “Assessment Of Wheat Quality” (MAK/5/004), University of Skopje, Macedonia, 17 October 2002

Technical Officer: Karin Nichterlein

Within the framework of a wheat improvement project, the Agency assisted the University of Skopje to establish a highly professional laboratory for wheat quality assessment with the potential to become an accredited laboratory. At the end of a training course for staff members of the Department of Genetics and Plant Breeding, Faculty of Agriculture, University of Skopje on wheat quality assessment, a National Seminar was organised to inform on quality assessment methods for wheat breeding and the processing industry and to present the services of the wheat quality laboratory. The seminar was attended by 35 participants from the Faculty of Agriculture, the Institute of Agriculture, the Ministry of Agriculture, companies of the milling and baking industry located in Skopje, Demir Kapija, Tetovo, Kichevo, Bitola and Kochani and seed trading companies in Skopje. The seminar was given by local scientists, Ms. Sonja Ivanovska, Mr. Ljupco Jankuloski, Mr. Milisav Ivanovski with support of an international expert, Dr. Erica Acs, Cereal Research Non-Profit Company, Szeged, Hungary.

FAO/IAEA/CLRRI National Training Course on “Marker-Assisted Selection And DNA Fingerprinting In Rice” (VIE/5/014), Cuu Long Delta Rice Research Institute (CLRRI), Cantho, Vietnam, 12-21 November 2002

Technical Officer: Karin Nichterlein

This training course was one activity to transfer molecular marker techniques to rice improvement programmes in Vietnam. It involved theoretical lessons and intensive practical exercises on the structure and role of DNA, PCR technique, enzymes, DNA isolation, dosage, dilution and storage, microsatellite markers, AFLP technique, strategy of marker-assisted selection. 14 participants from CLRRI, Omon, Institute of Agriculture of South Vietnam (VASI), Ho Chi Minh City (HCMC), Institute of Agricultural Genetics (AGI), Hanoi, Center for Nuclear Techniques, Biotechnology Division, HCMC, Nong Lam University of Agriculture, HCMC, University of Cantho, Biotechnology Department, Cantho and Western Highlands Agro-forestry Science and Technique Institute (WASI), Tay Nguyen were trained in the course. At the end of the course participants' projects on the use of AFLP to tag mutant traits at CLRRI (e.g. salt tolerance) and at AGI (e.g. aluminium tolerance and aroma) were presented and approaches for the development of mapping populations and mapping the mutant traits discussed. Three international trainers, Dr. Brigitte Courtois, Mr. Jean-Louis Noyer, Mr. Ange-Marie Risterucci from CIRAD, France and three national lecturers, Dr. Vuong Dinh Tuan and Dr. Nguyen Thi Lang from CLRRI and Dr. Vu Duc Quang from AGI taught at the course.

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

Technical Officer: Mirosław Maluszynski & Stephan Nielen

In response to the strong demand of scientists from Member States for sound theoretical and hands-on training on different aspects of molecular markers, their applications in characterizing mutations and their role in plant breeding, the 2nd Interregional Training Course on Mutant Germplasm Characterization using Molecular Markers was held at the Agency’s Laboratories at Seibersdorf. Twenty scientists from 20 different developing countries were selected to participate in the four week course, which covered - besides basic molecular techniques - some of the most important marker techniques such as RFLP, AFLP, SSRs, ISSRs and retrotransposon based marker technologies. As a new aspect chromosomal analysis using fluorescence *in situ* hybridisation was included into the training programme. This powerful technique enables the genetic linkage group to be joined with the physical chromosome, and allows chromosomes carrying useful genes or gene complexes to be followed through breeding programmes. Practical experience was also gained in DNA cloning and sequencing. In order to stimulate and facilitate the application of the acquired knowledge in their home institutes the participants were given the opportunity to conduct their own projects in the last week of the course. The following scientists participated in teaching different aspects of molecular markers:

Dr. Uri Lavi, ARO, Volcani Centre, ISRAEL

Dr. Petra Wolters, DuPont Company, USA

Dr. Suk-Ha Lee, Seoul National University, REPUBLIC OF KOREA

Dr. J.S. (Pat) Heslop-Harrison, University of Leicester, UK

Dr. Pierre J.L. Lagoda, Joint FAO/IAEA Division, Vienna, AUSTRIA

Dr. Stephan Nielen, Joint FAO/IAEA Division, Vienna, AUSTRIA

As a guideline for the laboratory work a manual with detailed protocols on molecular marker techniques was handed out to the participants. This manual was prepared on the basis of the protocols and experience of last years’ Training Course and is published now under the Agency’s Training Course Series. A hard copy with attached CD-ROM will be distributed, free of charge, to interested scientists from FAO and IAEA Member States. Requests for the manual should be sent by those interested to S. Nielen, Plant Breeding and Genetics Section, Joint FAO/IAEA Division of Nuclear Application in Agriculture, P.O. Box 100, Vienna, Austria, Fax: +43 1 26007 or by email: S.Nielen@iaea.org. An individual request is necessary as we plan to have all users’ addresses in order to distribute new additions and supplements.

FAO/IAEA/MRRC National Training Course on “On-Farm Trials Of Improved Finger Millet Mutants” (RAF/5/050) Misamfu Regional Research Centre (MRRC), Kasama, Zambia, 2-6 December 2002

Technical Officer: Karin Nichterlein

The plant breeders at Misamfu Regional Research Centre, Kasama, Zambia developed improved advanced mutant lines of finger millet with improved yield potential. In order to prepare an efficient on-farm evaluation of the mutant lines ‘FMM 165’ and ‘FMM 175’, in comparison to the variety ‘Nyika’ (parent variety) and a local cultivar of the location, this workshop was organised. Farmer representatives and extension staff from various finger millet cultivating locations were trained in the agronomic characteristics of the new breeding lines, selection of location and sites of on-farm trials, design of on-farm trials, crop management, collection of postharvest data, data submission, data analysis and reporting. Almost 40 participants attended the workshop. Mr. Subhash Gupta, from ICRISAT, India served as an expert to support local staff with teaching in the workshop.

FAO/IAEA National Workshop on “Black Pepper Improvement – Cell And Tissue Culture, Breeding And Induced Mutations And Molecular Markers” (SRL/5/034), Matala, Sri Lanka, 9-13 December 2002

Technical Officer: Mohan Jain

Black pepper is an extremely important export crop in Sri Lanka, and contributes greatly to the national exchequer. Drought is one of the major problems facing the black pepper production, which could be addressed by developing new drought tolerant varieties by induced mutations and biotechnology. There is little work done on black pepper tissue culture and mutagenesis in Sri Lanka. This was the first workshop on black pepper conducted under SRL/5/034 project, attended by 22 participants from all across the country, representing different institutes, organisations and universities. The participants had varied academic background, research experience and interests but lacked knowledge of recent developments in black pepper tissue culture and molecular marker analysis. The enthusiasm of participants to learn new scientific developments in molecular biology and biotechnology was an indicator of long discussions after every lecture. The Workshop dealt with plant tissue culture, *in vitro* mutagenesis, and molecular marker technologies (including practical demonstrations). On black pepper genetic improvement, N. Babu mainly covered: genetic resources, breeding for high yield and quality through selection and hybridisation, micropropagation for production, chemotaxonomy, biotechnological tools, and biotechnology for spices. On molecular biology, Madan Mohan gave lectures on: molecular markers I- general introduction and applications, molecular markers II- mapping and tagging of agronomically important genes, molecular markers III- marker-assisted selection in plant breeding, molecular markers IV- map based gene cloning of agronomically important genes, and molecular markers V- identification of genes based on bioinformatic tools. On induction of genetic variability, Mohan Jain delivered lectures on different approaches – somaclonal variation, induced mutations, somatic cell fusion, transgenics, bioreactors and micropropagation. Among local lecturers, Dr. A. Perera spoke on

GMO's in Sri Lanka to ban or boon; and Dr. R.S. Kularatne gave a demonstration on usage of software on gel documentation and cluster analysis.

Third and Final Meeting on “Regional Rice Mutants Multilocation Trials (RRMMT)”, (RAS/5/037), Can Tho, Vietnam, 9-13 December 2002

Technical Officer: Mirosław Maluszynski

The meeting was hosted by Cuulong Delta Rice Research Institute (CLRRI) Ministry of Agriculture and Rural Development, Omon, Can Tho. Ten rice breeders from 9 countries (Bangladesh, China, Indonesia, Korea, Malaysia, Pakistan, the Philippines, Thailand and Viet Nam) attended the meeting and presented results achieved through the implementation of the project. Dr. G. Eizenga (USDA-ARS) was invited to participate in the meeting as an expert on statistical evaluation of rice multilocation trials.

It should be strongly stressed that this short project (three years) brought important results in both components. Due to organization of 45 rice mutant trials conducted in nine countries over 19 locations in the region it was possible to identify 13 mutant varieties and 4 other accessions as highly performing in countries other than the country of their release and were introduced to breeding programs of participating countries. Additionally 21 entries, including 17 mutants, have been found as suitable for cross breeding programmes in various participating countries. Taking into consideration the very high yielding potential of selected mutant varieties and expression of very desired characters it should be expected that at least 5-7 new, high yielding rice varieties will be released during the next 3-5 years in countries of the Region bringing significant income to rice farmers. New, mutated germplasm (17 entries) introduced to the rice improvement programme of all participating countries brought many desired rice characters important for further breeding of varieties with such important characters as high yield, earliness, diseases and pest resistance and improved quality. According to participants the introduction of new rice germplasm was very desired and will contribute, in nearest perspective, to breeding of new varieties. All participants agreed that the way of organizing the germplasm exchange was very efficient and opened new possibilities in the transfer of desired genetic resources among countries of the Region. Implementation of this project also brought significant scientific results. For the first time it was documented that induced mutations may increase plasticity and general adaptability of particular varieties. This was possible to achieve due to the large scale of these regional multilocation trials.

D. STATUS OF EXISTING CO-ORDINATED RESEARCH PROJECTS

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

Technical Officer: Karin 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 from 19-23 May 2003, in Pretoria, South Africa.

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

Technical Officer: Mirosław 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 Plant Breeding and Genetics Newsletter No. 9)

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

Technical Officer: Mirosław 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: Mohan Jain

Improvement of tropical and subtropical fruit trees has been difficult using conventional breeding methods due to long juvenile period and large tree size. This challenge has been met with biotechnology and induced mutations. The aim of this CRP is to genetically improve of fruit trees with gamma-radiation induced mutations and biotechnology. During the 2nd RCM the following achievements were confirmed by the participants:

- 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 M₁V₄ 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.
- 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 their evaluation in identifying mutants.

(For more information see Plant Breeding and Genetics Newsletter No. 9)

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

<p>TC project on “Development of Drought and Heat Tolerant Canola Mutants” (PAK/5/038)</p>

Pakistan continues to meet the domestic edible oil requirements through huge imports at the cost of scarce foreign exchange. More than 1 million tons of vegetable oil for edible and industrial uses are imported annually. Although rapeseed (*Brassica napus*) and mustard (*B. juncea*) have been grown traditionally in the country for centuries their average yield is low. The development of high yielding Brassica species adapted to local conditions will contribute to an increase of the local production of vegetable oil, since Brassica species had already been grown on 0.32 million ha, i.e. over 54% of the total area under oilseed cultivation. The project was undertaken to combine Canola quality (e.g. double zero = low erucic acid content of the oil and low glucosinolate levels in the meal) with high yield and adaptation to drought and heat.

Breeding research of the Nuclear Institute for Food and Agriculture (NIFA), Peshawar, was previously supported through research contracts under two Co-ordinated Research Projects. These efforts led to the release of one improved rapeseed variety ‘Abasin-95’, a canola type with improved productivity, in 1996, which is currently grown on 3,000 ha in the North West Frontier Province. New breeding techniques (in vitro techniques, quality analysis for oil quality and glucosinolate content) were transferred to the Brassica oilseed programme through the previous TC project (PAK/5/034), by training staff and upgrading the breeding laboratories.

The project PAK/5/038, was established to extend breeding efforts, not only to oilseed quality but also to abiotic stress factors such as heat and drought tolerance. Training and expert services were provided in screening for drought and heat tolerance, disease resistance, optimization and standardization of quality assessment and doubled-haploid technique through microspore culture. Equipment and software were supplied through the project to transfer Near Infrared Reflectance Spectroscopy for the fast screening for quality traits. Two institutes were involved in the project, the Nuclear Institute for Food and Agriculture (NIFA), Peshawar, and the Atomic Energy Agricultural Research Centre (AEARC), Tandojam. They used mutation, hybridization and *in vitro* techniques, in order to incorporate tolerance to environmental stresses (e.g. drought, heat) into high yielding canola varieties.

After crosses among selected mutants of both mustard and rapeseed heterotic yield gain were observed in some hybrids. Doubled-haploid lines from *in vitro* mutagenesis are currently under evaluation. Out of 135 mutant and doubled-haploid lines of *Brassica napus* L. and *Brassica juncea* selected for drought and heat tolerance and evaluated in preliminary yield trials, one mutant line with very early maturity (one month earlier) and twenty mutant lines with early maturity and high yield were identified. Advanced yield trials of mustard confirmed the early maturity and improved yield of the mutant lines, whereas in the case of rapeseed, 60% of advanced mutant lines significantly out-yielded the local check varieties as well as the initial parent variety. The 24 most promising mutant lines of *B. juncea* and 18 mutant lines of *B. napus* are evaluated in Zonal Yield trials at NIFA, Peshawar and NIA, Tandojam, Sindh. The yield potential and environmental stresses especially the water deficit stress, of 38 rapeseed and mustard mutant lines are being tested in Advance Zonal Yield Trials Year 2002-03 in northern, central and southern parts of the country. The best performers will be recommended for official release as improved mustard and rapeseed varieties. The multiplication and dissemination to farmers of improved canola varieties with tolerance to heat and drought condition, will produce more edible oil for local consumption under rainfed conditions in Pakistan, thus providing farmers with an income and reducing the country's expenditures for oilseed imports.

F. ACTIVITIES AT THE PLANT BREEDING UNIT, SEIBERSDORF

Identification of Putative Rice Mutants with Salinity Tolerance

Salinity is a significant limiting factor to agriculture productivity, affecting about 900 million hectares of land surface on the earth. The existing problem is becoming more acute as a result of poor quality of irrigating water. In recent years the human population has risen dramatically and the food production rate is insufficient to keep pace with growth demand. To feed the world, scientists will have to bring modern agricultural methods to areas where they are not in use at present.

Rice is the most important food crop of one-third of the world's population and is very much affected by salinity. Developing improved varieties that can withstand salt affected soil remain open and impose major challenges to rice breeders. Breeding for salinity tolerance in rice is difficult. Considerable conventional breeding efforts to increase salt tolerance have been made but the progress in developing tolerance in rice has been slow because of the complex nature of tolerance. Mutation induction in combination with conventional breeding might open new frontiers for obtaining tolerant rice varieties.

In the year 1997, a project was initiated at the Plant Breeding Unit, to develop the techniques related to obtain salt tolerant rice varieties using gamma irradiation (^{60}Co) or chemical mutagens such as EMS.

To generate salt tolerant mutants one thousand seeds of the semidwarf and moderate salt tolerant rice variety Bicol and one thousand five hundred seeds of the semidwarf but salt susceptible rice variety IR29 were irradiated with dosages of gamma irradiation ranging between 100 to 400 Gy.

The mutated M_1 population was grown in a glasshouse till maturity and M_2 seeds (11,000 seeds for Bicol and 8000 seeds for IR29) were harvested and their seedlings screened for salt tolerance.

The hydroponics culture system developed by the International Rice research Institute, Manila, The Philippines (IRRI) with some modifications made at the Plant Breeding Unit were used for screening and selecting tolerant mutants. This technique allows the screening of large populations at an early seedling stage of development in a limited space.

The selection of seedlings tolerant to salinity was done using Yoshida's solution containing a salt concentration of 640mg of NaCl per litre (10dS/m). The response to salt was determined by the extent of the visible damage to the seedlings. The traditional rice variety Pokkali was used as a tolerant check, IR29 as a susceptible one, and non-irradiated parents as a control. The salinity stress was ended when the non-irradiated controls died. The salinity tolerance level of putative mutants was further confirmed by screening in the M_3 and M_4 generation.

As a result six mutants with higher salt tolerance than the parent variety Bicol and two salt tolerant from the salt susceptible variety IR29 were obtained in the year 2001. These plants are now being evaluated for their tolerance to salinity by IRRI under field conditions in Iloilo, Philippines.

Comparison of AFLP, SSR and ISSR Molecular Markers Techniques for Identification of Salt Tolerant Rice Mutant Lines

The characterization and identification of mutant plants requires a reliable marker system, which will be able to detect high levels of polymorphisms. Highly polymorphic multilocus marker systems like Amplified Fragment Length Polymorphisms (AFLP), Simple Sequence Repeats (SSR) and Inter-Simple Sequence Repeats (ISSR) are thought to be suitable for fingerprinting of mutant lines since they are quite abundant and reliable and have been used to assess genetic diversity in many plant species like maize, barley, sorghum, rice, banana and citrus.

To detect polymorphisms using AFLP, SSR and ISSR markers, genomic DNA from salt tolerant mutant lines developed at the Plant Breeding Unit and their parents were analyzed. For AFLP, 32 primer combinations, for SSR 28 primers and for ISSR 35 primers were used. Two mutants generated by gamma irradiation of the salt susceptible variety IR29 and 6 mutants from the moderately salt tolerant variety Bicol were studied. Polymorphisms were detected in the salt tolerant mutants developed from salt susceptible rice variety IR29 in all the markers techniques used. The polymorphisms were identified either by additional or by missing bands. These bands are now subject to further molecular analysis.

The used markers were not able to detect any polymorphisms of salt tolerant mutants originating from the variety, Bicol.

Musa Mutants to be Stored at the Repository (MMR) Established at Seibersdorf

From a population of 4000 plants derived from gamma irradiated shoot tips, 15 plants were selected for their tolerance to Juglone (5-Hydroxy-1, 4-naphthoquinone), a toxic metabolite of *Mycosphaerella fijiensis* and around 300 plants were selected based on aberrant morphology. All plants were phenotypically characterized and photos were taken of each one. Among the 15 plants with tolerance to Juglone (25 ppm) one was detected as aneuploid with 32 chromosomes instead of 33. A high throughput procedure for rapid and convenient detection of aneuploidy in triploid *Musa* using DNA flow cytometry was developed. From the population of plants derived from gamma irradiated shoot tips, plants were selected based on aberrant morphology, and their chromosome numbers were counted. At the same time, nuclear DNA content of all plants was measured using flow cytometry. In order to estimate chromosome number using flow cytometry, relative DNA content of plants with unknown ploidy was expressed as percent of DNA content of triploid plants. It was found that the classification based on flow cytometry fully agreed with the results obtained by chromosome counting. Thus flow cytometry is a convenient and rapid method for detection of aneuploidy in *Musa*.

Musa aneuploids are useful for gene discovery. It was already found in wheat that by inactivating genes promoting disease susceptibility, improvement of wheat for rust resistance was possible. Furthermore, mutants in general are particularly useful to link genetic to physical maps. It was thus decided to photograph all Grande Naine (AAA) mutants before reinitiating them *in vitro* and establishing a mutant repository. In addition, a mutant collection of the accession Calcutta 4 (AA), selected by the *Musa* genomics consortium as the reference material, is being developed by irradiating seeds with fast neutron or gamma rays.

Development of Cell Suspension Cultures in Rice and Plant Regeneration

Callus Induction and Maintenance

Seeds from two rice cultivars, Nipponbare and Taipei 309, were placed into MS (Murashige and Skoog 1962) basal medium and were supplemented with 2,4-D (2,4-dichlorophenoxyacetic acid, with concentrations of 0.5, 1.0, 2.0 and 3mg/l respectively), 0.5 mg/l Kinetin, 3% sucrose and solidified with 0.2% gelerite for callus induction. The cultures were then incubated in the dark at 26-28°C. Within three weeks the scutellar tissue produced two types of callus, embryogenic and non-embryogenic. Microscopic observation revealed the formation of globular structures.

Establishment and maintenance of embryogenic cell suspension cultures

The medium described by Ohira, Ojima and Fujiwara in 1973 (R2 medium) and second was described by Müller and Grafe in 1978 (AA medium).

One-and a half to two month-old established embryogenic calli (200-500mg fresh weight) was used to initiate suspension cultures in 40 ml liquid medium in a 250ml Erlenmeyer flask. The Erlenmeyer flasks were placed on a gyrotory shaker at 75 rpm. The cultures were incubated either in dark or in light at 27-28°C.

Irradiation treatment and plant regeneration

Small cell clusters were exposed to Gamma rays at dose of 100 Gy, placed in fresh liquid

medium for one week and then transferred to different regeneration media. Murashige and Skoog (1962) basic medium was used, 3% sucrose, solidified with gelerite (0.2%) and different hormonal concentrations being used for plant regeneration.

Anther Culture in Maize and Rice

In our experiments we used 8 maize hybrids from Moldova with different vegetative periods, belonging to the dental subspecies. We also used three varieties of rice Taipei 309, Nipponbare and IR-29.

To trigger anthers to go from their gametophytic stage of development to the saprophytic we used low temperature (7°C for 5, 10 and 14 days), stimulating doses of gamma radiation (10 and 15 Gy), electromagnetic field (EMF) with the following parameters (100 - 400 volts and 25 - 100 Ma).

In all cases the callus induction from anthers was higher when an electromagnetic field was used.

These preliminary studies will need to be repeated in order to confirm our results.

Services

Irradiation

There were 24 requests from 12 countries, comprised of 17 different crop species with a total of 85 varieties making a total number of 171 treatments.

Flow cytometry

There were 5 requests from 4 countries for ploidy determination using flow cytometry for 4 different crop species with a total of 155 measurements.

Mutant Repository (MMR)

Presently we have received the following mutant germplasm for their molecular characterization.

Seed propagated:

Received in 2001 from the Bangladesh Institute of Nuclear Agriculture. Some of these mutants were selected for their improvement in agronomic characteristics and others for their resistance to pest and diseases. The mutants are from the following commodities: Blackgram, Chickpea, Groundnut, Jute, Lentil, Mungbean, Mustards, Rice and Tomato.

Received in 2002 from R. Afza (Plant Breeding Unit), six salt tolerant mutants from the rice variety Bicol and two salt tolerant mutants from the salt susceptible rice variety IR29.

In vitro propagated:

Received in 2002 from N. Roux (Plant Breeding Unit), fifteen test tubes with banana plants selected for their tolerance to Juglone and three hundred banana plants (Grande Naine and Calcutta 4) with different mutations.

G. PUBLICATIONS

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Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. IAEA Training Course Series 19, Mutant germplasm characterization using molecular markers – a Manual. ISSN 1018-5518, Vienna, 2002.

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Roux, N.S., H. Strosse, A. Toloza, B. Panis and J. Dolezel, 2002. Potential of flow cytometry for monitoring genetic stability of banana embryogenic cell suspension cultures. First International Symposium on liquid systems for *in vitro* mass propagation of plants. Ås, Norway, 29 May-2 June 2002 (in press).

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

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**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. 10

Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture
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