PLANT BREEDING AND GENETICS NEWSLETTER

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

The fourth issue of the Plant Breeding and Genetics Newsletter is a report on our activities for the second half of 1999. Most of our work was concentrated on the development of new Coordinated Research Projects (CRPs) and on efficient implementation of Technical Cooperation Projects especially related to regional activities. Following the organization of the CRP on "Molecular characterization of mutated genes controlling important traits for seed crop improvement" the next CRP on "Mutational analysis of root characters in annual food plants related to plant performance" was also established with the participation of 21 institutes. Necessary documents were also prepared for the establishment of a new CRP dealing with induced mutations in tropical fruit trees. We are expecting to call for applications for this new project in February 2000. Our colleagues from Asia and the Pacific Region had an opportunity to discuss problems, achievements and future directions of mutation techniques and biotechnology for tropical and subtropical plant improvement during the FAO/IAEA seminar organized in Manila, The Philippines. The training of scientists from developing countries will be mainly achieved through the organization of regional training courses as interregional training courses, such as the Seibersdorf Training Course on Induced Mutations, is no longer in the programme of the Technical Cooperation Department. The following three regional courses were organized in the second half of the year: "Molecular characterization of genetic diversity of traditional and neglected crops selected for improvement through mutation techniques - AFRA" Pretoria, South Africa; "Hands-on experience on molecular and mutation techniques - RAF"- Seibersdorf, Austria and "Production and utilization of doubled haploid lines in rice breeding - RAS" - Suwon, Korea.

The wider involvement of molecular techniques in induced mutation projects stimulated us to pay more attention to the collection and dissemination of information related to the development and molecular characterization of various crop mutants obtained under FAO/IAEA projects. The Mutant Varieties Database is very close to having 2000 accessions but it is mainly a tool for plant breeders, similar to a newly organized database on mutagens and their successful doses. There is a need to establish an efficient information system, available to plant breeders but also to crop geneticists and molecular biologists, on various crop mutants generated under the FAO/IAEA projects or other activities, if information and seeds are available. We hope to be able to initiate this activity in the second half of 2000.

Activities of the Plant Breeding Unit of the FAO/IAEA Agriculture and Biotechnology Laboratory at Seibersdorf focused on the development of efficient methods for doubled haploid production with resistance to salinity in rice, activation of retrotransposable elements in rice as a tool for cloning genes of interest and on the effect of propagation methods on cytochimera dissociation. Dr. H. Bohlmann joined the Unit on 1 November, filling the post previously occupied by Dr. Paolo Donini. The activities of the Plant Breeding Section were supported by Dr. C.R. Bhatia who completed his one year assignment and by Dr. L. van Zanten who finished his 6 week temporary assignment as a consultant on information systems.

Miroslaw Maluszynski
Head, Plant Breeding & Genetics Section

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Dr. C.R. Bhatia (India) left the IAEA in September 1999, after a one year sabbatical, to take up a position with the Bangladesh Rural Enterprise and Agricultural Development (BREAD) Project, Dhaka, Bangladesh.
Dr. Holger Bohlmann (Germany) joined the Plant Breeding Unit on 1 November 1999.

B. FORTHCOMING EVENTS

Research Co-ordination Meetings

First RCM on "Mutational analysis of root characters related to drought tolerance to sustain crop production in arid and semi-arid zones", Vienna, Austria, 14-18 February 2000.

Second RCM on "Application of biotechnology and mutation techniques for the improvement of local food crops in LIFDCs", San Jose, Costa Rica, 26-30 June 2000.

First RCM on "Improvement of tropical fruits through mutations and biotechnology", Vienna, Austria, 25-29 September 2000.

Second RCM on "Molecular characterisation of mutated genes controlling important traits for seed crop improvement", Vienna, Austria, 2-6 October 2000.

Workshops

FAO/IAEA/UCR Workshop on "In vitro culture techniques for the improvement of vegetatively propagated tropical food crops", San Jose, Costa Rica, 1-5 July 2000.

C. PAST EVENTS

(Detailed reports on the events listed below are available on request from the Plant Breeding and Genetics Section).

3rd RCM On "Improvement Of New And Traditional Industrial Crops By Induced Mutations And Related Biotechnology", Corvallis, Oregon, USA, 2-6 August 1999

The Final Research Co-ordination Meeting under this FAO/IAEA Co-ordinated Research Programme was hosted by Prof. Steven Knapp, Oregon State University, Corvallis, Oregon, 2-6 August 1999. Fifteen scientists from 10 countries and one representative of FAO, Rome took part in the RCM. Fourteen scientific papers were presented summarizing results achieved under each research contract/agreement. A presentation was given on the Global Plant and Pest Information System by the FAO Representative. Results of the CRP and future trends were discussed.

It can be stated - on the basis of the presented papers - that induced mutations are frequently used to generate genetic diversity in oil and fibre crops in Bangladesh, Brazil, Canada, China, Germany, Greece, Hungary, India, Pakistan and the USA. Mutation techniques in combination with interspecific hybridization, embryo culture, doubled haploid techniques and genetic transformation have been applied to modify agronomic and quality traits of oilseed crops such as soybean, rapeseed, sunflower, linseed, cuphea and meadowfoam and fibre plants such as cotton and jute. Germplasm, isolated genes and DNA sequences for modification of fatty acid profiles have been exchanged among the participants.

New methodologies/approaches

Genetic transformation of soybean remains difficult and has been successful in only very few genotypes. The Chinese commercial variety of soybean, Hei Nong 35, developed from a cross with the mutant Heinong 16, has been identified as being very suitable for genetic transformation of soybean. A RAPD marker for resistance to race 4 of *Heterodera glycines* has been developed.

Microspore culture from rapeseed plants which were irradiated with 50 Gy led to the development of doubled-haploid lines with double zero quality ("Canola quality"). This indicates that the combination of radiation and doubled-haploid techniques can speed up the development of improved rapeseed germplasm. Doubled-haploid plants developed from mutant hybrids of rapeseed indicate a potential for fixation of mutant heterosis in rapeseed.

Gene transcripts associated with adaptation and tolerance to water-deficit and others related to CMS, restoration and semigamy were identified in cotton using differential display and were cloned. Cotton was transformed with three different lectin genes indicating tolerance to boll worm and aphids.

Main Conclusions and Recommendations

Tremendous achievements have been made during this CRP, in both public and private sectors regarding modification of oil content and composition of oilseeds using induced mutations and gene transfer from related species. These achievements lead to a variation of fatty acid crops, both for food and non-food uses.

The disease and pest resistance of relevant oilcrop species has to be substantially enhanced in order to stabilise production and realise the yield potential. Such approach contributes to sustainability through Integrated Pest Management (IPM) and hence less environmental degradation. Genetic variation for disease and pest resistance is not adequate. Therefore, ways of base-broadening respective genetic variation for resistance to biotic stresses are urgently required. New Agency projects should focus on alien germplasm via interspecific and intergeneric hybridisation, radiation or chemically induced mutations, identification and cloning of respective genes controlling disease reaction(s) of the host plant(s), directed mutagenesis of respective disease-related genes on a molecular level and transfer of wild type resistance genes or newly created alleles into the respective, susceptible crops.

In cotton, major emphasis should be given to the development of germplasm with tolerance to abiotic stresses (e.g. drought, heat, cool temperature) and improvement of carbon partitioning (harvest index).

The enhancement of genetic variability is in line with the base-broadening concept of the Global Plan of Action for Plant Genetic Resources for Food and Agriculture. Future CRPs in the area of industrial crops should focus on the production stability (tolerance to biotic and abiotic stresses) and concentrate on related plant species or genera, respectively, in order to be able to make use of similar genes and biosynthetic pathways within respective taxonomic groups (e.g., *Brassicaceae* or *Compositae* families).

The reports of this CRP are being edited and will be published as an IAEA Technical Document in 2000.

3rd RCM On "Radioactively Labelled DNA Probes For Crop Improvement", Vienna, Austria, 6-8 September 1999

Major Conclusions and Recommendations

The Agency should make DNA primers more readily available at lower cost and should continue to support the distribution of DNA clones. However, support is not needed for all crop species. For example, many wheat and maize clones may be obtained from the USDA-ARS (Albany, CA) and the USDA-ARS (Columbia, MO) centers. Curators at germplasm banks and genetic stock centers may already have responsibility as well as the financial support for maintaining and distributing the DNA clones and related material and information. DNA clones remain an important resource for research but their role has diminished with the advent of PCR-based marker systems. In developing countries, it is difficult for researchers to design and synthesize primers. Therefore, one recommendation from this CRP is to develop the means of enabling scientists in developing countries to obtain the necessary primers at a reasonable cost and in a timely manner.

Science and technology related to the utilization of DNA markers in crop improvement are advancing at an increasing rate. The rate and magnitude of the advancements make it difficult for researchers and research institution, anywhere, to review and assimilate the new information, knowledge and technology. Without special efforts in education, researchers and their facilities may become quickly out of date and disconnected from the opportunities afforded by the frontiers of science in this era. Thus, another recommendation is for the Agency to provide more frequent short-term (days or weeks instead of months or years in accordance with the goals of the session) training and educational opportunities in the principles and practice (theory and technical) of the utilization of DNA markers in crop improvement.

The Agency should also support the development of WWW sites devoted to the implementation of various DNA marker techniques and their use in crop improvement. Such sites could include detailed descriptions of methods, supplementary video or images, literature citations, sources of reagents, suggestions for appropriate data collection and interpretation, descriptions of applications, variations of the primary method and the many informal and often unwritten details that enhance the performance of a procedure or an analysis. We also recommend that the Agency take the steps needed to have their site listed as a link on WWW servers widely used by those involved in plant breeding and genetics. The Agency's web site need to be upgraded. Specifically, this can be achieved by expanding the existing web site of the FAO/IAEA to include useful links, an interactive question-and-answer forum to address specific problems of the implementation of techniques and to troubleshoot protocols. It should also provide a forum to publish results and improved protocols, share results and experiences, and materials between scientists.

3rd RCM On "Cellular Biology And Biotechnology Including Mutation Techniques For Creation Of New Useful Banana Genotypes", Colombo, Sri Lanka, 4-8 October 1999

The third FAO/IAEA Research Co-ordinated Meeting (RCM) on "Cellular biology and biotechnology including mutation techniques for creation of new useful banana genotypes" was held in Colombo, Sri Lanka, 4-8 October, 1999 with sixteen participants from Austria, Belgium, Colombo, Czechoslovakia, Cuba, Guyana, France, Germany, Malaysia, Philippines, Sri Lanka and the USA.

Banana is a poor man fruit crop in tropical and subtropical countries. It has the potential to become a cash crop for improving the economic status of farmers. This could be achieved by producing resistant cultivars against diseases like sigatoka, Fusarium wilt and other pests like nematodes. Biotechnology including induced mutations and gene technology together with conventional methods could assist in overcoming these problems in developing new banana cultivars.

During the last four years of this RCM, several achievements were made including development of biotechnological tools for plant regeneration, useful new mutated banana clones, screening protocols for disease resistance and linkages with participating research groups that facilitated exchange of SSR primers and technology transfer. Genetic engineering protocols for transferring useful genes were developed in banana. Dissociation of cytochimera and karyological changes in embryogenic cell suspension culture, somatic seedlings or micropropagated plant material was successfully performed with flow cytometry. Furthermore, *in vitro* screening of nematode resistance; early screening technique for Fusarium wilt, and purification of *Mycosphaerrella fijiensis* toxin and demonstration of their necrotic effect on leaves were established. A new banana mutant highly resistant to Fusarium wilt disease was isolated. Selective repetitive DNA sequences were physically mapped in *Musa* chromosome by *in situ* hybridization (FISH) *C*onsiderable progress was made in application of molecular marker techniques. Twenty PCR-based simple sequence repeat (SSR) markers were developed for *Musa* and found a high degree of polymorphism between two genomes. The "long and narrow leaf" somaclonal variation mutation was linked to *in vitro* activation of retrotransposable element. Similarly, amplified fragment length polymorphism (AFLP) was successfully used in distinguishing the A and B genomes and some known somaclones and irradiated mutants of banana.

The reports of this CRP are being edited and will be published as an IAEA Technical Document in 2000.

1st RCM On "Molecular Characterization Of Mutated Genes Controlling Important Traits For Seed Crop Improvement", Vienna, Austria, 4-8 October 1999

General Conclusions

For decades, the mutations and methods fostered by the Plant Breeding and Genetics Section have been utilized in crop improvement programmes in many species around the world. In any circumstance, mutations provide a rapidly inducible source of genetic variation that

potentially expands the genetic repertoire and adaptation of crops. Depending on the crop and region of the world, they have been and remain a critical and a primary source of genetic variation.

More recently, the value of mutagenesis and mutations has increased because they have been appreciated as essential components of genome projects in model systems such as yeast, *Drosophila*, mice, *C. elegans*, zebrafish, and *E. coli*. Now, this approach has spread to the plant science communities of Arabidopsis, rice, wheat, maize, barley, petunia and others. Within five years, we will know the complete DNA sequence of genomes of Arabidopsis and rice. Sooner, we will know the expressed sequence of hundreds of thousands of other genes from wheat, maize, tomato, barley and other crops.

Sadly, DNA sequence alone will not suffice. Crop species are especially formidable for such research: most crop genomes are polyploid (unlike animals) and they contain tens of thousands of expressed and duplicated genes (duplicate in structure if not function). The DNA sequence of a gene, the primary product of genome projects, provides only a clue of the gene's identify and does not reveal the gene's native biological function(s). Rather, the critical information regarding gene function is revealed by concerted analyses of phenotypic and allelic variation and gene expression facilitated by mutations ('Phenomics').

Today, nearly all mutations are of interest to basic and applied biologists (e.g. molecular biologists, biotechnologists and plant breeders) because the new methods render all genes amenable to concerted evaluations. Mutations in all genes and an allelic series of mutations at a locus are now an exploitable resource for basic and applied aspects of crop improvement. For crop species, the time has arrived to create, organize and share a rich collection of characterized mutations.

Various methods of mutation induction were discussed. It was pointed out that gamma radiation is the preferred choice of radiation for inducing mutations in plants. Of the various chemicals available to induce mutations it was felt that MNH and EMS or sodium azide could be applied. In addition to radiation and chemical mutagenesis we also discussed the possibility of using other methods for inducing mutations. These included insertional mutagenesis using transposons.

All the Principal Investigators agreed to abide by the agreement for exchange and sharing of research materials and technology. The setting up of web page for exchange of ideas was also discussed. We felt that IAEA could take up this responsibility which would be very beneficial to all the participants of the CRP program. Since the participants are from different geographical regions of the world, it was felt that we could help each other in screening their mutants under different climatic conditions.

The reports of this CRP are being edited and will be published as an IAEA Technical Document in 2000.

FAO/IAEA Seminar on "Mutation Techniques and Molecular Genetics for Tropical and Subtropical Plant Improvement in Asia and the Pacific Region", Makati City, the Philippines, 11-15 October 1999

The Seminar was hosted by the Department of Science and Technology - Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (DOST-PCARRD), Philippine Nuclear Research Institute (PNRI), the University of the Philippines at Los Banos (UPLB) and the Department of Agriculture - Bureau of Agricultural Research held at the ACCEED Conference Center, Makati City, Metro Manila, Philippines from 11-15 October 1999. The main objective of this Seminar was to present and discuss the current status and future directions of mutation techniques and related molecular genetic approaches in the Region. The Seminar focused on the application of these techniques in the areas of plant research and crop improvement. A total of thirty papers from PR China, India, Indonesia, Japan, Malaysia, Mongolia, Myanmar, Pakistan, the Philippines, Sri Lanka, Thailand, the USA, Vietnam and CIMMYT, FAO/IAEA, and ICRISAT were presented and discussed by 45 participants. Additionally, the current breeding and molecular research for rice improvement at IRRI was presented during a visit to the Plant Breeding and Molecular Genetics Laboratory, IRRI.

Available Molecular and Biotechnology Techniques

- Development and utilization of minisatellite sequences for DNA fingerprinting and linkage analysis in rice.
- Development and utilization of PCR technology for DNA fingerprinting of crop species using a wide variety of primer techniques.
- Use of tissue culture system to activate inherent retrotransposon system in rice which resulted in the production of mutant phenotypes.
- Development of symmetric and asymmetric protoplast fusion, where donor of cytoplasm is irradiated to suppress nuclear components, between cultivated and wild rice species; specifically, involved in the transfer of rice blast resistance gene from wild to cultivated rice through asymmetric fusion.
- Development and application of fluorescence *in situ* hybridization analysis including GISH in the genomic analysis of somatic hybrids resulting from protoplast fusion.
- Molecular localization of a new *tgms* gene on rice chromosome 2.
- Application of SSR, AFLP and RGA marker techniques for DNA fingerprinting and diversity analysis using non-radioactive detection systems including silver staining.
- Application of isoenzyme techniques to detect polymorphism in tea clones.
- Characterization of genetic diversity of coconut by inverse sequence tagged repeats (ISTR) DNA analysis.
- The use of doubled-haploid techniques to accelerate breeding programs in *indica* rice.
- Agrobacterium-mediated transformation of *Brassica napus* using the transposable element Ac resulted in the possible resistance to *Sclerotinia* (stem rot).
- Application of RAPD marker technology in the selection of mutants.

Utilization of Induced Mutations for Genetic Improvement of Food Crops and Ornamentals

- In rice, corn and wheat, the application of mutation techniques has resulted in improved varieties for high yield, stress prone areas, and new cropping systems.

- Generation of variation for breeding purposes in *Dendrobium phalaenopsis*, *Chrysanthemum*, *Curcuma*, red ginger, *Canna*, *Dracaena* and *Muraya exotica* (Kamuning) for leaf color, flower size, color, and shape, and plant height through gamma radiation induced mutation and *in vitro* techniques.
- Development of gamma induced tomato mutants with high yield, resistance to bacterial wilt, and with improved fruit quality.
- Generation of EMS-induced tomato cotyledon mutants; screening made for narrow petioles (*npc*) and polycotyledon (*poc*) phenotypes; the trait is recessive mutation as confirmed by genetic segregation analysis.
- Release of mutant-derived varieties of food legumes. Development of a high yielding, beanfly resistant mungbean variety, CNM-BER 8709-5, through gamma ray irradiation. Development of improved rust resistant and lodging tolerant soybean through induced mutations.
- Development of screening methodology for use in the evaluation of chickpea mutant populations using traits related to drought tolerance (e.g., root and canopy traits, crop water use efficiency and water loss) rather than yield as parameters.
- Modification of agronomic traits and oil quality in oil seed crops (*Brassica* oilseeds, sunflower, sesame).
- *In vitro* mutagenesis for induction of variation in banana for crop duration and morphological traits through multiple shoot tip and cell suspension cultures.
- Mutation induction studies in roselle and tea have been initiated.
- Chromosome differentiation (karyotyping) and agronomic characterization of different Mongolian *Allium* species for biodiversity evaluation was conducted.

Conclusions

The five-day seminar clearly demonstrated the significant progress and impact of the application of mutation techniques in the development and utilization of improved varieties of various economically important crops. The integration of related molecular genetic techniques in the characterization of mutant derived lines and varieties clearly illustrated the potential of these techniques in complementing and accelerating breeding programmes through marker-aided selection, diversity analysis, and fingerprinting for plant variety protection.

Scientists from the participating countries in the Region believe that seminars of this type and technical visits to established research centers and institutions are very informative and essential and are therefore highly desirable in the future. The activity provided scientists with the rare privilege to be acquainted with and updated on recent advances on induced mutations, tissue culture, and molecular techniques of the participating countries. The seminar gave the participants the opportunity to meet, interact, and discuss with other scientists available and relevant technologies. It is envisioned that the recommendations raised from this Seminar will be met with positive consideration by the FAO/IAEA and other key players of the crop improvement programs in the Region (NARSs, CGIAR institutes and IPGRI).

Regional (AFRA) Training Course On "Molecular Characterisation Of Genetic Diversity Of Traditional And Neglected Crops Selected For Improvement Through Mutation Techniques", Pretoria, South Africa, 6-17 September 1999

The Workshop was hosted by the ARC - Roodeplaat Vegetable and Ornamental Plant Institute (VOPI), Pretoria, Republic of South Africa for scientists from national research teams participating in the AFRA III-18 project with evaluation of germplasm collections and development of mutant generations of selected traditional and neglected crops. The purpose of this workshop was to train at least one breeder per team in various molecular techniques (RAPDs, RFLP, AFLP, micro satellites, comparative mapping) for characterising diversity in selected traditional and neglected crops. Lectures and practical classes given by ARC staff and two international experts addressed these areas. Thirteen participants from Algeria, Cameroon, Egypt, Ethiopia, Ghana, Libya, Morocco, South Africa and Sierra Leone were trained during the course.

RAF/5/035 Regional Training Workshop on "Hands-on Experience on Molecular and Mutation Techniques", Seibersdorf, Austria, 13-24 September 1999

The workshop was a training activity of the regional project on "Control of bayoud disease in date palm". It was held in the Plant Breeding Unit, Seibersdorf, Austria from 13-24 September 1999. Eight crop scientists from Algeria, Morocco and Tunesia were trained in the use of chemical and physical mutagens, molecular techniques such as RAPD, AFLP and RFLP analysis for assessment of genetic diversity in date palm and identification of the *Fusarium* pathogen causing the bayoud disease. The training will enable them to integrate recent molecular tools in their date palm improvement programmes.

D. STATUS OF EXISTING CO-ORDINATED RESEARCH PROJECTS

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

This CRP was initiated in 1998 with the objective to overcome 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: quinoa (*Chenopodium quinoa*), cocoyams (*Colocasia esculenta, Xanthosoma* spp.), yams (*Dioscorea* spp.), grain and vegetable amaranths (*Amaranthus* spp.) Bambara groundnut (*Vigna subterranea*), grasspea (*Lathyrus sativa*), okra (*Abelmoschus esculentus*), bitter potatoes (*Solanum jucepzukii, Solanum ajanhuiri*) and naranjilla (*Solanum quitoense*). At present there are 18 participating institutes from Bolivia, Costa Rica, Ecuador, France, Germany, Ghana, India, Indonesia, Mexico, Slovakia, South Africa, Syria and Thailand including an agreement holder from IPGRI based at ICARDA. It is planned to hold the next Research Co-ordination Meeting in Costa Rica from 26-30 -June 2000.

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

This CRP was initiated in 1994 with the general aim to integrate 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 readily be regenerated via somatic embryogenesis for large-scale plant production, which is ideal for *in vitro* mutagenesis to the selection of mutants with desirable agronomic traits. Flow cytometry analysis of nuclear DNA content in Musa showed that Musa A and B genomes differ in size. Since 1996, Belgium has become an important contributor to this CRP. Twenty institutions world-wide are involved. The 3rd RCM was held from 4-8 October 1999 in Colombo, Sri Lanka.

Mutational Analysis Of Root Characters In Annual Food Plants Related To Plant Performance

This CRP was initiated this year with the overall objective to assist Member States in the application of mutation techniques and related biotechnology to generate and utilise mutants for the identification of root properties and genes for improvement of productivity and sustainability of crop plants. At present there are 21 participating institutes in this project**.** It is planned to hold the first RCM in Vienna, from 14-18 February 2000.

Molecular Characterization Of Mutated Genes Controlling Important Traits For Seed Crop Improvement

This CRP was initiated at the beginning of 1999 with the aim to assist Member States in the application of molecular genetics of mutated genes to improve production in both major cereals and related under-utilised crops. More specific objectives were to: (a) collectively develop, characterise and data base mutant collections of key crops for application by CRP members and the world scientific community; (b) to molecularly characterise new or existing mutants affecting key agronomic traits in major crops using comparative approaches in under-utilised crops with a view to their eventual isolation. The first RCM was held in Vienna from 4-8 October 1999 and was attended by 19 research contract/agreement holders. The second RCM is planned for 2-6 October 2000, also in Vienna.

E. NEW CO-ORDINATED RESEARCH PROJECTS

Improvement Of Tropical And Subtropical Fruit Trees Through Induced Mutations And Biotechnology ***(project not yet approved)***

This project will have the specific objective to develop and characterize highly performing induced mutants of tropical fruits. Up to 12 research contracts and 5 research agreements are expected to be awarded. In addition, it is foreseen that two technical contracts will be awarded to facilitate scientific activities in the following areas: developing of plant regeneration and propagation systems of recalcitrant tropical fruit crops, and molecular characterization of mutants with diseases and pest resistance.

F. TECHNICAL CO-OPERATION PROJECTS

In keeping with past practice, in this issue of the Plant Breeding and Genetics Newsletter we will again highlight the activities and achievements in a Technical Co-operation (TC) Project. The model project in Peru is reported on below:

PER/5/024:

The use of induced mutations for improvement of barley was initiated in 1979 under the IAEA TC project. The co-operation of the Cereals Programme with the IAEA has been continued up till now. In 1986, when the ARCAL VII programme was initiated, the staff actively participated in training and transferring of modern biotechnology to ongoing breeding programmes. In 1990, seeds of barley variety 'Buenavista' (also developed by UNALM) were treated with gamma rays. ^{60}Co source from the Instituto Peruano de Energia Nuclear were used for the treatment. The high frequency of various mutations was observed in M_2 generation after treatment with 300Gy. Naked (hull-less) grain mutation was very often observed in this and following generations. From this treatment a semi-dwarf, two-rowed, hull-less and hanging spike mutant was selected. After selection work and evaluation of this mutant line in multilocation trials a new mutant variety was released in April 1995 under the name 'UNA-La Molina 94'. The parent variety 'Buenavista' was already well adapted to highland conditions. Nevertheless, the mutant variety improved this characteristic through more still straw (increased resistance to lodging), hanging spikes (increased resistance to hail damage) and earliness (about 2-3 weeks earlier than parent variety). This last character is especially important as a shorter vegetation period can help to avoid adverse climatic factors such as frost, drought and hail.

The new barley mutant variety 'UNA-la Molina 95' is especially suitable for cultivation on elevations above 3,000 meters. At such elevations, where about 7 million people live, only a few crops can be cultivated. The new barley variety can be cultivated on marginal land and bring an economically justified income. High nutritional value of this naked barley should significantly improve food security and food self-sufficiency in these remote areas. The high suitability of 'UNA-La Molina' variety for the food processing industry, is also opening a new market for local farmers.

On the basis of these results a new model project was established on "Introduction of barley and other native crop mutant cultivars to Peruvian highlands". The Cereals Programme of UNALM has taken responsibility as the main counterpart and co-ordinator of this project. The IAEA began collaboration with this department more than 15 years ago. The collaboration developed on the basis of several Technical Co-operation projects, participation in ARCAL VII project and through Research Contracts in a regional FAO/IAEA Co-ordinated Research Programme on "The use of mutation techniques for improvement of cereals in Latin America".

La Corporacion Backus, Maltaria Lima supports this project through organisation of five demonstration plots in two State Departments (La Libertad and Huancavelica). This counterpart also helps in large scale selection of barley mutants for improvement of malting quality. As this company is also involved in food processing, it is a potential recipient of naked barley, quinoa and kiwicha seeds. It is expected that during the next few years the market for naked barley will reach more than 25,000 t. This means that a barley growing area of about 12-15,000 ha will be necessary to meet these requirements. The company is also interested in genetic improvement of quinoa. As a large seed type of quinoa is necessary for food processing, the company is importing this material from Bolivia. Peruvian farmers grow small seed type landraces, better adapted to local conditions.

IPEN will participate in this project through a seed irradiation service. It is expected that new irradiation experiments will be initiated to continue development of new mutant varieties in all three crops being the subject of this project. This collaboration is especially important as radio-sensitivity tests for various landraces of quinoa and kiwicha have to be performed. It should be noted that there is a lack of scientific information related to the radio-sensitivity of these two crops.

The outputs of this project are:

Commercialization of the first naked barley mutant variety in Peru through extension of this variety on the highlands, and at the end of the project to reach 20,000 ha of production on farmer's fields. Development of quinoa and kiwicha radiation induced mutants with desirable characters for breeding new varieties. Establishing practices for rapid extension of new varieties and in this way to sustain production of cereal crops in the highlands. Simulation of an integration of nuclear techniques and modern biotechnologies in agricultural research -- especially in plant breeding. Short and long-term objectives of this project are directly oriented towards the Peruvian farmers living in Andean highlands.

Current Operational Projects are:

BGD/5/019	Extension services to farmers on promising mutant varieties
COL/5/017	Mutation breeding of plantain and rice
COS/5/021	Radioactive probes for plant disease diagnosis
COS/5/023	Improved mutant varieties of rice and banana
CPR/5/010	Induced mutations for improvement of rice
CPR/5/011	Improvement of cotton and rapeseed through induced mutations
CPR/5/013	Induced mutations to improve rice quality
ECU/5/020	Resistance to disease in cacao and babaco

ELS/5/008	Improvement of potato through *in vitro* mutation breeding
ETH/5/011	Improvement of tef through mutation breeding
GHA/5/026	Improvement of cassava through mutation breeding
GUA/5/012	Mutations and biotechnology for crop improvement
INS/5/026	Mutation breeding of bananas
INS/5/027	Mutation breeding of ornamental plants
IRA/5/007	Mutation techniques for crop improvement
IRQ/5/011	Nuclear techniques in cereal production
IRQ/5/015	Induction of mutations in crops through *in vitro* culture
KEN/5/021	Improved drought resistance of crops by induced mutations
MAG/5/008	Mutation techniques and biotechnology for rice and cassava
MAK/5/004	Mutation and doubled haploid techniques to improve wheat
MAL/5/021	Mutation breeding and biotechnology for plant improvement
MLI/5/014	Field performance of selected mutants of sorghum and rice
MON/5/009	Nuclear techniques to improve production of wheat and legumes
MYA/5/008	Mutation breeding in grain legumes
PAK/5/033	Development of leaf curl tolerant varieties of cotton
PAK/5/035	Development of salt tolerant varieties of basmati rice
PAK/5/039	Pest resistant chickpea through induced mutation
PER/5/024	Introduction of barley and other native crop mutant cultivars
PHI/5/027	Mutation breeding of priority agricultural crops
RAF/5/029	Nuclear techniques in plant breeding and biotechnology (AFRA X)
RAF/5/035	Control of bayoud disease in date palm
RAF/5/042	Development of improved crop varieties (AFRA III-18)
RAS/5/037	Mutational enhancement for genetic diversity in rice (RCA)
RLA/5/035	Evaluation of cereal crop mutants (ARCAL XXIA)
SRL/5/030	Mutation breeding in bananas and plantains
SUD/5/023	Improving cotton and sugar cane crops (phase II)
THA/5/045	Radiation induced mutations for bean and chrysanthemum
URT/5/020	Improving productivity of basic food crops in Tanzania
VEN/5/018	Genetic improvement of fruits and pepper
VIE/5/013	Improvement of basic food crops through induced mutations
VIE/5/014	Rice mutant varieties for saline land
ZAM/5/020	Improvement of beans through mutation breeding

G. ACTIVITIES AT THE PLANT BREEDING UNIT, SEIBERSDORF

Activation Of Retrotransposable Elements In Rice And Insertional Mutagenesis As A Tool For Cloning Genes Of Interest

Our investigations are dealing with the activation of retroelements by stress conditions. The effect of tissue culture on the activation of the rice retrotransposon Tos 17 was shown by Hirochika et al. (1996). We have generated DNA probes based on the sequences of Tos 17 and two other potentially inducable retrotransposons in rice (Tos 10 and Tos 19). Using these probes in Southern hybridizations to DNA from Taipei 309 plants derived from radiosensitivity tests, the integration of new transposon copies into the genome as a consequence of gamma irradiation was shown. The number of new copies per plant was found to vary between 1 and 5, which is less than the copy number reported for tissue culture induction. This is of advantage for the aim of gene tagging by insertional mutagenesis because the identification of an affected gene will be easier than with multiple new integrations. On the other hand only 10 % of the analysed plants exhibit transposed Tos 17 copies. That means that too many plants are needed for a tagging strategy. Current experiments are aimed at the combination of different stresses to increase the number of plants with transposed elements. These experiments are mainly focused on the transcription of the transposon sequence and the level of its mRNA. Northern blots and RT-PCR are used for this analysis.

Since the role of transposable elements as a reason for somaclonal variation is often discussed, the retrotransposon sequences were also used for analysis of variants which were derived from anther culture of the indica variety Pokkali. These so-called gametoclonal variants are characterised by semi-dwarfness and photoperiod insensitivity. Using Tos 17 as a probe, a polymorphism in the number of restriction fragments was detected. The gametoclonal variants did not show a 3.5 kb band which was present in the seed derived control as well as in the phenotypically normal anther culture derived plants. The DNA methylation status of the controls and the gametoclones was checked. It was proved that the missing band was not due to changes in the methylation pattern at that specific site. The most likely reason for the missing band could be the aberration of a chromosomal fragment which bears a transposon site. If this site is also linked to the phenotypic change has to be verified by crossing experiments. As a molecular approach the sequences adjacent to the 3.5 kb band in the normal plants are going to be isolated by inverse PCR and further analysed by subsequent sequencing and homology search.

The Effect Of Propagation Methods On Cytochimera Dissociation

In-vitro mutagenesis of multicellular meristems of *Musa* spp. has a major limitation of a high degree of chimerism. Usually, repeated vegetative propagation must be carried out to dissociate chimeras but detailed studies as to the number of cycles required (e.g. three) has yet to be verified. In general, mutated cells are difficult to monitor, however mutations which result in a change in genome number may be an exception in this respect since they can easily be induced by colchicine treatment and can be detected by flow cytometry. Colchicine treatment induced ploidy chimerism (mixoploidy), and chimera dissociation was assessed by micropropagating with three different propagation systems (shoot-tip culture, multi-apexing culture and corm slide culture).

The results showed that when using shoot-tip culture, during three subcultures just after colchicine treatment, the average percentage of cytochimeras was reduced from 100% to 36% and from 100% to 24 % when propagating by the corm slide culture technique whereas the multi-apexing technique allowed a reduction of the average percentage of cytochimeras from 100% to 7% after the same number of subcultures. Nevertheless, none of the systems led to complete elimination of chimerism. Presumably, a combination of a high proliferation rate and production of adventitious buds could explain why the multi-apexing technique favours chimerism dissociation. The fact that 7% of the Pisang Mas shoots propagated through the multi-apexing technique remained chimeric suggests that the shoots may have axillary and/or an adventitious origin. In some instances adventitious shoots develop in conjunction with axillary shoots.

The results have shown that flow cytometry is a suitable method for monitoring cytochimera dissociation. One way to dissociate chimerism faster is by using the multi-apexing technique. Nevertheless, in periclinal chimeras, another propagation system based on a single cell origin will be needed. By mixoploidy induction and flow cytometry detection, it would be possible to verify the unicellular (or multicellular) origin of any propagation system. Understanding of genetic mosaicism was previously important mainly in the area of mutation induction. Biotechnology has advanced so rapidly that the knowledge of factors causing genetic mosaicism and chimerism will be important in many areas including genetic engineering.

Radiation Service

The Plant Breeding Unit of the Agency's Laboratories in Seibersdorf has for many years provided a cost free service of treatment of plant material with fast neutrons. The actual treatment was carried out by the Austrian Research Center, Seibersdorf. Their reactor was closed down at the beginning of July 1999 and we are no longer able to provide radiation service with fast neutrons. The radiation service with gamma radiation is not affected by these changes.

Radiation service statistics (1999):

requests	74
gamma ray treatments	29
fast neutron treatments	45
treated species	30
recipient Member States	20
seed samples	72
in vitro material	2

H. PUBLICATIONS

Maluszynski, M., Ahloowalia, B., Ashri, A., Nichterlein, K. and van Zanten, L. (1999) Induced mutations in rice breeding and germplasm enhancement. Proceedings of The International Rice Commission, 19th Session, Cairo, Egypt, 7-9 September 1998, 194-204.

Maluszynski, M. (1999) Crop germplasm enhancement through mutation techniques. In: Rutger, J.N., J.F. Robinson and R.H. Dilday (Eds.), Proceeedings of the International Symposium on Rice Germplasm Evaluation and Enhancement, Stuttgart, Arkansas, 74-82.

Kodym A, Zapata-Arias F.J. (1999) Natural light as an alternative light source for the *in vitro* culture of banana (*Musa acuminata* cv. 'Grande Naine'). Plant Cell, Tissue and Organ Culture 55: 141-145.

Mulu Ayele, Dolezel J., Van Duren M., Brunner H., and Zapta-Arias F.J. (1996) Flow cytometric analysis of nuclear genome of the Ethiopian cereal Tef (*Eragrostis tef*). Genetica 98: 211-215.

Roux, N.S., Dolezel J. and Zapata-Arias F.J. (1999) Cytochimera dissociation through shoot-tip culture of mixoploid bananas", Plant Biotechnology and *In Vitro* Biology in the 21st Century (Proc. 9th Int. Conf. Jerusalem, 1998), Current Plant Science and Biotechnology in Agriculture, Vol. 36 (Altman A, Ziv M, Izhar S, Eds) Kluwer Academic Publishers, Dordrecht, Boston (1999) 255-258.

In vitro techniques for selection of radiation-induced mutations adapted to adverse environmental conditions. TECDOC (in press)

Induced mutations for sesame improvement. TECDOC (in preparation)

Radiation induced mutations and other advanced technologies for the production of seed crop mutants suitable for environmentally sustainable agriculture. (External publication by Kluwer Academic Publishers --- in preparation)

Induced mutations in connection with other biotechnology for crop improvement in Latin America. TECDOC (in preparation)

Radioactively labelled probes for crop improvement. TECDOC (in preparation)

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.):

Plant Breeding and Genetics Newsletter

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International Atomic Energy Agency
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