PLANT BREEDING AND GENETICS NEWSLETTER

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

This year seems to be very promising for the Plant Breeding and Genetic sub-Programme. At the demand of geneticists, plant breeders, and more recently molecular geneticists for information on released mutant varieties of specific crops, the FAO/IAEA Mutant Varieties Database (MVD) was transferred to the web site and is now available through Internet under the following URL: *http://www-mvd.iaea.org* . The idea to collect and transfer information on crop varieties developed with the use of mutation techniques to plant breeders was conceived at almost the same time as the establishment of the Plant Breeding and Genetics Section (PBG), Joint FAO/IAEA Division. The first classified list of induced mutant varieties was presented by Sigurbjörnsson at the Pullman Symposium, and published in 1969. Since the first issue of the MBNL (May, 1972) information on newly released mutant varieties was published at the end of each issue under the title "List of Mutant Varieties". However, fast developments in personal computer technology, together with the large number of suitable software, gave opportunity to organize a database on IBM PC using "DbaseIII Plus" software. Since 1987 the MVD has been available for the staff of the Plant Breeding and Genetics Section. Today it is available for anyone who is interested in officially released mutant varieties in crop and decorative and ornamental plants. I would like to express many thanks to all plant breeders who supported us with relevant information and especially to previous and current staff of our sub-programme who greatly contributed to collection of all data. The full list of 2252 mutant varieties has been published in the Mutation Breeding Review No. 12 (December 2000) to close this period of collecting data on mutant varieties. Such condensed but full information on mutant varieties should help geneticists, molecular biologists and plant breeders to asses the value of mutation techniques in germplasm enhancement, and stimulate the use of induced variation.

However, the widespread use of valuable mutants or mutant varieties has been limited by non-availability of seeds and propagules. Rapid and free access to such mutants is essential to determine their value and use in various locations. In addition, mutants of crops are becoming very valuable in functional genomics, (i.e. associating a set of DNA fragments with gene function), and proteomics (associating gene sequences with their resultant proteins), technology only recently developed. Hence, it is essential to collect, preserve, and propagate mutants of important crops for unrestricted access by plant breeders and geneticists around the world. Moreover, collections of such mutants would maintain germplasm diversity of the local cultivars. In an age where free access to genetic material is becoming more and more restricted, it is important that public agencies facilitate, as far as possible, the wide distribution and utilisation of genetic resources. To meet this demand the Plant Breeding and Genetics sub-Programme initiated work on establishment of the Crop Mutant Germplasm Register and Repository with the aim to collect, catalogue, preserve and facilitate the exchange of mutants and mutant varieties of major food crops and neglected crops for global use in future plant breeding, genetic, molecular research, and in genomics. A Register database, accessible via the Internet, containing useful information on all mutant accessions has been created with the help of S.L. Lee, M. Marsella (Consultants) and I. Ferris (FAO/IAEA). A germplasm Repository of mutants and parent varieties of crop plants will soon be established at the FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf and connected with the DNA fingerprinting service. We hope to finish all preparatory work before the end of the year. The first mutant accessions of linseed (from Dr. G. Rowland – Canada) and rice (Dr. Jin Wei – China) have already arrived.

As one of the most important future events I would like to inform you of the FAO/IAEA Interregional Training Course on Mutant Germplasm Characterisation Using Molecular Markers. The course will be held at the Plant Breeding Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria from 1 - 25 October 2001. Twenty participants from developing Member States of FAO and IAEA have already been selected from more than 60 candidates.

We have also initiated preparations for the International FAO/IAEA Symposium on the Use of Mutated Genes in Crop Improvement and Functional Genomics, which will be held in Vienna, Austria from 3-7 June 2002. It is foreseen that the Symposium will establish working linkages between molecular genetics and plant breeders applying mutation techniques. This should be achieved through focusing the symposium discussions on economic impact of mutated genes, induced biodiversity and germplasm enhancement, mutational analysis of crop tolerance to stresses, crop plant improvement in developing countries in the era of molecular breeding, mutation techniques in functional genomics, and mutant databases and exchange. You will find more information about the Symposium on page 7 of this issue of the Newsletter or under the IAEA Homepage (http://www.iaea.org/worldatom/Meetings/next_year.shtml).

Miroslaw Maluszynski

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B. FORTHCOMING EVENTS

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

Technical Officer: M. Maluszynski

This Training Course will be composed of lectures and exercises on various molecular marker techniques and especially: new horizons in rice breeding; hybridization and amplifiction-based DNA markers with hands-on training on SSRs and SNPs markers; construction of molecular maps; DNA fingerprinting of germplasm/mutants; general strategies of genome mapping; molecular tagging of genes governing agronomic traits; PCR based markers and their utilization in rice breeding; marker assisted selection in rice breeding; application of molecular markers in cloning of agronomically important genes and in functional genomics.

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

Technical Officer: M. Jain

The fourth and final FAO/IAEA Research Coordination Meeting will be held in Leuven, Belgium, 24-28 September 2001. Sixteen participants will present their results of the last six years work. The working material of this meeting would preferably be published as a book (if quality of submitted papers allows) and could also be linked electronically to INIBAP.

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

Technical Officer: K. Nichterlein

The purpose of this Workshop is to train skilled plant breeders/technicians belonging to the national research team of project RAF/5/042 on "Development of improved crop varieties". Training will include application of induced mutations, *in vitro* techniques and drought screening techniques in crop improvement programmes. Skills are required to use induced mutations in combination with *in vitro* techniques to create new variations for desired agronomic traits, and to use improved drought screening techniques in segregating populations to identify and evaluate mutants with improved drought tolerance. Lectures and practical classes given by regional/international experts will cover these areas. Preference will be given to candidates from participating institutes not previously involved in training activities of the project.

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

Technical Officer: M. Maluszynski

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

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

Nature of the course: The course will entail lectures and practical laboratory exercises covering the theory and use of DNA markers with particular emphasis on their applications in plant breeding and in utilisation of crop plant mutants. The course will include substantial hands-on training and the topics covered will include molecular biology theory, DNA extraction and purification, the polymerase chain reaction (PCR), nucleic acid electrophoresis, commonly-used DNA markers (RAPD, AFLP, SSR, DAF, ISSR), applications of DNA markers (marker-assisted selection, DNA fingerprinting, linkage analysis, genetic mapping principles and computer software) and to enhance the utilisation of plant mutants.

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

Technical Officer: J. Zapata-Arias

The aim of this meeting is to present and discuss the results of the research of the Plant Breeding Unit, Seibersdorf and of the Technical Contract counterparts in Brazil and Cuba on the use of natural light as alternative light source in tissue culture of tropical and subtropical crops. Guidelines on implementing low cost tissue culture technology in developing countries will be prepared for publication.

International FAO/IAEA Symposium on the "Use of mutated genes in crop improvement and functional genomics", Vienna, Austria, 3-7 June 2002

Technical Officer: M. Maluszynski

Background Information

The application of gamma rays and other physical and chemical mutagens for crop improvement in the past 70 years has increased crop biodiversity and productivity in different parts of the world. The

number of officially released crop mutant varieties has already exceeded 2200. A large number of these varieties are food crops released in developing countries. Some of them were obtained as infrequent mutation of specific genes responsible for agronomically important plant characters. This has resulted in the widespread use of these mutated genes in plant breeding programs throughout the world and has brought about an enormous economic impact, e.g. in barley, sunflower, soybean, rice and many other crops. The Symposium would inventory the use and economic impact of "super mutations" in improvement of crop production and address this message to plant breeders.

Moreover, induced mutations have recently become the subject of molecular investigations leading to descriptions of the structure and function of related genes. Mutated genes have therefore become valuable material to plant molecular biologists for the analysis of fine structure and organization of genetic material in crop species. They will play a critical role in understanding of function, isolating and incorporating genes responsible for plant productivity and resistance to biotic and abiotic stresses and in this way significantly influence crop production, especially in developing countries. The Symposium will establish working linkages between molecular geneticists and plant breeders applying mutation techniques to accelerate work on functional genomics of staple food crops, develop strategies for rapid application of molecular genetics technologies in breeding programmes and on development of crop mutant germplasm collections.

List of Topics

The programme of the Symposium consists of invited lectures and contributed papers. Papers on the following topics will be considered for presentation at the Symposium: Economic impact of widespread mutated genes; Induced biodiversity in plant breeding programmes; Mutational analysis of tolerance to abiotic stresses; Mutational analysis of crop resistance to diseases and insects; Mutations and mutated genes in functional genomics; Crop plant improvement in developing countries in the era of molecular breeding; Mutant databases, seed collections and exchange.

Participation

All persons wishing to participate in the Symposium have to complete a Participation Form and send it to the competent official government authority for subsequent transmission to the IAEA. A participant will be accepted only if the Participation Form is transmitted through the government of a Member State of the sponsoring Organizations or by an organization invited to participate. The costs for the organization of the conference are borne by the sponsoring Organizations. No registration fee is charged to participants. The Participation Form and other documents are available at the Secretariat or under the IAEA Homepage http://www.iaea.org/worldatom/Meetings/next_year.shtml

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The Scientific Secretary of the Symposium is Mr. M. Maluszynski, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture (telephone extension 21619, e-mail: M.Maluszynski@iaea.org). Symposium organization is provided by Ms. Hildegard Schmid, Division of Conference and Document Services (telephone extension 21316 & 21311, e-mail: Hildegard.Schmid@iaea.org).

C. PAST EVENTS

DAE-BRNS Symposium on the "Use of nuclear and molecular techniques in crop improvement", Mumbai, India, 3-8 December 2000

Technical Officer: K. Nichterlein

The symposium was jointly organized by the Board of Research in Nuclear Sciences and the Nuclear Agriculture and Biotechnology Division of the Bhabha Atomic Research Centre in Mumbai, India. Fifty papers were presented during seven sessions with 120 participants of various research institutions from India. The organizers invited the Technical Officer to deliver a lecture on "Achievements and trends of using induced mutations in crop improvement". The aim of the symposium was to consolidate the achievements of over four decades of research on induced mutations in plant breeding in India and to pool resources including molecular techniques the country for further substantial improvement of crop productivity. Results of previous breeding efforts using mutation techniques, the release of improved crop mutants as official varieties were reported in various papers: soybean variety 'Macs 450' with high yield for cultivation in the Southern Zone, the dew bean variety 'CAZRI Moth-1' with high yield under arid and semi-arid conditions, black gram variety 'TAU94-2' with high yield and resistance to yellow mosaic virus released for Southern Zone, other black gram varieties 'TAU-1', 'TAU-2' and 'TPU-4' released for the Central Zone, groundnut varieties 'TG-3', 'TKG-19A', 'TAG-24' and 'TG-26' with desirable traits such as earliness, wide adaptability, large seeds and high harvest index. Promising lines under advanced evaluation are castor hybrids with improved yield and resistance to *Fusarium* wilt, a dwarf mutant of cowpea with potential for rice fallows, and cowpea mutants with large seeds or high pod numbers contributing to increased yield. With regard to the role of induced mutations in future crop improvement in India it was emphasized by an invited lecturer that more focused and intensive efforts are needed for research on modification of root characters, nodulation of legumes, oil quality, host-pathogen interactions, response to photoperiod and gene silencing in transgenics in order to bring out new varieties that benefit farmers. Other invited lecture stressed the potential of transgenics, molecular markers and genomics research in increasing crop productivity in future.

FAO/IAEA Workshop on "*In vitro* mutagenesis and molecular marker analysis of ornamental plants", Bangkok, Thailand, 18-22 December 2000

Technical Officer: M. Jain

The objective of this Workshop was to impart training in plant tissue culture, *in vitro* mutagenesis and molecular marker analysis. This Workshop was attended by 40 participants,

with half attending only theory and the other half attending both theory and practical demonstrations. Most of the participants had research interest in plant tissue culture and plant improvement, including induced mutations and somaclonal variation. The few participants who were interested in molecular markers were engaged in the use of molecular markers in their research projects. Prof. Saxena, Canada, covered extensively plant tissue culture – history, techniques and applications with a case study of geranium, African violet, and some medicinal plants. He also covered commercialization of tissue culture. The Technical Officer gave two lectures on induction of genetic variability by tissue culture and mutagenesis. The Thai counterpart has isolated several mutants in ornamental plants. Some of the mutants have been officially released and they are going to be included in the FAO/IAEA mutant database.
Prof. Gustafson, USA gave lectures on genetic mapping, cytological mapping, *in situ* hybridization, and molecular markers. Molecular marker technology will be helpful to the counterpart for molecular characterization of ornamental plant mutants, and establishment of molecular database of each mutant.

FAO/IAEA Regional Training Workshop on "*In vitro* protocols and mutant selection for using Bayoud toxin" (RAF/5/035), INRA, Marrakech, Morocco from 13-17 November 2000

Technical Officer: M.Jain

The objective of this Workshop was to give training in tissue culture and mutagenesis, molecular biology, mutagenesis and Bayoud disease toxin. The Workshop was attended by nine participants from Algeria (3), Tunisia (3), and Morocco (3). Since plant tissue culture is an important component of the date palm project, two lectures were delivered on application of plant tissue culture technologies and *in vitro* mutagenesis. The various tissue culture technologies were introduced and their possible applications in plant improvement including induced mutations and somatic cell hybridization and somaclonal variation. All participants showed great interest in lectures on micropropagation, cryopreservation and somatic embryogenesis. Somatic embryogenesis technology has taken long strides in conifers, especially in plant regeneration, bioreactor for somatic embryo production, somatic embryo encapsulation or somatic seed, and cryopreservation for long-term storage. A recent report on date palm somatic embryogenesis has indicated 5% variation in regenerated plants, which is at the acceptable level. This report is very encouraging to us in the use of somatic embryogenesis in date palm. Since the origin of somatic embryogenic cultures is from a single cell, there won't be any serious problem of chimerism after irradiation, and *in vitro* selection against Bayoud toxin becomes convenient. The Tunisian group has developed an excellent system of somatic embryogenesis in date palm. During the discussion, they agreed to train Moroccan and Algerian scientists in somatic embryogenesis

In molecular biology, the main focus was on molecular marker applications; genome mapping; genetic mapping of disease resistance traits; and application of molecular genetics in genetic diversity assessment, conservation, restoration and sustainable management of plant genetic resources. There is a tremendous potential for using molecular biological tools in date palm improvement and conservation. Scientists from all three participating countries would require training in molecular markers. Lectures were given on Bayoud disease: its origin, spread and control; Bayoud pathogen and its toxin *Fusarium oxysporum* f. sp. *albedinis*; *in vitro* and *in*

vivo selection against Bayoud toxin; characteristics of Bayoud toxin and production; and extraction, purification and chemical structure of toxins. The Morocco group has already developed technology for Bayoud toxin isolation and production, and also for screening mutants against this toxin. Participants discussed the transfer of Bayoud toxin to Tunisia and Algeria. This is a long-standing issue and has resulted in slowing down the progress of this project. Finally, the Moroccans have agreed to supply Bayoud toxin to Tunisians and Algerians and help in screening of irradiated date palm material against Bayoud toxin.

International Symposium on tropical and subtropical fruits, Cairns, Australia, 26 November to 1 December 2000

Technical Officer: M. Jain

The organizers of this conference invited the Technical Officer to speak on "Induction of mutations in fruits of tropical and subtropical regions". Since the IAEA has an on-going CRP on tropical and subtropical fruits, this conference was helpful in gaining updated information on recent progress and forged new links with international scientists and private growers. The Symposium was divided into several themes: pre-harvest production, genetics and breeding, entomology, plant pathology/pre-harvest, post harvest, plant tissue culture/biotechnology, economics/marketing, conservation of plant genetic resources, processing/product development and technology transfer/application of research to industry. There were 250 participants from 35 countries. The number of oral presentations was 130 and 72 posters were presented. There were several presentations on micropropagation of pineapple, papaya, avocado, banana, coconut and Annona. None of the presentations indicated commercialization of micropropagated planting material of fruit crops. Even though there is strong resistance from consumers towards transgenic fruit, emphasis is still being given on transgenic research. Consequently, radiation-induced mutation activities have taken the back seat. IAEA should continue in its efforts to support induced mutations in obtaining useful mutants in fruit crops as a result of progress made in plant regeneration by tissue culture–organogenesis and somatic embryogenesis.

AFRA Regional Project Co-ordination Meeting of RAF/5/042 "Development of improved crop varieties", Arusha, Tanzania, 19-23 February 2001

Technical Officer: K. Nichterlein

This meeting was held to review results achieved under the regional project RAF/5042 and to prepare a new project document for the period 2002-2006. The meeting was attended by 14 project co-ordinators from Cameroon, Egypt, Ethiopia, Ghana, Kenya, Libya, Madagascar, Mali, Morocco, Sierra Leone, South Africa, Sudan, Tanzania and Zimbabwe.

Project achievements

Varieties released

Cassava: One mutant variety of cassava ('Tekbankye') was released in Ghana under the national program in 1998.

Sesame: Three varieties of sesame ('Taka 1', 'Taka 2' and 'Taka 3') were officially released in Egypt in 2000. The test for these varieties indicated that they are high yielding, disease resistant, insect resistant with an economic return higher than standard varieties. In addition, the Ministry of Agriculture released two mutant varieties ('Tushki' and 'Shandawill 3') deriving from the same breeding populations as the varieties above.

Sorghum: Eight mutant varieties of sorghum developed from local varieties were officially registered and released in Mali in 1998 under a national TC project and extended under the regional project. They have been improved for panicle length, number of grain per panicle, plant height (reduced by 0.5-1.0 m), lodging resistance, grain quality, plant cycle (earliness), striga tolerance and grain yield with the support of the Agency's coordinated research and TC projects.

Dissemination of released mutant varieties

Ghana: The released cassava mutant Tekbankye is currently being multiplied for distribution to farmers both in Ghana and interested AFRA Member States. This is being carried out both *in vitro* and *in vivo*. The *in vivo* multiplication is being carried out by the Root and Tuber Crop Improvement Programme, which is sponsored by the International Fund for Agricultural Development (IFAD), Government of Ghana and IAEA TCP GHA/5/026. The materials are currently in secondary multiplication sites from where cuttings will be made available to Ghanaian farmers. The *in vitro* multiplication for distribution for recipient's country outside Ghana is being carried out in the Tissue Culture Laboratory at the Biotechnology and Nuclear Agriculture Research Institute. Currently there is enough material available *in vitro* for distribution for regional exchange to interested countries.

Egypt: Seeds of the three new sesame mutant varieties (Taka 1, Taka 2 and Taka 3) were disseminated 3 years ago to 20 sesame growers/year to raise breeder seed and for demonstration trials. In the year 2001 all the available seeds from these varieties will be cultivated in about 50 feddan (21 ha) for further multiplication under the farmer fields to raise certified seed (about 50 growers).

Mali: Three of the eight registered mutant varieties namely 'FAMBE', 'TIEDJAN' and 'GNOME' have been multiplied under this regional project for extension. These varieties were grown in 1998-99 by 507 farmers, in 11 villages, on 1,499 ha in CMDT Fana region. No reliable statistical data are available on their dissemination in other regions of Mali. Seed of these mutants has been exchanged with other countries. Currently these mutants are in evaluation trials in Ghana and in other countries of the West and Central African Network on Sorghum (ROCARS).

Mutant varieties in the pipeline

Banana: Two mutant clones of desert banana with higher yield and good quality are submitted for official release in Sudan in 2001.

Barley: Five drought tolerant mutants of barley in Libya were provided to the Agricultural Research Centre (ARC) in 1997/98 to expedite their release. Two mutant lines of barley with improved yield will be submitted to National Variety Release Committee in Tanzania in 2002 provided the results of micro-malting tests in 2001/2 are positive.

Cassava: Four cassava mutant clones with improved cooking quality for release in Ghana in 2002.

Cocoa: One virus resistant cocoa mutant clone developed in Ghana for possible release in 2003.

Lentil: Three mutants of lentil with high yields, improved biological nitrogen fixation and moderate tolerance to fusarium wilt are submitted for registration in Morocco in 2001.
Safflower: Two spineless mutants of safflower with high yields, high oil content and high oil quality will be submitted to National Variety Trials in the 2002/2003 season.
Sorghum: Three new mutants improved for their late maturing, adapted to south of Mali (Guinean area), and two medium maturing varieties adapted to the Soudanian/Sahelian area, which improved for lodging resistance, grain quality and grain yield components have been submitted for registration in 2001. Two more mutants with improved drought tolerance and dwarf ness have been developed and it is planned to protect them.
Wheat: One wheat mutant line (Km 14) is recommended for release in drought-prone environments in Kenya in 2001.

Promising mutant materials
Promising mutant materials have been reported in various crops from different countries (see summary report of each country). These materials are under preliminary evaluation and require a series of rigorous tests before they are considered as candidate varieties.
In all projects the testing and evaluation of mutant lines/clones suffers from limited funding by the national budget for recurrent costs (labour, consumables). It is stressed that this may lead scientists to concentrate on projects that also cover recurrent expenditure. It was suggested to explore possibilities to receive funds for advanced projects from the Common Funds for Commodities (CFC) to supplement funding of projects in areas for which the Agency cannot provide funding.

Breeding for drought tolerance
Drought is still a major constraint to crop production in Africa and genetic improvement of drought tolerance is complex requiring advanced technologies and well-trained manpower. Shortage of trained manpower and research fund are mentioned as limiting factor in most African countries. However, the Agency has organized training courses and workshops on the subject, and screening techniques for drought have been developed in the past five years. Some of the drought screening techniques transferred to programmes in the region include wooden box method, irrigation schemes, and rain out shelters. Participating countries are at different implementation stages in this project and some countries did not fully benefit from the previous training and establishment of drought screening facilities for various reasons. Hence, the Agency is expected to help upon request from those countries that have not established such facilities. It was also emphasized that host institutions of participating countries are required to allocate fund to run the project.

Improvement of neglected/underutilized crops
Not all countries were able to initiate projects due to lack of funding at the national level where funding goes to major crops only. It was agreed that only projects with government support might be sustainable and without national funding projects should not be initiated. So far, germplasm accessions have been acquired, evaluated and ealy mutant generations have been produced in cocoyam (Cameroon), white lupine (Egypt), noug and tef (Ethiopia), African yam bean (Ghana), bambara groundnut (Madagascar), African rice (Mali), amaranths (South Africa), lablab (Tanzania).

D. STATUS OF EXISTING CO-ORDINATED RESEARCH PROJECTS

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

Technical Officer: K. Nichterlein

This CRP was initiated in 1998 with the objective of overcoming major constraints to increase productivity of neglected and underutilized crops by genetic improvement, in order to enhance the economic viability and sustain crop species diversity, and in future to benefit small farmers. Mutation techniques in combination with biotechnology are applied for the improvement of various vegetatively and seed propagated crops: cocoyams (*Colosasia esculenta, Xanthosom*a spp.), yams (*Dioscorea* spp.), grain and vegetable amaranths (*Amaranthus* spp.), Bambara groundnut (*Vigna subterranea*), grasspea (*Lathyrus sativa*), okra (*Abelmoshus esculentus*), bitter potatoes (*Solanum jucepzukii*, *Solanum ajanhuiri*) and naranjilla (*Solanum quitoense*). At present there are 16 participating institutes from Bolivia, Costa Rica, Ecuador, France, Germany, Ghana, India, Indonesia, Slovakia, South Africa, Syria and Thailand including an agreement holder from IPGRI based at ICARDA. It is planned to hold the third Research Co-ordination Meeting in Vienna, 27-31 May 2002.

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

Technical Officer: M. Jain

This CRP was initiated in 1994 with the general aim of integrating radiation induced mutations, *in vitro* culture and molecular genetics methods into the conventional breeding of banana to induce desirable variation such as disease resistance, dwarfism and earliness, and also to promote the development of methods for large-scale and rapid multiplication of the mutants/segregants through micropropagation and somatic embryogenesis. Plants can be readily regenerated via somatic embryogenesis for large-scale plant production, which is ideal for *in vitro* mutagenesis and the selection of mutants with desirable agronomic traits. Since 1996, Belgium has been an important contributor to this CRP. Twenty institutions worldwide are involved. The 4th and final RCM will be held in Leuven, Belgium, 24-28 September 2001.

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

Technical Officer: M. Maluszynski

This CRP was initiated in 2000, with the overall objective of assisting Member States to apply mutation techniques and related biotechnology to generate and utilise mutants for the identification of root properties and genes for improvement of crop plants. At the present time there are 21 participating institutes in this project.

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

Technical Officer: M. Maluszynski

This CRP was initiated in 1999 with the aim of assisting Member States to apply molecular genetics of mutated genes for improving production in both major cereals and related under-utilised crops. More specifically to collectively develop, characterise and data-base mutant collections of key crops for application in breeding programmes and to molecularly characterize new or existing mutated genes affecting key agronomic traits in major crops using comparative approaches in under-utilized crops with a view to their eventual isolation.

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

Technical Officer: M. Jain

This CRP was initiated in 2000 and the first RCM was held in October 2000. The overall objective is to generate and characterize radiation induced and natural genetic diversity in tropical and subtropical fruit trees for improving nutrition balance, food security, and enhancing economic status of growers in Member States. It is planned to hold the second RCM in Orlando, Florida, in June 2002.

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
ETH/5/011	Improvement of TEF through mutation breeding	K. Nichterlein
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
GUA/5/012	Mutations and biotechnology for crop improvement	M. Maluszynski
INS/5/026	Mutation breeding of bananas	K. Nichterlein
INS/5/027	Mutation breeding of ornamental plants	M. Jain
INS/5/030	Sustainable agriculture development in Yogyakarta	M. Jain
INS/5/031	Mutation breeding of horticultural crops	M. Jain
IRQ/5/015	Induction of mutations in crops through *in vitro* culture	M. Jain
JOR/5/008	Establishment of in vitro mutagenesis laboratory	M. Maluszynski
KEN/5/021	Improved drought resistance of crops by induced	M. Maluszynski

	mutations	
MAG/5/008	Mutation techniques and biotechnology for rice and cassava	M. Maluszynski
MAK/5/004	Mutation and doubled haploid techniques to improve wheat	K. Nichterlein
MAL/5/024	In vitro mutagenesis for horticultural crop plants	M. Jain
MYA/5/010	Development of improved rice with tolerance to drought	K. Nichterlein
PAK/5/035	Development of salt-tolerant varieties of basmati rice	K. Nichterlein
PAK/5/038	Development of drought and heat tolerant canola mutants	K. Nichterlein
PAK/5/039	Pest resistant chickpea through induced mutation	K. Nichterlein
PAK/5/040	Improvement of heat-tolerant semi-dwarf bread wheat	K. Nichterlein
PER/5/024	Introduction of barley and other native crop mutant cultivars	M. Maluszynski
PHI/5/027	Mutation breeding of priority agricultural crops	S. Nielen
RAF/5/035	Control of bayoud disease in date palm	M. Jain
RAF/5/042	Development of improved crop varieties (AFRA III-18)	K. Nichterlein
RAF/5/049	Field evaluation of bayoud-resistant date palm mutants	M. Jain
RAS/5/037	Mutational enhancement for genetic diversity in rice	M. Maluszynski
RLA/5/035	Evaluation of cereal crop mutants (ARCAL XXIa)	M. Maluszynski
ROK/5/033	Quality improvement of major crops and integrated plant nutrition management in the low-input agri-cultural system.	M. Maluszynski
SRL/5/034	Radiation-induced mutations for black pepper improvement	M. Jain
SRL/5/036	Virus screening of improved banana mutants for large-scale dissemination	S. Nielen
SUD/5/026	Improvement of the productivity and sustainability of industrial crops	S. Nielen
URT/5/020	Improving productivity of basic food crops in Tanzania	M. Maluszynski
VEN/5/018	Genetic improvement of fruits and pepper	M. Jain
VIE/5/014	Rice mutant varieties for saline land	K. Nichterlein
YEM/5/003	Applying nuclear techniques for improvement of crop yield	K. Nichterlein
ZAM/5/022	Crop improvement through *in vitro* mutation techniques	K. Nichterlein

TC project Control of Bayoud disease in date palm (RAF/5/035)

In Saharan and Sub-Saharan regions of Africa and the Middle East, date palm is a very important fruit tree and in some areas, it provides food, shelter and fuel to the people. Bayoud disease of date palm is epidemic in this region and became a major production constraint. The FAO/IAEA started this project in 1995, to overcome susceptibility to Bayoud disease through genetic improvement, using *in vitro* propagation and selection, in combination with physical mutagen treatment. High quality and susceptible Bayoud disease date palm cultivars were used to isolate disease resistant mutant lines and dwarfs. The counterparts from Algeria, Morocco and Tunisia are part of this project. IAEA has provided them tissue culture facilities including

chemicals and equipments and expert advice. This project has made substantial progress in date palm plant regeneration from somatic embryogenesis and organogenesis .

The Moroccan counterpart has been successful in developing plant regeneration protocol via organogenesis by using offshoots and young inflorescence as explants from selected date palm trees. From irradiated tissue, over 100 plantlets have been established in the soil and have been growing in the greenhouse for the past two years. These plantlets are ready for screening against Bayoud disease in the greenhouse. Bayoud toxin is already available, having been isolated from *Fusarium oxysporum* f. sp. *albedinis* by Dr. Sedra in Marrakech. However, the number of plantlets is very low for mutant selection, due to a major problem of hardening *in vitro*-grown date palm plants. It is essential to maintain temperature control and high humidity for hardening *in vitro*-grown plants in the greenhouse before their transfer to the field. The greenhouse had problems of temperature control during the summer and blockage of fog system due to salt deposition in water pipes. An efficient thermal screening system has been installed to reduce incident light and subsequent heating, especially during hot season. High light flux at the early stage of acclimatization may cause inhibition of photosynthesis resulting in either poor quality or death of plants. The screen is made of a plastic matrix covered with thin aluminum foil, which is UV-proof. The newly installed Fog system has very fine water particles, which creates a true aerosol around the plantlets and stays on the leaves about 60 minutes longer. As a result, water consumption is reduced. Another advantage of this system is that the substrate is not flooded with water, especially at the time of fertilization during acclimatization. The new facility is made of stainless steel, which resists corrosion and makes cleaning the fog system easy. The increase in relative humidity was efficient and within 2 minutes 100 percent humidity level was achieved. By the installation of an acclimatization greenhouse, the tissue culture laboratory at INRA, Marrakech is in a position to harden *in vitro*-grown date palm plants. This group has already developed a plant multiplication system of date palm by organogenesis and technology for Bayoud toxin production and selection system against this toxin. Now they are in position to multiply gamma irradiated plant material in large numbers and select mutants resistant to Bayoud disease.

Prof. Drira's group at the University de Sfax, Faculty of des Sciences, Sfax, Tunisia has developed a somatic embryogenesis system in date palm. The conversion of somatic embryos to plantlets is very low due to poor quality of embryos. These embryos differ in quality with zygotic embryos. They have made some progress in improving the germination rate by changing the osmotic potential of the culture medium, and also by desiccation of somatic embryos before germination. However, establishment of somatic embryo derived plants (somatic seedlings) in the soil is still very poor. Non-synchronized somatic embryo development and precocious germination of somatic embryos in the liquid medium are major reasons for the poor success of regenerating large number of somatic seedlings. The age (2-3 year-old) of the cultures is another factor in maintaining embryogenic potential of somatic embryogenic cultures. Therefore, cryo-storage of embryogenic cultures would be helpful in maintaining embryogenic potential of the cultures for an unlimited period of time. They have plants growing in the greenhouse coming from the irradiated material. So far, they are unable to screen them due to unavailability of Bayoud toxin from Morocco. The Morocco group agreed to supply Bayoud toxin to the Tunisians and Algerians. Now this group should be able to screen plants derived from irradiated material and irradiated cell suspension against Bayoud toxin.

F. ACTIVITIES AT THE PLANT BREEDING UNIT, SEIBERSDORF

Improvement of *Musa* ssp. through mutagenesis

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

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

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

DNA Fingerprinting Service

Molecular markers are valuable tools for plant breeding and genetics in both practical aspects and basic research. Many different types have been developed during the last years. The most frequently used markers are all based on PCR because they require only limited amounts of DNA and are fast. The most sensitive method currently in use is AFLP (Amplified Fragment Length Polymorphism) which has been developed by Vos *et al.* (Nucl. Acids Res. 23, 1995, 4407-4414). This method can be applied to any species without prior sequence information. DNA is restricted (usually with 2 different restriction enzymes) and synthetic adapters are ligated to the ends which serve as starting points for two rounds of PCR amplification. The second amplification round, also called selective amplification, uses primers which extend for a few bases into the unknown template DNA thus resulting in amplification of only a subset of fragments which can then be separated by electrophoresis. Using various combinations of primers with specific “selective” bases different patterns (fingerprints) of DNA fragments can be obtained.

The Plant Breeding Unit is currently establishing a DNA fingerprinting laboratory. We have acquired a MultiPROBE II robotic workstation from Packard Bioscience and a 3100 Genetic Analyser from Applied Biosystems that will help us to automate the AFLP procedure. For that, one of the selective primers has to be fluorescently labelled. The 3100 genetic Analyzer is able to automatically calculate the size of the separated fragments using fluorescently labelled DNA standards.

Once the DNA Fingerprinting Laboratory is fully operational it will be announced under what terms a DNA Fingerprinting service can be used by Member States.

Detection of androclonal variation in anther-cultured rice lines using RAPDs

Random amplified polymorphic DNA analysis was used to determine the occurrence and extent of variation in rice (*Oryza sativa* L.) plants regenerated from anther culture. Androclonal variation in morphologically uniform progenies was detected using 40 10mers oligonucleotide arbitrary primers. Among 27 plants from nine anther culture derived lines, variation was detected in three plants from two lines by two primers, namely UBC 160 and UBC 209. Primer UBC 160 amplified a polymorphic band on one of the three progenies from DH-34, while UBC 209 detected polymorphisms on two out of three progenies from line DH-58. Apart from these, the amplification products were monomorphic across all the regenerants from anther culture derived plants. Out of 40 tested primers, no difference in the banding pattern was observed in three seed derived plants.

Services

Radiation treatment with ^{60}Co

9 individual requests from 7 different countries

treatments: 163	plant species: 13
16 *in vitro*	3 *in vitro*
147 seed samples	10 seed samples

Ploidy determination using flow cytometry

3 individual requests from 3 different countries

plant species: 2

Training

The following scientists visited and/or received training:

Mr. M. Rahman	Bangladesh	June 2001
Ms. S. Begum	Bangladesh	January 2001 – July 2001
Mr. Oh Ki-Won	Korea	December 2000 – May 2001
Ms. N. Rakotoarisoa	Madagascar	October 2000 – April 2001
Mr. M. Khan	Pakistan	March 2001 – June 2001
Ms. B. Parejo Rey	Spain	April 2001
Ms. S. Reuben	Tanzania	January 2001

G. PUBLICATIONS

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- Minocha, R and S. M. Jain, 2000. Tissue culture of woody plants and its relevance to molecular biology. In: Molecular Biology of Woody Plants. Vol. 1. S. M. Jain and S. C. Minocha (Eds.). Kluwer Academic Publishers, The Netherlands. Pp 315-359

- Nichterlein, K., H. Bohlmann, S. Nielen, and M. Maluszynski, 2000. Achievements and trends of using induced mutations in crop improvement. DAE-BRNS Symposium, Bhabha Atomic Research Center, Mumbai, India. Pp 27-35.

- Nichterlein, K., Maluszynski, M., and Jain, M., 2001: Improvement of oilseeds and modification of oil quality through induced mutation techniques. Mutation Techniques and Molecular Genetics for Tropical and Subtropical Plant Improvement in Asia and the Pacific Region. Report of an FAO/IAEA Seminar, Makati City, The Philippines, 11-15 October 1999, 15-17. IAEA, Vienna.
- Nichterlein, K., 2000: Induced mutation in improvement of major and neglected crops - achievements and current applications. Proceeding of International Tef Workshop, Addis Ababa, Ethiopia. In print

Working Material: Report of an FAO/IAEA Seminar held in The Philippines, 11-15 October 1999, "Mutation techniques and molecular genetics for tropical and subtropical plant improvement in Asia and the Pacific Region"

Working Material: Report of the 3rd FAO/IAEA Research Co-ordination Meeting held in Sri Lanka, 4-8 October 1999, "Cellular biology and biotechnology including mutation techniques for creation of new useful banana genotypes"

TECDOC No. 1195: Sesame improvement by induced mutations

TECDOC No. 1216: Induced mutations in connection with biotechnology for crop improvement in Latin America

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):

|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|

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L. Extent of acceptance by growers:

- **Commercial value:** _____
- **Hectares of cultivation:** _____
- **Other:** _____

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

Name of person contributing this information: _____

THANK YOU FOR YOUR KIND COLLABORATION!

Plant Breeding and Genetics Newsletter

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