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Joint FAO/IAEA Division of Nuclear  
Techniques in Food and Agriculture  
and FAO/IAEA Agriculture and Bio-  
technology Laboratory, Seibersdorf

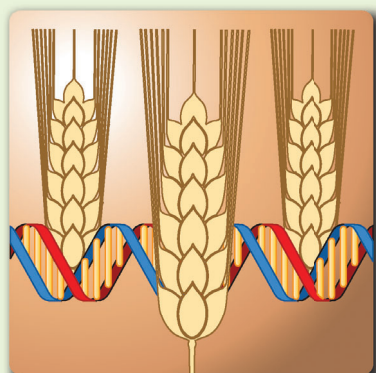
# Plant Breeding & Genetics Newsletter

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*Neglected crops: grain amaranth.*

## A. TO THE READER

The activities of the Plant Breeding and Genetics sub-Programme concentrated, during the last six months, on organization of a new Coordinated Research Programmes and numerous training courses. To establish and implement the CRP on "Effect of Mutagenic Agents on DNA Sequence in Plants" a consultants meeting was held in Vienna, July 2003 with participation of four experts: G. Caetano-Anolles and M. Dizdar (USA), M. Gale (UK) and I. Szarejko (Poland). As a result we were able to implement this CRP with the participation of 12 institutes from nine countries. Similarly, another consultant meeting was held in November to initiate a new CRP on "Identification and pyramiding of genes responsible for crop quality characters and resistance to quality affecting stresses." With the help of invited experts – G. Bryan (UK), F. Correa-Victoria (CIAT) and M. Giband (France), necessary documents have been prepared and will be presented to the IAEA authorities for approval. It is expected that the CRP will be implemented in the beginning of 2004.

Rapid development of molecular markers technology generated strong interest in identification and characterization of mutated genes. To meet this expectation numerous training courses and workshops were organized in the second half of the year, mainly related to regional Technical Cooperation projects implemented in Asia and Africa. Among them were training courses on:



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- Application of induced mutations and biotechnology for crop improvement, organized by Horticultural Crop Research and Development Institute in Peradeniya, Sri Lanka
- Selection methods for low phytic acid mutants in rice, Hangzhou, China
- Methodology for multi-location trials and selection of mutants tolerant to abiotic stresses, ICRISAT, India
- Standardization of crop breeding methods for the improvement of drought tolerance, Lusaka, Zambia
- First workshop on Improvement of plant salt tolerance for sustainable food and feed production in saline lands, Bangkok, Thailand.

It was also possible to organize the 3<sup>rd</sup> Interregional Training Course on Mutant Germplasm Characterization using Molecular Markers. Twenty participants from all regions of the world participated in this event organized as usually in Seibersdorf, Austria.

The last two years we have been very much involved in collecting and evaluating data related to the economic impact of mutant varieties released in numerous countries of the world. The related publication has been finally prepared and approved for publication in scientific journal 'Euphytica' under the title 'Global impact of mutation-derived varieties.' We hope that this publication will help our counterparts in successful presentation of project proposals dealing with mutation assisted plant breeding.

For many years, the Joint FAO/IAEA Division has supported and coordinated research that focuses on development of more efficient doubled haploid production methods and their applications in breeding of new varieties and basic research. The production of doubled haploids has become a necessary tool in advanced plant breeding programmes for many crop species. Requests from researches and trainees for systematized protocols dealing with the production of doubled haploids in various species were instrumental in initiating work on the Manual in 2001. This idea was strongly supported by scientists working under the EU large scale program 'COST 851' on 'Gametic cells and molecular breeding for crop improvement'. The book 'Doubled haploid production in crop plants. A Manual', edited by M. Maluszynski, K. Kasha, B. Forster and I. Szarejko has been published by Kluwer Academic Publishers and is already being distributed to our counterparts in developing countries.

The activities and results achieved by the Plant Breeding and Genetics Sub-programme has been recognized by the authorities of the Agency. The following staff members from our sub-programme received from the Director General, M. ElBaradei, the Distinguished Service Award at a ceremony that took place in the Staff Assembly on 26 November 2003:

'Miroslaw Maluszynski and Rownak Afza - for their outstanding work in plant mutation, a technology used as part of the world effort to alleviate hunger, poverty and food insecurity by producing crop varieties with higher yields and better resistance to drought, salinity and pests. The use of this nuclear technique has brought very great economic and social benefits to Member States of the Agency and FAO. Mr. Maluszynski has been the driving force and vision behind the Agency's programme of co-ordinated research, technical co-operation and training activities. His scientific and technical expertise and his leadership have helped the Agency gain the highest trust from national and international experts and decision makers in this field. Ms. Afza has provided a high level of technical support for plant breeding research as well as for training courses by providing guidance in the use of plant tissue culture and mutation induction techniques as well as the more recently introduced molecular techniques. She has also played a significant role in the development of salinity-tolerant lines of rice through mutation induction and subsequent screening'.

The following are changes within the team of the Sub-programme:

Dr. Brian Forster (UK) joined the Plant Breeding Unit in August 2003 on a temporary assignment.

After 21 years of service in the Plant Breeding and Genetics Section, including almost 13 years as Section Head, I will retire at the end of December 2003. I would like to thank all counterparts from Coordinated Research Projects and Technical Cooperation Projects who were working with me on their implementation, for their enthusiastic and stimulating work on application of induced mutations for crop improvement in their countries. I would especially like to thank my colleagues from the Plant Breeding and Genetics Section at the Vienna International Centre and the Plant Breeding Unit at Seibersdorf for their high motivation and very efficient work in implementation of our Sub-programme. I will continue my work on identification and characterization of mutated genes at the Department of Genetics, University of Silesia, Katowice, Poland.

*Mirosław Maluszynski*

## B. STAFF

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

		<b>E-mail</b>	<b>Phone (431) 2600</b>
	+		
James D. Dargie	Director	<a href="mailto:j.dargie@iaea.org">j.dargie@iaea.org</a>	21610

### **Plant Breeding and Genetics Section**

Mirosław Maluszynski	Head of Section	<a href="mailto:m.maluszynski@iaea.org">m.maluszynski@iaea.org</a>	21619
Mohan Jain	Technical Officer	<a href="mailto:s.m.jain@iaea.org">s.m.jain@iaea.org</a>	21623
Stephan Nielen	Technical Officer	<a href="mailto:s.nielen@iaea.org">s.nielen@iaea.org</a>	21618
Pierre Lagoda	Technical Officer	<a href="mailto:p.lagoda@iaea.org">p.lagoda@iaea.org</a>	21626
Qing Yao Shu	Technical Officer	<a href="mailto:q.y.shu@iaea.org">q.y.shu@iaea.org</a>	21617
Kathleen Weindl	Secretary	<a href="mailto:k.weindl@iaea.org">k.weindl@iaea.org</a>	21620
Katayon Allaf	Secretary	<a href="mailto:k.allaf@iaea.org">k.allaf@iaea.org</a>	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	<a href="mailto:c.rigney@iaea.org">c.rigney@iaea.org</a>	28267
Chikelu Mba	Plant Biotechnologist	<a href="mailto:c.mba@iaea.org">c.mba@iaea.org</a>	28268
Rownak Afza	Senior Technician	<a href="mailto:r.afza@iaea.org">r.afza@iaea.org</a>	28208
Andrea Kodym	Technician	<a href="mailto:a.kodym@iaea.org">a.kodym@iaea.org</a>	28213
Mirta Matijevic	Technician	<a href="mailto:m.matijevic@iaea.org">m.matijevic@iaea.org</a>	28317
Guenter Berthold	Technician	<a href="mailto:g.berthold@iaea.org">g.berthold@iaea.org</a>	28414
Andreas Draganitsch	Technician	<a href="mailto:a.draganitsch@iaea.org">a.draganitsch@iaea.org</a>	28414
Franz Zwiletisch	Technician	<a href="mailto:f.zwiletisch@iaea.org">f.zwiletisch@iaea.org</a>	28414
Elisabeth Swoboda	Secretary	<a href="mailto:e.swoboda@iaea.org">e.swoboda@iaea.org</a>	28281

## C. FORTHCOMING EVENTS

<b>Research Coordination Meeting on the “Effects of Mutagenic Agents on the DNA Sequence in Plants” Vienna, Austria, 1-5 March 2004</b>
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Technical Officer: P. Lagoda

The first RCM for this CRP will be held at the Vienna International Centre (Vienna, Austria) with 13 participants from Bulgaria, China, Columbia, India, Korea, The Philippines, Poland, South Africa, United Kingdom and USA.

Starting in the 1970's, the International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization (FAO) of the United Nations sponsored extensive research on mutation induction to enhance breeding of food and industrial crops that resulted in the introduction of new varieties of rice, wheat, barley, apples, citrus, sugar cane, banana, and others (more than 2300 officially released new varieties in the FAO/IAEA Mutant Varieties Database). The application of mutation induction to crop breeding has a tremendous economic impact on agriculture and food production that is currently valued in billions of dollars and millions of cultivated hectares.

The technology proved particularly beneficial for the manipulation of asexually propagated crops. In vegetatively propagated crops common to horticulture, commercial breeders have developed efficient protocols to generate new forms of variation in plants such as Bermuda grass, Japanese pear, sweet potato, peppermint, potato, sweet cherry, carnation, chrysanthemum, rose, alstroemeria, poinsettia and African violet. By changing only one or two traits, clones with proven consumer acceptance and known production requirements can be introduced to markets with minimum expense.

With the advent of modern techniques of molecular biology and the potential to move specific genes from one organism to another in the 1980's, research on mutation induction for breeding of crops became largely ignored for crops reproduced as seed. Still, mutation induction coupled to selection remains the most "clean" and inexpensive way to create varieties by changing single characters without touching the general phenotype. Controversies focusing around the potential or possible hazards or risks of spreading and consuming genetically modified organisms (GMOs) are pushing more and more countries to take action to limit the consumption of transgenic crop varieties. The market implications due to potential concerns over transgenic crops are incalculably lowering the overall benefits of introducing GM crops. On the contrary, the same countries have not taken action against crops developed through mutation induction enhanced breeding. It comes therefore as no surprise, that recently commercial companies are very urgently showing their renewed interest in mutation induction techniques.

Mutation induction to enhance breeding of plants involves exposure of plant parts to treatment with mutagens (chemical or physical) and selection of desirable changes in treated plants. Where breeders have been using mutation induction to broaden the genetic base of germplasm, and use the mutant lines directly as new varieties or as sources of new variation in crossbreeding programmes, the precise nature of the mutations induced has generally not been a concern. Intuitively a conservative level of small base pair rearrangement and deletion is probably ideal. Nowadays, the applications of mutation techniques have expanded beyond

direct use in breeding to novel applications in gene discovery and reverse genetics. This explains the "renaissance" of mutation induction techniques in fundamental science and biotech firms, a renewed interest in induced mutation in novel reverse genetics and gene discovery technologies. But in order to attain optimum efficiency in these new high-throughput applications we really do have to understand the nature of induced mutations.

However, while the agronomic potential of induced mutations is well understood, the precise effects of different mutagenic agents on the DNA sequence in plants have never been described.

Mutagenic agents can be classified into three categories: physical (e.g. gamma rays), chemical (e.g. ethyl methane sulphonate) and biological (e.g. transposons). At present, limited data are available on the scope of genetic effects at the molecular level in plants and on the specificity of these different categories of agents. Effects include DNA damage, base pair changes, small insertions and deletions (indels), and chromosomal rearrangements. Moreover, much less is known about how mutation and epigenetic processes, such as methylation, activation of retroelements, and high-order DNA structure, interact. Currently, we also lack knowledge on how the different mutation events are physically distributed along the genome, and how mutation induction affects spontaneous mutation rates. We need to explore how different mutagens alter frequencies, rates and patterns of the different types of mutation events.

Until recently, traditional mutation induction for crop improvement has been restricted to broadening the genetic base of germplasm. This was met without explicit knowledge of the molecular bases underlying the mutation process. Nowadays, mutation techniques have expanded beyond direct use in breeding because of developments in applied molecular genetics and genomics. In order to develop new varieties, gene and gene function discovery require molecular tools that reflect insertional mutation activity and the construction of mutation libraries.

This demands increased activity of known transposable elements (e.g. retrotransposons) and the generation of moderate deletions (about 10-100 kb in length) at very high rate, respectively. This is believed to be preferentially accomplished by various mutagenic treatments. Alternatively, we need to discover new alleles with innovative methods. Reverse genetic approaches such as targeting induced local lesions in genomes (TILLING) and other techniques can be used for this purpose. In these applications, the generation of gene knockouts demands a relatively large pool of base pair substitutions and/or small deletions spanning not more than a few base pairs.

Clearly, different kinds and levels of mutations are required for these different applications. In the past, this could not be achieved because of lack of technological tools and resources. Mutation frequencies and rates at the molecular level were difficult to measure and mutation types and patterns could not be identified without comprehensive knowledge of sequences or high-throughput profiling approaches of entire genomes. Current advances in genomics coupled with rapidly growing sequence databases, as well as advances in DNA analysis and understanding of DNA damage and repair processes will, for the first time, enable an extensive study and understanding of the effects of the mutation induction process at the molecular level for plant breeding purposes.

At present 12 participants from nine countries participate in this project.



**National Training Workshop on “Hardening and Commercialization of In-vitro Plants, Disease Indexing, and Greenhouse Technology in Date Palm” (RAF/5/049), Sfax, Tunisia, 1-5 December 2003**

Technical Officer: S.M. Jain

Bayoud disease has caused heavy damage to date palm production in Saharan and Sub-Saharan regions of Africa - caused by *Fusarium oxysporum* f.sp. *albedinis*. So far, the selection of mutant date palm plants against this fungus has been done against the toxin, isolated from *Fusarium oxysporum* f.sp. *albedinis*. Large populations of mutated plants are needed for the selection of disease resistant plants under greenhouse conditions. Only selected mutant plants against fungal toxin are transferred in the field for final evaluation. For large-scale mutant plant multiplication, initially selected mutant plants are micropropagated to undergo further tests in the greenhouse with fungal spores. The selected mutant plants are transferred to the field for final evaluation for disease resistance. During large-scale *in vitro* plant multiplication, disease indexing is important for preventing large-scale contamination and to find the solution for preventing it, e.g. addition of antibiotic in the culture medium. Furthermore, the management for handling large populations of *in vitro* hardened plants is very important in the greenhouse, more or less on the commercial level. The maintenance of greenhouse environment, e.g. humidity, temperature, light etc., is essential for hardening, plant growth and maintenance, and selection against fungal spores.

The main purpose of this course is to update knowledge in mutation induction, greenhouse technology, disease indexing, *in vitro* mutant plant multiplication and hardening plants, molecular markers, and field transfer of *in vitro* plants. There will be a total of 10 participants from Algeria, Morocco, and Tunisia.

**National Training Workshop on “Biotechnology, Induced Mutations, and Molecular Markers for Genetic Improvement of Crop Plants” (YEM/5/003), Dhamar, Yemen, 20-24 December 2003**

Technical Officer: S.M. Jain

In Yemen, agriculture is an important economic sector, engaging over 75% of the country's population, and contributing greatly to the national exchequer. Farmers grow different types crops including vegetables, fruits, cereals, legumes and industrial cash crops. Water shortage is one of the major problems facing the sustainable agriculture production, and also cost of production becomes enormously high. Even though agricultural production has gone up, the cost of production per unit area is very high. Therefore, it has become essential to reduce water consumption in bringing down the cost of production. This could be done to finding new ways to irrigate and develop new drought tolerant cultivars with high yield potential by induced mutations and biotechnology. In Yemen, there is hardly any work done on tissue culture and molecular markers. Somatic embryogenic cell suspension cultures would be ideal for radiosensitive studies as well as plant regeneration from irradiated material. Molecular markers such as AFLPs would assist in mutant characterization of genetic biodiversity.

This is the first time this type of training course will be organized in Yemen. Its main purpose is to update current knowledge in crop breeding, induced mutations, tissue culture and molecular markers. Fifteen participants are expected.

## D. PAST EVENTS

<b>Consultants Meeting on “Effect of Mutagenic Agents on DNA Sequence in Plants” Vienna, Austria, 7-9 July 2003</b>
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Technical Officer: P. Lagoda

The objective of the above meeting was to formulate a proposal for a new CRP on the effects of mutagenic agents on the DNA sequence in plants. The following scientists were invited to participate:

- \* Mr. Gustavo Caetano-Anolles (University of Illinois, Department of Crop Sciences, USA)
- \* Mr. Miral Dizdar (National Institute of Standards & Technology, USA)
- \* Mr. Michael Gale (Comparative Genetics Unit, John Innes Centre, UK)
- \* Ms. Iwona Szarejko (Silesian University, Department of Genetics, Poland)

Mr. G. Caetano-Anollés is a worldwide-accepted crop expert on evolution of macromolecular structure, evolution of transcript networks and evolutionary role of spontaneous mutations among others.

Mr. M. Dizdar 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 Mr. M. Gale, who contributed his outstanding expertise in co-linearity and microsynteny in crops, Ms. I. Szarejko adding her exceptional knowledge, experience and know-how in mutation induction for breeding and the collegial input of the sub-programme staff assisting at the meeting, the consultants team was very efficient in fulfilling the challenging task of planning a new CRP on the effects of mutagenic agents on the DNA sequence in plants.

Physical, chemical and biological mutagenic agents cause genes to mutate at rates above the spontaneous baseline, thus producing a range of novel traits and broadening the genetic diversity of plants. The use of induced mutants in breeding has had a profound impact on world agriculture and more than 2300 new crop varieties, all carrying novel induced variation, have now been officially registered (IAEA Mutant Varieties Database). This has all been achieved largely in the absence of knowledge of the precise changes induced at the DNA level. Indeed there still is very little understanding of the nature of the mutations induced by different mutagens. With the advent of molecular genetics and genomics, induced mutations are finding new applications in modern plant breeding. Reverse genetics and deletion library methodologies capable of discovering new genes and their modes of action are often underpinned by variation induced by both physical and chemical mutagens. However, the efficiency of these new methods will be enhanced only when the type, frequency and distribution of mutations in a range of crop species can be predicted, and ideally directed.

Today, with the range of technologies available to the scientific community to assay variation in DNA sequence and the availability of a vast amount of crop plant DNA sequence, including



the complete sequences of both *Arabidopsis* and rice, these questions can now be approached. This Coordinated Research Project sets out to define the type, frequency and patterns of molecular changes induced by the range of physical and chemical mutagens in a range of crop

<b>Training Course on “Application of Induced Mutations and Biotechnology for Crop Improvement” Ganoruwa &amp; Peradeniya, Sri Lanka, 5-16 August 2003</b>
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Technical Officer: M. Maluszynski

The IAEA/RCA Regional Training Course on Application of Induced Mutations and Biotechnology for Crop Improvement has been organized under the Agency project RAS/5/040 and hosted by Horticultural Crop Research and Development Institute (HORDI). Sixteen participants from nine countries (China, India, Indonesia, Korea, R. of, Pakistan, The Philippines, Thailand, Viet Nam and Sri Lanka) attended the Training Course.

The importance of induced mutations for crop breeding in Sri Lanka was underlined by Mr. R. Hewamanna, the Chairman of the Sri Lanka Atomic Energy Authority who made an interesting speech on nuclear applications in agriculture and especially in plant breeding. The course was divided into two parts, one week each: mutation techniques and production of doubled haploid plants (lecturers: I. Szarejko and M. Maluszynski), and application of molecular markers for characterization of mutated germplasm (lecturers: U. Lavi and C. Mba). Additionally, local speakers presented the status of plant breeding and applications of mutation techniques and molecular markers in Sri Lanka.

All lectures and practical exercises in the first week of the training course were implemented as planned. This was mainly possible due to excellent and timely organization of the training course by local staff, especially by Ms. Ranjani Peiris – the course director. Numerous scientists from HORDI participated voluntarily in lectures and practices expressing strong interest in applications of induced mutations and double haploids in breeding local crops. All radiation sensitivity tests developed during the training were carefully analysed by participants. It was possible to demonstrate the most important approaches leading to evaluation of critical dose of radiation and chemical mutagens even in these very simple conditions using normal, basic equipment of regular plant breeding stations.

As in other countries, there is an observed renaissance in the use of mutation techniques in plant breeding. Officially released mutant varieties of rice, tomato, sesame, bean and groundnut are grown in Sri Lanka. Some mutant rice varieties greatly contributed to production of this crop in the country. Recently, a new rice variety developed after gamma rays treatment has been released with the name PD-3. This variety has such desired characters as earliness, limited shattering, and cold tolerance. The variety has been very well accepted by farmers. Another rice mutant variety, ‘MI-273’ released in 1972 is still widely cultivated. Currently, HORDI is developing an intensive programme with improvement of horticultural crops using induced mutations.

The second week of the training course was also very successful. The lectures and exercises on applications of molecular markers in plant breeding with the use of induced mutations, presented by experts, brought to participants not only practical knowledge on basic methods in molecular biology but also on possible applications of molecular markers in speeding up selection of desired mutated genotypes.

**Regional Training Course on “Selection Methods for Low Phytic Acid Mutants in Rice”  
Hangzhou, China, 29 September – 3 October 2003**

Technical Officer: P. Lagoda

This Regional Training Course was organized at the Institute of Nuclear Agricultural Science (INAS), Zhejiang University (ZJU) in Hangzhou, China (Training Course Director Mr. Dianxing WU) with the participation of Mr. Victor Raboy (ARS/USDA).

The Training Course was open to rice geneticists and breeders from IAEA Member States in Asia and Pacific Region taking part in RAS/7/014 – Component II Project on “Induced mutations for rice with low phytic acid content”, who have been actively involved in application of mutation techniques for rice improvement.

High yielding rice varieties with low phytic acid are very important to fortify micronutrient malnutrition in children, women and elders. To this end, breeders need a method to select low phytic mutants from large populations in a short time. This can be done by evaluation of a total concentration of organic, phytic acid phosphorous and inorganic phosphorous (Pi). By knowing the Pi content in seed, one can expect/indicate the phytic acid content. High Pi means low phytic acid. Application of this method in low phytic mutant selection will be of great importance in solving a country’s micro-nutrient problem.

Through this training course, the 11 participants from China, Indonesia, Pakistan, Thailand and Viet Nam were provided with a working knowledge on methodologies in induction and selection of low phytic mutants in rice. The practical exercises gave them hands-on experience in applying the taught analytical techniques on their own mutant material.

The training course was implemented through lectures and laboratory exercises. The theory lectures covered methodologies on induction of low phytic acid mutants; basic knowledge on phytic acid content in seeds and its physiological function; and results of induced mutation projects for development of low phytic mutants in other crop species. The ecological aspects of introduction of low phytic crops for large-scale production and reduction of pollution were highlighted. The laboratory exercises concentrated on rapid and comparatively low-cost although high-throughput screening methods for low phytic rice mutants.

In round-table discussions, the different needs the individual participant's laboratory infrastructure to be upgraded (if necessary) in order to implement the learned techniques were assessed and discussed.

**National Training Workshop on “Mutation and Molecular Breeding in Crop Improvement” (INS/5/030 and INS/5/031), Jakarta, Indonesia, 29 September – 3 October 2003**

Technical Officer: S.M. Jain

This National Training Workshop was organized together with both INT/5/030 and INT/5/031 TC projects. Project INT/5/030 deals with an increase in overall crop production by integrating newly developed drought tolerant crops (e.g. sorghum in the existing cropping system). Project INT/5/031 is concerned with the development of commercially viable induced mutant varieties of horticultural crops such as cut flowers, garlic and citrus. A total of 15 participants attended this course. The syllabus of this course included subjects such as molecular and conventional breeding, and tissue culture and induced mutations in both horticultural and food crops. In molecular biology, recently developed technologies were discussed (e.g. QTL analysis, marker-assisted selection, linkage mapping). A practical demonstration was given on DNA isolation, PCR analysis and tissue culture. This course would benefit scientists interested in the use of molecular and mutation tools in breeding programs.

**Reporting Workshop on “Enhancement of Genetic Diversity in Food, Pulses & Oil Crop Establishment of Mutant Germplasm Network” Manila, The Philippines, 27-31 October 2003**

Technical Officer: Q.Y. Shu

Seventeen plant breeders from nine countries (China, India, Indonesia, Korea, Pakistan, The Philippines, Sri Lanka, Thailand and Viet Nam) participated in the meeting. A technical and field visit was made to International Rice Research Institute (IRRI) and IPB/UPLB. Three groups of RMMTs (e.g. food crops, legumes, sesame) were initiated and are now being implemented in the region. Some interesting data have already been observed. For instance, the mungbean RMMT, in which 19 mutant and parent varieties were tested in 12 locations of seven countries, has completed the first run of trial in most locations, many mungbean entries from other countries showed high yield potential but all are susceptible to a virus disease in Pakistan. Substantial progress has been made in enhancement of genetic diversity through induced mutation. Four new mutant varieties have been registered during the past year or are pending this year in the participating institutions.

It was agreed that China will host the 3<sup>rd</sup> IAEA/RCA RTC in Beijing and Korea will host the 2<sup>nd</sup> Report Meeting in Suwon.

<b>3<sup>rd</sup> Interregional Training Course on “Mutant Germplasm Characterization using Molecular Markers” Seibersdorf, Austria, 6-31 October 2003</b>
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Technical Officer: S. Nielen

After successful implementation of the first two courses and enriched with the experiences gained and recommendations received from previous participants the 3<sup>rd</sup> Interregional Training Course on Mutant Germplasm Characterization using Molecular Markers was held at the Agency's Laboratories in Seibersdorf. From more than eighty applications twenty scientists from Chile, China, Costa Rica, Cuba; Egypt, India, Iran, Kazakhstan, Kenya, Kuwait, Malaysia, Morocco, Peru, Philippines, Poland, Sri Lanka, Sudan, Tunisia, Uruguay, and Viet Nam were selected to participate in the four week course. Through lectures, an introduction was provided on genomics, genomes and genes, as well as into the principle of various molecular markers and current applications. The practical exercises covered, besides basic molecular techniques, RFLP, AFLP, SSRs, ISSRs and retrotransposon based marker technologies. Additionally, physical mapping strategies such as localization of retroelements on *Musa* BAC clones and localization of repetitive elements on chromosomes using fluorescence in situ hybridization (FISH) were part of the practical programme. Participants were encouraged to bring to the course their own plant material, seeds or extracted DNA in order to apply, during the last week, the techniques learned during the course. All participants appreciated this kind of independent project work and most of them could take valuable results home. For the organizers of the course the participant's projects also served as an indicator for the success of the training. The following scientists participated in teaching different aspects of molecular markers:

Mr. Uri Lavi, ARO, Volcani Centre, ISRAEL  
Mr. Günter Kahl, Johann Wolfgang Goethe-University, Frankfurt, GERMANY  
Mr. J.S. (Pat) Heslop-Harrison, University of Leicester, UK  
Mr. Chikelu Mba, Joint FAO/IAEA Division, Vienna, AUSTRIA  
Mr. Brian P. Forster, Joint FAO/IAEA Division, Vienna, AUSTRIA  
Mr. Pierre J.L. Lagoda, Joint FAO/IAEA Division, Vienna, AUSTRIA  
Mr. Stephan Nielen, Joint FAO/IAEA Division, Vienna, AUSTRIA

Again, most of the laboratory exercises were based on the manual ‘Mutant Germplasm Characterization using Molecular Markers’, published under the Agency's Training Course Series. A hard copy will be distributed, free of charge, to interested scientists from FAO and IAEA Member States. Requests for the manual should be sent 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](mailto: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. A new chapter on evaluation of molecular data is in preparation.

**Consultants Meeting on “Identification and Pyramiding of Genes Responsible for Crop Quality Characters and Resistance to Quality Affecting Stresses” Vienna, Austria, 3-5 November 2003**

Technical Officer: Q.Y. Shu

A Consultants Meeting was held in Vienna with the aim to develop a new Coordinated Research Project (CRP). Dr. Glenn Bryan of the Scottish Crop Research Institute, United Kingdom, Dr. Fernando Correa-Victoria of Centro Internacional de Agricultura Tropical (CIAT), Colombia, and Dr. Marc Giband of CIRAD-CA were invited to the meeting, which was also attended by Technical Officers of the Plant Breeding and Genetics Section and the Plant Breeding Unit.

Crop quality improvement is gaining unprecedented importance in both developed and developing countries. The improvement of quality characters in crop plants has great potential to alleviate problems caused by poverty and malnutrition through both direct (food quality and quantity) and indirect effects that affect farmers' social and economic status (income stability, etc). During the meeting, various aspects of 'crop quality' and biotic and abiotic stresses having a negative impact on product quality were defined, the advent of molecular marker techniques were reviewed; and the strategies on pyramiding genes contributing to quality characters by marker assisted selection (MAS) were formulated.

The objectives of this CRP focus on the development and transfer between participants of methodologies and technologies for the identification and tagging of genes contributing to important crop quality characters and their pyramiding to generate improved breeding material using molecular marker assisted selection.

**Regional (RCA) Training Course on “Methodology for Multi-location Trials and Selection of Mutants Tolerant to Abiotic Stresses” (ICRISAT), India, 10-14 November 2003**

Technical Officer: Q.Y. Shu

The Training Course was hosted by the International Crop Research Institute for Semi-Arid and Tropics (ICRISAT), and attracted 12 participants from China, India, Indonesia, Republic of Korea, the Philippines, Sri Lanka, Thailand and Viet Nam. Three scientists from the Bhabha Atomic Research Center, Mumbai, also participated. R Serraj of ICRISAT acted as the course director. Overall, the course addressed three major objectives:

- Enhance the capacity of scientists to design and carry out multi-location trials, and to analyze and evaluate the data.
- Familiarize the participants with screening tools and methods for the selection of crop varieties resistant to abiotic stresses.
- Perform hands-on practical training in experimental design, biometrics, analysis of variance and G-by-E analysis.

**1st Co-ordination Meeting on “Improvement of Plant Salt Tolerance for Sustainable Food and Feed Production in Saline Lands” (INT/5/147), Bangkok, Thailand, 10-14 November 2003**

Technical Officer: S.M. Jain

This meeting was jointly organized by the Land Development Department and Office of Atoms for Peace, Bangkok, Thailand and the IAEA. Altogether 10 scientists from China, Costa Rica, Cuba, Guatemala, Iran, Morocco, Pakistan, Thailand, Tunisia, and Viet Nam participated in the meeting. Each participant presented a country report highlighting the current status of research, available facilities and infrastructure. A hard copy of proceedings and lectures presented at the meeting were distributed to each participant on the last day of the meeting. The final report of the meeting was prepared, which contained work plan, current status of the technology, requirements of each participating country, and general recommendations, which are as follows:

- Establishment of a gene bank and database of all salt tolerant mutant lines in participating countries obtained in this project for breeding purposes.
- Exchange of information related to developed techniques, markers, salinity, screening procedures, etc.
- Exchange of mutant lines/varieties among participating countries in diverse environments is recommended.
- Invitation to farmers to participate in selection of segregating populations in easily approachable saline areas.
- Preparation of a technological package (improved soil, water and nutrient management) for optimum production with developed salt tolerant mutants.
- Publication of the results in peer national and international journals.
- Implementation of detailed activities, in 2005.
- To provide a project progress report to IAEA twice a year.
- To characterize the generated germplasm by using molecular techniques.

In 2004, two training courses will be organized: a) Seed irradiation and screening techniques for salt tolerance, in Pakistan; and b) Application of Induced Mutations and Biotechnology for Crop Salt Tolerance Improvement, in China.

**Regional (AFRA) Workshop on “Standardization 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 was a follow up activity of previous training events related to development of drought tolerant germplasm, which is one of the major components of the regional AFRA project RAF/5/050 ‘Increasing Production of Nutritious Food through Mutation Breeding and Biotechnology’. The Workshop was hosted by the University of Zambia (UNZA) and the National Institute for Scientific and Industrial Research (NISIR) and attended by 19 participants from Algeria, Ethiopia, Kenya, Libyan Arab Jamahiriya, Madagascar, Mauritius, Sierra Leone, South Africa, Sudan, Tunisia, United Republic of Tanzania and Zambia.

The workshop started with presentations by all participants describing the progress and results obtained so far under component one of the project (Germplasm development and evaluation of major food crops for drought prone areas in Africa), and the work plans for future project activities. Significant progress was noted in the implementation of the projects of all countries participating from the beginning. It was of particular interest that the first pre-release of a finger millet mutant in Zambia was achieved. The mutant is characterized by higher yield due mainly to changes in plant architecture (branched and open fingers). Lectures on drought screening and mutation induction/biotechnology were provided by the invited resource person, Mr. S. Gupta, India, and the Technical Officer.

Main objective of the workshop was the preparation of guidelines for a manual on drought screening techniques. Three working groups were formed focusing on legumes, cereals, and root and tuber crops. Experience and know-how in drought screening techniques were exchanged in the groups and each group prepared a document on the traits of interest for selection, applicable techniques for screening including detailed protocols, and advantages and disadvantages of the particular techniques. As a conclusion those traits were identified that are considered by the group as being most important as a measurement to determine tolerance to drought stress conditions. The drafted documents after editing will serve as the basis for compiling the envisaged manual. Further experience and results within the individual projects will be monitored and evaluated in order to enable estimations on how the selected traits are correlated to yield.

<b>Regional Workshop on “Monitoring of Food Fortification Programmes Using Nuclear Techniques” Bangkok, Thailand, 1-5 December, 2003</b>
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Technical Officer: P. Lagoda

The purpose of the workshop is to report on the selection efficiency for low phytic acid (LPA) mutants in rice and lessons learned. Further, contingencies and limitations of the different participating institutes from China, Indonesia, Pakistan, Thailand and Viet Nam, will be discussed.

The Agency’s objective is to promote the use of nuclear techniques for development purposes. Nutrition is a top priority within the health sector, in particular in poorer countries, and a global development co-operation priority, as improved nutrition not only fight against malnutrition but also to alleviate poverty. Bio-fortification of staple foods is the most affordable and sustainable intervention strategy to control micronutrient deficiencies. Food bio-fortification has the greatest potential to improve the nutritional status of a large number of people.

Plant breeding can provide a longterm, sustainable approach to reducing one of the most significant public health problems in Asia, mineral micronutrient (iron and zinc) deficiency. With plant breeding it is possible to increase the density of iron and zinc in rice foods, or decrease the amount of the major anti-nutrient, phytic acid. These two approaches are complementary. Once consumed in foods, phytic acid binds iron and zinc in the intestinal tract, and the resulting salts are not well digested. Instead these salts are excreted. There is one important difference in the impact of phytic acid on iron versus zinc. The phytic acid in a meal negatively impacts the retention of iron in that meal. However, the phytic acid in a meal negatively impacts retention of both the zinc in the meal and zinc in endogenous pools in the body. Thus dietary phytic acid contributes to both iron and zinc depletion and deficiency.



Therefore it makes excellent sense to use induced mutations to reduce seed phytic acid in combination with genetic approaches to increasing seed minerals.

A simple and straightforward mutation approach to reducing seed phytic acid has been well documented. Research first conducted by the United States Department of Agriculture's Agricultural Research Service (USDA-ARS), and continued by many private sector breeding and biotechnology companies, has shown that *low phytic acid (lpa)* or "Low Phytate" mutations can be readily isolated in a number of important species. To date such mutations have been isolated in maize, barley, wheat, rice and soybeans. These mutations cause reductions in phytic acid phosphorus ranging from 50% to greater than 95%. The most progress has been made with maize and barley. The reduction in seed phytic acid P is accompanied by an equivalent (in terms of phosphorus) increase in seed inorganic P, so that the sum of phytic acid P and inorganic P remains relatively unchanged, as compared with normal rice seeds.

The reporting workshop is open to the project counterparts from the five participating countries, actively involved in rice breeding and scientific work on bio-fortification either in laboratory or "field" implementation.

#### **E. STATUS OF EXISTING CO-ORDINATED RESEARCH PROJECTS**

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

Technical Officer: Q.Y. Shu

This CRP was initiated in 2000, with the overall objective of assisting Member States to apply mutation techniques and related biotechnology to generate and utilize 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 final RCM is planned in Turkey in the fall of 2004.

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

Technical Officer: P. Lagoda

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-utilized 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. Tentatively, the last RCM is to be held in September 2004, in Portugal.

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

Technical Officer: S.M. Jain

Radiation induced mutations have been quite effective in producing useful mutants, resulting in great economic gains. In fruit crops, there has been little work done in mutagenesis. There has been an excellent interaction between developed and developing countries in this CRP, especially in technology and knowledge transfer. This project has made good progress in utilizing various strategies in order to recover mutants that address 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.

The tested crops were: citrus, mango, jujube, guava, cashew, avocado, papaya, litchi, annona, pitanga, carambola and jaboticaba; and mutants are being evaluated in the field. For example, in papaya M2 field evaluation, 1000 trees were evaluated for traits such as plant vigour, fruiting character, yield, fruit quality, sex segregation and resistance to Malformed top disease (MTD). Total soluble solids (TSS) increased as high as 16.5% as compared to control 13-15%. Flesh colour of fruits was changed from orange-red to yellow type. Generally, Eksotika papayas segregate 2 hermaphrodite:1 female in normal population. In M2 population, a small number of male trees appeared as a result of hermaphrodite MH gene mutated to male gene M. Among irradiated tree population, several trees showed MTD resistant ratings between 0-1 as compared to control 4. There is an excellent chance of getting MTD resistant mutant lines.

The 3<sup>rd</sup> and final RCM will be held in South Africa during the 1st week of October 2004.

## **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 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.

During the first year of project implementation significant progress has been made with regard to the generation of mutated plant material in rapeseed, tomato, and cotton. Chromosome and chromosome arm specific markers that will be useful for physical mapping of quality related genes have been localized on chromosomes of *Chenopodium quinoa* and Brassica species using fluorescent *in situ* hybridisation (FISH). The karyotypes of different Capsicum species have been established using molecular cytogenetics. Good progress has been made towards generating probes that will be used for development of physical maps in banana, aimed at localization of quality related genes. As well underway is the construction of a BAC library for Papaver.

## Effect of Mutagenic Agents on DNA Sequence in Plants

Technical Officer: P. Lagoda

This CRP started in October 2003 with 13 participants from Bulgaria, China, Colombia, India, Korea, The Philippines, Poland, South Africa, United Kingdom and USA. The main objective of this project is to exploit new developments in DNA analysis and genomics to define types, frequencies, rates and patterns of mutation induced by the different mutagens. This will generate a knowledge base that will guide and assist future users of these technologies for mutation induction-related applications. The CRP will focus on physical mutagens such as gamma radiation, fast neutron, and X-rays. Selected chemical mutagens will be used to compare the relative efficiency of both types of mutagenic agents. The effect of these mutagens will be evaluated on genetically homogeneous seed and vegetatively propagated plant material. Specific objectives include:

1. Determining total levels of DNA damage at the M1 level (treated seed, pre- and post-germination assays).
2. Determining types, frequencies, rates and patterns of mutations in M2 generations, in:  
(a) the whole genome, and  
(b) targeted sequences.
3. Preparation of protocols and guidelines for the use of particular mutagens for specific purposes in crop improvement and genomics.
4. Determining the chemical and molecular basis for differential radiation-sensitivity in closely related plant varieties.
5. Determining the baseline spontaneous rate using a select plant system, as an inherent indicator of genotype mutagenicity.

The first RCM will be held in the first week of March 2004, in Vienna (Austria).

## F. 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	S. Nielen
CPR/5/013	Induced mutations to improve rice quality	Q.Y. Shu
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 <i>in vitro</i> culture	S.M. Jain
JOR/5/008	Establishment of <i>in vitro</i> mutagenesis laboratory	S. Nielen
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	<i>In vitro</i> 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	S. Nielen
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
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 XX1a)	S. Nielen
ROK/5/033	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 <i>in vitro</i> mutation techniques	S. Nielen

## **G. ACTIVITIES AT THE PLANT BREEDING UNIT, SEIBERSDORF**

### **Molecular characterization of *Musa* putative mutant germplasm**

There is an on-going effort at PBU to develop diagnostic markers for valuable mutations and apply them in high throughput genotyping systems of plants that are of importance to member states. We have developed a population of induced mutants in banana through irradiation of meristematic regions. After tissue culture and micropropagation the population was screened for juglone (SIGMA) tolerance, a synthetic toxin used as a surrogate in controlled environment assays for tolerance to banana leaf spot or black sigatoka disease, caused by *Mycosphaerella*. This is a major production constraint and can reduce yield by up to 50%. Promising juglone resistant mutants have been identified in glasshouse tests at PBU. Also, earlier assays of these mutants using flow cytometry seemed to indicate a whole chromosomal deletion in one of the mutants. We are therefore using molecular markers to screen these mutants and the parental line with a view to developing molecular tags for the resistance and possibly also confirming the deletion. Several molecular markers including Simple Sequence Repeats, Random Amplified Polymorphic DNAs and Amplified Fragment Length Polymorphisms are being used in these assays.

Preliminary results are promising, as several molecular markers have discriminated between the parental line and the mutants. We are at the stage of validating these results after which the next step would be the cloning and sequencing of the polymorphic bands thereby turning them into sequence characterized amplified region (SCAR) markers. Such markers could then be applied in marker-aided selection (MAS) as well as in several functional genomics studies to elucidate the nature of resistance to this disease.

### **Molecular genetic fingerprinting of sesame world collection**

In furtherance of our mandate of working on neglected crops, we have recently started a collaborative on sesame with the Dept. of Field Crops, Faculty of Agriculture, Akdeniz University, Antalya, Turkey. This is aimed at a detailed molecular genetic fingerprinting of the world collection of sesame being housed in this Institute. In addition to an elucidation of the genetic diversity in this germplasm, it is hoped that another output would be an understanding of the genetic architecture of this population, such as heterotic groups, and that this would help in the selection of parents for use in the genetic improvement of this crop.

### **Molecular characterization of rice germplasm from China**

Different molecular marker systems are being used to characterize eight rice varieties, including two mutants, from China. These are putative parental lines for use in the development of new superior rice varieties in China. This is in collaboration with the Chinese institute, Zhejiang Academy of Agricultural Sciences (ZAAS), Zhejiang, China.

### **End sequencing of *Musa* Bacterial Artificial Chromosome clones**

We are continuing with the end sequencing of the Bacterial Artificial Chromosome (BAC) clones so far received from Genomics Resource Centre for the Consortium at the Institute of Experimental Botany of the Academy of Sciences of the Czech Republic, Olomouc, Czech Republic.

### **Multiplication of *Musa* putative mutant germplasm for field-testing**

We have prepared over 500 *in vitro* plantlets of putative banana mutants for shipment to the National Banana Research Program, Kawanda Agricultural Research Institute of the National

Agricultural Research Organization (NARO), Uganda. They will be field-tested in this black sigatoka disease endemic region for field resistance to this disease.

### **Irradiation Services**

The 20 batches of irradiation services provided during the period June to December 2003 are broken down as follows:

Number of requests	20
Number of species	32
Number of varieties	79
Number of treatments	219
Number of countries	20

### **Ploidy Determination**

The ploidy levels of a total of 127 banana samples derived from cell suspension cultures were determined during the period under review. These were from Katholic University, Lueven, Belgium.

### **Mutant Germplasm Repository**

No new entries were received between June and December 2003 so the status of the mutant germplasm repository remains a total of 3004 accessions distributed over three crops from three countries.

### **Fellowship Training and Scientific Visits**

<b>Name</b>	<b>Country</b>	<b>Subject Area</b>	<b><i>Period</i></b>
Labbe, Genevieve	France	Banana Molecular Biology	2003-06-01 to 2003-09-01
Jin, Wei	China	Rice Molecular Biology	2003-08-01 to 2004-01-31
Çağiran, Musa İlhan	Turkey	Sesame Molecular Biology	2003-11-10 to 2004-05-10

### **Training Course**

We hosted the 3<sup>rd</sup> FAO/IAEA Interregional Training Course on “Mutant Germplasm Characterization Using Molecular Markers”, 6-31 October 2003. A total of 20 participants drawn from 20 countries in Africa, Asia, Europe and Latin America participated in this 4-week exercise that exposed the trainees to several aspects of theoretical and applied molecular biology, cytogenetics and induced mutagenesis.

## H. PUBLICATIONS

1. Dris, R. and S.M. Jain (eds.), 2003. Production practices and quality assessment of food crops: 1. Preharvest practice. Kluwer Academic Publisher, The Netherlands.
2. Dris, R. and S.M. Jain (eds.), 2003. Production practices and quality assessment of food crops: 2. Plant mineral nutrition and pesticide management. Kluwer Academic Publisher, The Netherlands.
3. Dris, R. and S.M. Jain (eds.), 2003. Production practices and quality assessment of food crops: 3. Quality handling and evaluation. Kluwer Academic Publisher, The Netherlands.
4. Dris, R. and S.M. Jain (eds.), 2003. Production practices and quality assessment of food crops: 4. Postharvest treatment and technology. Kluwer Academic Publisher, The Netherlands.
5. Jain, S.M. and M. Maluszynski, 2003, Induced mutations and biotechnology in improving crops. In: *In vitro* application in crop improvement: Recent progress. A. Mujib et al (eds.). Oxford & IBH Publishing, New Delhi, India. pp 167-200.
6. Jain, S.M., 2003. Improvement of plant salt tolerance for sustainable food and feed production in saline environments. Proceedings, 1<sup>st</sup> INT/5/147 Project Coordination Meeting on Developing salt-tolerant crops for sustainable food and feed production in saline lands, Bangkok, Thailand, pp 6-9.

### **Plant Breeding and Genetics Newsletter No. 12**

The PBG Newsletter is prepared twice a year by the Plant Breeding and Genetics Section,  
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**PLEASE COMPLETE THIS REGISTRATION FORM AND SEND IT TO THE PLANT BREEDING AND GENETICS SECTION AT THE FOLLOWING ADDRESS:**

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

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

**A. Latin name of species:**

\_\_\_\_\_

**English name:** | | | | | | | | | | | | | | | |

**B. Name of new variety (cultivar):**

\_\_\_\_\_

C. Year of release from breeder: | | | | |

**D. Place and Date of official approval:** \_\_\_\_\_

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

**mutant**

**1.** | | | | | | | | | | | | | | | | | | | |

**yes / no**

2. | | | | | | | | | | | | | | | | | | | | | |

**yes / no**

3. | | | | | | | | | | | | | | | | | | | |

**yes / no**

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

**mutation derived**

**1.** 

**yes / no**

2. 

**yes / no**

3. 

**yes / no**

**G. Kind(s) of mutagenic treatment:**

**H. Doses(s) and/or concentration(s):** | | | | | | | | | | | |

**I. Year of mutagenic treatment:** | | | | |

**J. How was the variety bred:**

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

\_\_\_\_\_

\_\_\_\_\_

**address:** | | | | | | | | | | | | | | | | | | | | | |

\_\_\_\_\_

\_\_\_\_\_

**L. Extent of acceptance by growers:**

- Commercial value: \_\_\_\_\_
- Hectares of cultivation: \_\_\_\_\_
- Other: \_\_\_\_\_

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

**Name of person contributing this information:** \_\_\_\_\_

**THANK YOU FOR YOUR COLLABORATION!**