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# PLANT BREEDING AND GENETICS



# NEWS LETTER

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in Food and Agriculture  
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International Atomic Energy Agency  
Vienna



CO-ORDINATED  
RESEARCH  
PROJECTS

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TO THE READER.....	1
A. STAFF .....	2
B. FORTHCOMING EVENTS.....	3
C. PAST EVENTS .....	3
D. STATUS OF EXISTING CO-ORDINATED RESEARCH PROJECTS .....	11
E. NEW CO-ORDINATED RESEARCH PROJECTS .....	14
F. ACTIVITIES AT THE PLANT BREEDING UNIT, SEIBERSDORF .....	18
G. PUBLICATIONS .....	21

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## TO THE READER

Dear Reader,

This is the first issue of the Plant Breeding and Genetics Newsletter. The Newsletter will inform you about current activities of the FAO/IAEA sub-programme on plant breeding and genetics which is implemented by the Plant Breeding and Genetics Section of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture (Vienna) in close collaboration with the Plant Breeding Unit of the FAO/IAEA Agriculture and Biotechnology Laboratory (Seibersdorf). The work of this sub-programme goes back to 1964 when, with the establishment of the Joint FAO/IAEA Division, activities were initiated to realise the potential of different types of ionizing radiation as well as radiomimetic compounds to induce agronomically useful mutations in crop plants.

Interest in the use of mutation techniques to generate and select desired genetic variation in crop species has increased significantly during the last decade. This has been mainly due both to the substantial success in applying *in vivo* mutation techniques in breeding new, improved varieties and to the new opportunities for induced mutations in vegetatively propagated crops using *in vitro* techniques and the advances made in rapid, often non-destructive, mass-screening methods. Also, recent developments in plant molecular genetics have indicated that induced mutations are probably the most effective approach to generate desired genetic polymorphism in plant characters and hence mutational analysis of plant structure and function has become the main interest of many laboratories. On the basis of these developments the Section now implements activities within the framework of the following general mandate: To support national plant breeding programmes and enhance biodiversity through applying mutation techniques and modern biotechnologies to improve local, often neglected major food crops in marginal and stress-prone areas, and to domesticate plant species with potential value for food or export products in partnership with National Agriculture Research Institutes and International Research Centres.

Already in the early 70's the need was expressed by numerous co-operators for circulation of a Newsletter on induced mutations and their applications in plant breeding and genetics, and the Section followed this up by publishing the first issue of its Mutation Breeding Newsletter (MBNL) in May 1972. Over the following 20 years, the MBNL was edited by Alexander Micke, the then Section Head (1969-1991). Over the years the MBNL, developed into a scientific journal and due to increasing number of scientific papers, essentially no space was left to provide researchers with information about the activities of the sub-Programme on plant breeding and genetics. This information gap has become increasingly apparent as our activities have expanded into the application of *in vitro*, molecular methods and other related biotechnologies in Co-ordinated Research Projects, Training Courses or Technical Co-operation projects. To fill the information gap we have therefore decided to publish this Plant Breeding and Genetics Newsletter (PBGN) every 6 months so that you have more information about current and planned activities of the FAO/IAEA Plant Breeding and Genetics sub-Programme. It is expected that in the near future, it will also be available on the Joint Division's home pages on the Internet. I would like to emphasize, however, that this Newsletter does not replace the MBNL. In fact, the MBNL will continue to publish scientific papers related to the application of mutation techniques in plant breeding and genetics.

Mirosław Maluszynski

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## B. FORTHCOMING EVENTS

Third and final Research Co-ordination meeting on "In vitro Techniques for Selection of Radiation Induced Mutants Adapted to Adverse Environmental Conditions". 17-21 August 1998, Shanghai, China.

Third and final Research Co-ordination meeting on "Induced Mutations and Other Advanced Technology for Production of Crop Mutants Suitable for Environmentally Sustainable Agriculture". 24-28 August 1998, Stuttgart, Arkansas, USA.

Third and final Research Co-ordination meeting on "Induced Mutations in Connection with Biotechnology for Crop Improvement in Latin America". 5-9 October 1998, Lima, Peru.

## C. PAST EVENTS

**Final RCM of the FAO/IAEA/Italy Co-ordinated Research Project on Improvement of Basic Food Crops in Africa through Plant Breeding, Including the Use of Induced Mutations. Naples, Italy, October 30-November 3, 1995.**

This Co-ordinated Research Project, funded by the Italian Government, was initiated in 1989. The primary objective was to breed improved varieties of staple food crops of Africa with emphasis on the indigenous species and their local cultivars. Twenty persons participated from Burkina Faso, Cameroon, Côte d'Ivoire, Ethiopia, Ghana, Kenya, Liberia, Mali, Nigeria, Tanzania, Uganda, and 14 scientific reports were presented.

These included reports from 10 Research Contract holders from Africa, 3 Technical Contract holders from Italy and an update on research carried out at the FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf. The proceedings of the meeting are available as IAEA TECDOC-951 and can be obtained free of charge by writing to the Section. We hope that this document will be of value to researchers, students and policy makers alike in their endeavor to promote plant breeding and increase food production in Africa.

### Workshops

Under a regional Technical Co-operation project (RAF/5/035) on the control of Bayoud disease of date palm, three workshops were held. The first was on "Mutation induction and selection by using *in vitro* techniques", 6-17 November 1995 at FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf. The second was on "Development of *in vitro* techniques for mutant selection in date palm", and was held at INRAT, Degache, Tunisia, 20-25 May 1996. Seven researchers from Tunisia, Algeria and Morocco participated. The third workshop on "Use of molecular techniques for mutant selection", was held at INRA, Marrakech, Morocco, 10-21 March 1997. Six participants from Tunisia, Algeria and Morocco participated and it included lectures and laboratory exercises on DNA isolation, restriction, amplification, PCR and RAPD techniques.

### **Consultants Meeting on Bayoud Disease of Date Palm, IAEA, Vienna, 25-29 March 1996.**

Date palm plays an important role in the economic and social life in the Sahara. There is an old saying that "without date palm there is no life in the Sahara". Bayoud disease of date palm is caused by *Fusarium oxysporum* f.sp. *albedinis* (F.o.a). This disease has already destroyed 15 million date palm trees in North Africa and has become a trans-national problem requiring a trans-national solution, involving a free exchange of information and experience between participating countries and international technology transfer.

This consultants meeting was held to discuss Bayoud disease in depth and make recommendations to control it. The group considered current knowledge on the

fungus including: biotypes; the spread in North Africa; methods of early detection; rapid diagnostic techniques in mature palms; tests for resistance based on pathological, genetic and molecular methods; fungal toxin characterization to differentiate between biotypes and for co-culture experiments. The group made recommendations on the short and long term strategies to breed for resistance to Bayoud, including induction of variation for resistance to Bayoud in well established date palm varieties using induced mutation and *in vitro* techniques. Recommendations were made to develop procedures to select for resistance to Bayoud based on toxin co-culture *in vitro*. A document based on the recommendations was published.

### **Regional Workshop on Evaluation of Promising Cereal Mutants. INTA, Argentina, 21-25 October 1996.**

This workshop aimed at training scientists from national institutions on planning and conducting multi-location trials of promising cereal mutants. It covered the principles of designing and organizing regional multi-location trials, seed multiplication, exchange of mutant germplasm, collection of data related to agronomic performance of mutant lines, statistical evaluation of the data and analysis. The workshop programme consisted of lectures and round table discussions. The participants presented reports on the implementation of the

programme agreed during an earlier workshop held in Campinas, Brazil, from 4-8 December 1995. The workshop provided a forum for discussing problems related to exchange of germplasm among the participating countries. Biological tools for shortening breeding cycles were also discussed. The participants also presented their work plans for the next year of the project which were then discussed to establish a uniform design of the trials and procedure for evaluation of mutants. A report on this workshop is in preparation.

**Final RCM of the FAO/IAEA Co-ordinated Research Project on The Application of DNA Based Marker Mutations for Improvement of Cereals and Other Sexually Reproduced Crop Species, Vienna, Austria, 4-8 November 1996.**

In recent years, great strides have been made in the development of molecular markers suitable for studies of sexually propagated crop species. Markers that are based on the polymerase chain reaction (PCR) technique are suitably polymorphic for applications in breeding programs and will be used increasingly in crops important in both developing and developed countries.

The most commonly used DNA markers are restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNAs (RAPDs). Another marker technology, known as simple sequence repeats (SSRs) or "micro-satellites", has many desirable features, including the use of a PCR reaction instead of a blotting procedure, co-dominant inheritance, and genome-specificity in polyploids, but is expensive

and time-consuming to develop due to the requirement for DNA sequencing. Yet another new marker technique has recently become popular, namely Amplified Fragment Length Polymorphisms (AFLP). The AFLP technique is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA. Finally, the newest type of DNA marker is known as the "biochip". These are high-density oligonucleotide arrays bound to a solid microchip. The method can be used to characterize the spectrum of sequence variation in a population and can be applied to the analysis of many genes in parallel. Since biochips are still highly experimental and expensive to design, it may be some time before they are widespread in crop genetic systems.

*The conclusions and recommendations from this project are the following:*

- Different types of molecular markers are best suited to different types of mapping and breeding applications. Choosing the most appropriate marker system is essential for success. Effective planning and execution in the capture and management of marker data is also critical.
- Genetic markers, such as phenotypically-identified macro-mutants (those which have a large effect on the phenotype) and cytological markers can provide opportunities for scientists to assess the agricultural significance of a gene or character. Integrating this information with that derived from molecular markers makes macro-mutants even more significant for practical purposes, as shown for tomato and potato. Integration of cytological information with DNA markers enhances the understanding of genome architecture.
- The identification of mutations with linkage to RFLP, PCR or DNA fingerprinting markers has further increased the practicability of using mutants as exemplified in downy mildew resistance genes in lettuce where the relation between deletion breakpoints and molecular markers has provided greater resolution than meiotic recombination.
- Integrated mapping resources - including comparative maps between related crop species, genome databases, and clone banks - have been created. These resources enhance the utility of molecular markers in plant breeding and mutation analysis.
- In the long-run, the most powerful use of molecular markers in plant breeding may be the ability to clone genes known only by phenotype. It must be realized, however, that the cloning of genes of agronomic interest is currently an expensive, demanding enterprise which is only possible in the most advanced and well funded laboratories.
- Types of cloning strategies such as transposon tagging that directly mark sites, rely on efficient

transposon activity in the species of interest and have worked well in maize and tomato, however this may not be generally applicable. *Agrobacterium* mediated transformation is being utilized in a number of crop species, but its applicability is limited to crops where efficient transformation and regeneration and efficient means of screening for the desired mutants are available.

- The end result of identification of genes of economic interest is their application in the production of commercial materials. Transformation utilizing genes of economic interest is now a reality for many crops. A potential use of physical mutagenesis is to remove unwanted selective markers in the later stages of transgenic line evaluations.

- The primary obstacles to widespread use of markers are the lack of facile marker systems and the resources required to support their use. The advantages provided by markers are many but they depend on numerous variables such as crop biology, trait(s) undergoing selection, resources, and type of cultivar. However, it has become clear that markers should be considered as an option under many circumstances and aspects of crop improvement.

- Monoclonal and polyclonal antibodies have been routinely used for strain identification with viruses. It helps the assessment of the homogeneity and stability of the pathogen population, introduction of new strains and purity of inoculum. However, there are limitations to extending these techniques to other pathogens and the development of PCR-based DNA markers would make important contributions to crop improvement by clarifying the genetic architecture and repertoire of the pathogen populations.

- The majority of agriculturally important characters are multigenic, strongly influenced by environment and expensive to evaluate directly. Enormous progress has been made by DNA marker techniques, but the inability to describe and select for specific quantitative trait loci (QTL) may limit the breeders' ability to make progress in the future.

- Given the rather recent development of the suite of enabling technologies, adoption and implementation of molecular techniques has been very rapid. Many other cultivars will be developed through these methods for traits such as grain quality (starch properties, oil quality, protein quality), disease resistance, insect resistance, exploitation of heterosis (through engineered nuclear male sterility). These methods not only facilitate the production of novel cultivars, but also the production of cultivars in far less time.

- However, many challenges remain for complex (multigenic) characters such as drought-, cold- and salt tolerance, and yield. Considerable progress has been made using DNA markers to tag agronomically valuable genes in many crop species and thus the foundation has been established for using those markers and mutations for more rapid cultivar development in species largely ignored by the private sector. Virtually all phases of plant breeding, including selection of parents, prediction of progeny performance, progeny selection and varietal identification, have been affected by these tools. The prospects are very good for markers to positively affect the rate of genetic gain. The critical component then, will be maintenance and creation of genetic variation through the various methods.

The final technical document of this CRP was published at the end of 1997.

**FAO/IAEA Regional Training Course on Molecular Approaches, Mutations and Other Biotechnologies for the Improvement of Vegetatively Propagated Plants, Kuala Lumpur, Malaysia, 28 October - 8 November 1996.**

15 trainees from China, India, Indonesia, Malaysia, Pakistan, Philippines, Republic of Korea, Sri Lanka, Thailand and Vietnam participated in this course which covered the use of chemical and physical mutagens and their mode of action; the induction of mutations and selection under *in vivo* and *in vitro* conditions; micro-propagation methods for separation of chimeras and rapid multiplication;

and the use of molecular methods and DNA markers in mutant identification and selection. These topics were handled by the lecturers Dr. O. Kamra (Canada), Dr. R. Litz (USA), Dr. G. Kahl (Germany), Dr. Mohammed Bin Osman, Dr. Mak Chai, Dr. Normah, Dr. Nazir and Dr. Farida Shah (Malaysia). We thank the lecturers and the course director Dr. Zakri for their excellent contributions to the success of this course.

**Consultants Meeting on Biotechnology in the Next Millennium, Vienna, Austria on 2-6 December 1996.**

Consultants from Colombia, Germany, Pakistan, South-Africa and representatives from FAO and UNIDO exchanged visions and discussed the past lessons and future strategies for rapid integration of biotechnology in crop improvement for the developing countries. During the meeting an overview was given on the research strategy and programme activities of the Plant Breeding and Genetics Section of the Joint FAO/IAEA Division together with a broad overview on past developments on biotechnologies in the African, Asian and Latin American regions

The recommendations from this meeting can be used as guidelines for biotechnology activities in developing countries. These concern the creation of awareness on national and regional levels, the commitment of national institutions to biotechnology programmes, priority setting, the optimal utilization of already existing capacities, capacity building, and networking at the regional and international level. More specific guidelines were formulated on technologies such as *in vitro* and molecular techniques, and the development of human resources. The aspect of Intellectual Property Rights and the issue of biosafety was included in relation to the development of transgenics.

**First Co-ordination Meeting of the Regional Technical Co-operation Project on Development and Promotion of Improved Mutant Crop Varieties. Biotechnology and Nuclear Agriculture Research Institute, Ghana Atomic Energy Commission, Legon Accra, Ghana, 3-7 February, 1997.**

Project co-ordinators from Algeria, Cameroon, Côte d'Ivoire, Egypt, Ethiopia, Ghana, Kenya, Libya, Madagascar, Mauritius, Morocco, Sudan, South Africa, Sierra Leone, Tanzania, and Zaire participated in the meeting and planned the national and regional activities under the



project, especially the evaluation of mutant lines/clones developed under previous technical co-operation projects, research contracts or national programmes. It was agreed that the following mutant lines/clones will be evaluated in 1997/98: sesame and safflower in Egypt, cotton and banana in Sudan and cocoa in Ghana. Work plans were prepared and discussed.

**Final RCM of the FAO/IAEA Co-ordinated Research Project on Use of Novel DNA Fingerprinting Techniques for the Detection and Characterization of Genetic Variation in Vegetatively Propagated Crops. Bhabha Atomic Research Centre, Mumbai, India, 24-28 February 1997.**

During this CRP, experience was gained in building productive collaborations between research scientists and breeders using biotechnology as the common language allowing the breeder to explain his problems and the research scientist to propose strategies to resolve them. As a spin-off, the breeders and research scientists have been endowed with capacities to transfer the knowledge and know-how. The monologue between "Biodiversity Centers" and "Biotechnology Centers" has merged into a profitable dialogue. The pressure on modern breeders caused by the urgent demand for more and better disease resistant and high yielding food crops, increases the need for continuous improvement of plant varieties. The basis of genetic improvement is the optimization of gene interactions and is based on genetic diversity, which can be obtained from several sources, including: natural populations; products of sexual crosses (via recombination, segregation and selection); spontaneous mutations (aneuploidy, polyploidy, other mutations); induced mutations (physical and chemical); and insertional mutagenesis (transposable elements, T-DNA, retroposons).

DNA markers are integrally connected with the success of molecular breeding. Moreover, juvenile markers, i.e. markers evaluated at early stages of plant development, are needed by breeders in developing countries to identify new gene sources in the available biodiversity, to select parents in order to increase heterosis, to decrease the number of backcross generations, for gene introgression breeding programmes, for marker assisted selection (MAS) and, ultimately, for gene isolation and transfer via map-based or deletion-based cloning. Fortunately, sufficient numbers of powerful genetic markers have been developed in human biology.

PCR technology has boosted the scientific output and yield of this worldwide cooperative research. Based on PCR technology, neither a complex laboratory infrastructure nor highly trained and skilled staff are required to effectively genotype entire breeding populations with large numbers of individuals, as proven by marker assisted cattle breeding. PCR analysis is very simple, robust and reliable: a minimal quantity of total genomic DNA, even partially degraded, is enough to act as the template for multiplex amplifications. Germplasm assessment and genotyping for identification purposes (breeder's rights, variant authentication, conformity test) can be achieved with polymorphic loci (genetic fingerprinting). The homogeneity of *in vitro* mass propagated material can easily be monitored using recurrent checks during the whole process. PCR assays may be readily automated with no further human interference between sampling *in situ* (in the fields) and analysis "in silico" (on the computer screen), which will allow the transfer of molecular markers to the "grass roots".

### *Achievements of this CRP:*

Various molecular marker technologies were used to recognize genetic diversities in vegetatively propagated crops. The building of this research network permitted the transfer of technology and the enrichment of human and genetic resources available to individual projects where technologies of DNA profiling (RFLP, RAPD, SSR, AFLP, DAF, and RAMPO) were evaluated. Cost, convenience, reliability and information content were recognized as key criteria for selecting an appropriate profiling technology.

The association of markers and morphological traits led to the generation of maps in several species which allow the exploitation of alternative life cycles of vegetatively propagated plants. In this fashion, where linkage analysis (detection of linkage disequilibrium) is possible, there are no technological barriers to extend these strategies to the vegetatively propagated crops of interest and to genetically engineer agronomically important genes.

This CRP prepared the ground for advanced research by developing and applying techniques for the characterization of genetic diversity, taxonomic and phylogenetic relationship, cultivar identification, and in exceptional cases, the preparation of preliminary core maps in banana and yam. The techniques and markers can be used directly to continue research towards mapping and characterization of mutations and agronomically interesting traits in vegetatively propagated crops.

### *Results*

- Molecular markers have proven potential for identifying genetic variability in vegetatively propagated crops. Therefore this successful technology and its use should be continued.
- The multiplicity of DNA marker technologies should be maintained and expanded if required by a specific problem, as each technology (RAPD, RFLP, AFLP, DAF, STMS, RAMPO) has already provided useful results in a range of crops. Despite differences in cost, reliability, and need for prior sequence information, no single technique provides clear-cut experimental advantages.
- The network established in the present CRP has been effective and valuable, and should be maintained and strengthened either through organizational (future CRPs, other granting agencies, national programmes, institutional sources) or other means (e-mail, germplasm exchange, collaborative projects, exchange of personnel).

A final technical document is now being prepared for publication.

### **Regional Planning Workshop of Principal Investigators on Drought Tolerance. Centre Régional de la Recherche Agronomique du Rif, Station Centrale des Radioéléments, Tangier, Morocco, 12-16 May 1997.**

Plant breeders and plant physiologists nominated as principal investigators on drought tolerance from Algeria, Ethiopia, Kenya, Madagascar, Morocco, Sierra Leone, Sudan and Tanzania participated. During the workshop the first year's programme was planned for development and evaluation of drought tolerant mutant germplasm of cereals and legumes. Lecturers from ICRISAT and IITA participated in the workshop. It was also agreed that each country will focus activities on improvement of drought tolerance of one crop only with regional relevance: Algeria (barley), Ethiopia (chickpea), Kenya (wheat), Madagascar (groundnut), Morocco (durum wheat), Sierra Leone (maize), Sudan (groundnut) and Tanzania (barley). It was agreed that a multidisciplinary approach is needed and that breeders should

therefore collaborate closely with agronomists, physiologists and soil scientists in order to understand specific drought problems and establish drought selection sites for reproducible results. Trait-based selection for drought tolerance should be given high priority focusing on a few selected traits. Suitable selection techniques developed in International Agricultural Research Centres and other institutes should be adapted to national programmes to facilitate the identification of drought tolerant mutants.

**15<sup>th</sup> IAEA/FAO Interregional Training Course on Advances in Plant Mutation Techniques. Agriculture and Biotechnology Laboratory, Seibersdorf, 20 May to 27 June 1997.**

26 participants from Algeria, Argentina, Armenia, Belarus, Bulgaria, China, Cuba, El Salvador, Ethiopia, Ghana, India, Islamic Rep. of Iran, Kenya, Rep. of Korea, Myanmar, Nigeria, Pakistan, Peru, Poland, South Africa, Sri Lanka, Sudan, Tunisia, Uganda, Uruguay and Vietman were trained in basic aspects of genetics and breeding with special regard to their application to mutation induction and selection. Advances in *in vitro* techniques and molecular techniques were covered in relation to their use combined with mutation techniques. The course was planned and implemented to cover major problems and technical aspects of plant improvement in both seed and vegetatively

propagated crops. In addition to lectures by the staff of the Plant Breeding & Genetics Section/Unit, lectures were also given by Dr. A. Ashri and Dr. U. Lavi (Israel), Dr. S. Daskalov (Bulgaria), Dr. J. Dolezel (Czech Republic), Dr. Z. Kaczmarek (Poland), Dr. G. Kahl (Germany), Dr. A. Lebeda and Dr. Z. Ohnoutka (Czech Republic), Dr. H. Brunner, Dr. A. Micke and Dr. Schiessendopler (Austria), Dr. I. Szarejko (Poland) and Dr. A.M. van Harten (Netherlands). Special thanks to these persons for the time and effort they invested to make this course a success.

**Third and final RCM of the FAO/IAEA Coordinated Research Project on 'Induced Mutations for Sesame Improvement'. University, Bangkok, Thailand, 6-10 April 1998.**

16 participants from Australia, Bangladesh, China, Egypt, India, Israel, Kenya, Pakistan, Republic of Korea, Sri Lanka, Thailand Turkey, Uganda and USA attended this meeting which was held at the Kasetsart University in Bangkok, Thailand. The participants presented the final achievement on their individual projects and formulated conclusions and recommendations on the use of induced mutations for sesame improvement.

The main characters for improvement were determined, and for each of them a detailed description was given. Wherever possible the trait was split into components and recommendations were given for the mutagen treatment and selection criteria. The main characters described are yield potential, harvest index, seed quality, oil quality, disease resistance, shatter resistance, uniform maturity and male sterility. Selection criteria were defined for all these characters.

The use of induced mutations has proven to be a promising technique for the genetic improvement of sesame since many participants reported obtaining several mutants with desirable characters like indehiscence, determinate growth, increased seed yield, disease resistance and water logging resistance. In some, they reached the stage of regional or national yield trials for their mutant lines prior to possible release of new varieties. For example the participant from the Republic of Korea, Dr. C.W. Kang, recently officially release a mutant derived sesame variety 'Pungsankkae' which was obtained after crossing of a local Korean variety with Dr. Ashri's determinate mutant 'dt-45' from Israel. Future release of new mutant varieties is expected in the coming two years in countries such as Bangladesh, Egypt, Pakistan, Rep. of Korea. A local demonstration field was organized with accessions of mutant lines from the various participating countries. Observations, plant development and breeding objectives were discussed in the field. A wide variety of confirmed mutant lines was produced in this project and a database of these will be organized at the Plant Breeding and Genetics Section. It was recognized that exchange of useful germplasm was and remains very valuable and should be encouraged as much as possible. An assessment was made of the achievements and recommendations were made for future coordinated research efforts. A final technical document will be prepared for publication.

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

##### **Induced Mutations in Connection with Biotechnology for Crop Improvement in Latin America**

The project started in 1994 following the recommendation of plant breeders and geneticists involved in applications of mutation and related biotechnologies for crop improvement. The first Research Co-ordination Meeting was held in October, 1994 in Guatemala and concentrated on choosing the best approaches to solve

The final RCM is planned for 1998. At the moment the programme has 13 participants from Bolivia, Brazil, Chile, Colombia, Cuba, Ecuador, Guatemala, Mexico, Uruguay and Venezuela. Increased crop productivity has become the highest priority in most countries of the Latin America Region and among factors limiting this, the most important and most common are: soil aluminium toxicity, salinity, drought, lack of plant available

breeding problems in seed and vegetatively propagated crops. The second RCM was held in October, 1996 and was devoted to evaluation of results coming from development of new mutated generations and characterization of previously developed mutants with desired traits.

phosphorous in soil but also disease and pest susceptibility. Modern biotechnology, including induced mutations, offers an enormous possibility to breed desired varieties in a relatively short time. Additionally, both these techniques make available the breeding of some vegetatively propagated crops, which until now were improved mainly on a selection basis from natural or cultivated populations.

### ***In vitro* Techniques for Selection of Radiation Induced Mutants Adapted to Adverse Environmental Conditions**

The first RCM of this CRP was held in 1994 in Vienna, and the second in Cairo, Egypt, in 1996. It is planned to hold the 3rd RCM in Shanghai, China in August, 1998. At the moment the project has 10 participants from Bangladesh, China, Colombia, Egypt, Ghana, India, Netherlands, Pakistan, Peru and the USA.

The participants in this CRP are working on the improvement of potato, sweet potato, garlic, sugarcane, pineapple and alfalfa by combining *in vitro* techniques with induced mutagenesis to

select for resistance to salinity, freezing, heat, drought, and water-logging depending upon the particular adverse condition prevailing in their country. They reported results of radio-sensitivity tests on *in vitro* cultured plant material such as micropropagated plants and organogenic or embryogenic callus cultures. In addition, reports were presented on the modifications of culture media required to regenerate and multiply local varieties and to carry out *in vitro* selection for specific stress conditions.

### **Induced Mutations and Other Advanced Technology for Production of Crop Mutants Suitable for Environmentally Sustainable Agriculture**

At the moment the CRP has 20 participants from Belgium, Bulgaria, Brazil, Canada, China, Costa Rica, Japan, Pakistan, Poland, Sri Lanka, Turkey, UK and the USA. The second RCM was held in San Jose - Heredia, Costa Rica in 1995, and the final RCM is planned for August, 1998. At the beginning of the project it was recommended that breeders should pay more attention to the proper plant "ideotype" for each species being bred for sustainable crop production. More elaboration is needed on the proper characters related to earliness, vigor, abiotic stress tolerance, and on characters related to suitability for inter-cropping production. It was also pointed out that the use of doubled haploids (DH) needs to be increased in all breeding programmes as it has become very clear that the production of DH lines is capable of speeding up variety production in areas of sustainable agriculture. It was felt that rapid screening techniques are the key to plant improvement when developing mutants for production under stress conditions. It was concluded that there was a clear need for cheap, reliable co-dominant molecular marker systems. PCR clones that are species- or genome-specific (i.e. micro- or mini-satellites) should be developed, and the technology for their development should be made cheap, reliable and easy to utilize in most breeding programmes. Information about past and existing varieties of all crops and ornamentals produced by induced mutations was requested.

### **Radio-actively Labeled DNA-probes for Crop Improvement**

The first RCM under this programme was held in Vienna in 1995, and the second RCM was held in San José, Costa Rica in 1997. At the moment the programme has 8 participants from Costa Rica, Germany, UK and the USA.

Recent advances in DNA-based technologies offer the opportunity for molecular genetic techniques to positively affect plant breeding and genome diversity characterization in developing countries. The use of linked DNA markers makes possible the large scale application of indirect selection for important agronomic traits. This project helps to foster international co-operation for transferring modern biotechnology to developing countries with

the active participation of leading laboratories.

An important activity in this project is the promotion of probes and primers including:

- a) RFLP anchor probe sets for i) wheat, barley, pearl and foxtail millet, for ii) maize, rye, and rice, for iii) sorghum, and for iv) mungbean, cowpea, common bean and soybean.
- b) Primers for mapping and fingerprinting like arbitrary primers which can be used in genetic mapping and marker-assisted breeding, taxonomy and systematics, differential gene expression. The CRP participants recommended that distribution of Operon primers is limited to qualified developing countries with an appropriate level of technical competency to successfully utilize such primers. A set of 10 conventional 8-mer primers is available for distribution upon request. Other more specialized primer sets (e.g. 11-mer and 12-mer mini-hairpin primers) can also be made available.
- c) Primers for yam, chickpea, banana, plantain and phytopathogenic fungi
- d) Primers for virus detection.

*(for more information on this project, see the WWW page on the Internet under the following URL: <http://www.iaea.or.at/programmes/d2/radprobe.htm>)*

### **Improvement of New and Traditional Industrial Crops by Induced Mutations and Related Biotechnology**

The application of induced mutations and related biotechnologies to oil crop and fiber plant improvement programmes is the major emphasis of this CRP. It was established in early 1995 following recommendations of several expert consultancies to include both known industrial crops and potential industrial crops in the process of domestication. At present research activities of 16 participating institutes from Bangladesh, Brazil, Canada, China, Germany, Greece, Hungary, India, Pakistan, Spain, Turkey and US, are being co-ordinated. The current research related to improvement of oil crops as industrial crops is oriented towards the following objectives:

- a) Development of enhanced germplasm of sunflower, linseed, soybean, rapeseed, *Cuphea*, borage, cotton and jute for traditional and non-traditional, industrial and non-industrial use. This work will be accomplished by using induced mutations, natural genetic variants, engineered genes, classical breeding approaches and biotechnological methods;
- b) Development of mutagenic approaches for additional species and screening procedures for agricultural and industrial requirements;
- c) Development of suitable genotypes adapted to new areas and for new needs - agricultural and industrial;
- d) Assessment and demonstration of the potential of induced mutations to affect critical steps in various biosynthetic pathways leading to modifications in oil quality and other metabolites;
- e) Enhancement of regional and interregional co-operation in the area of inducing mutations by radiation and other means to obtain faster breeding progress.

The Second Research Co-ordination Meeting (RCM) under the FAO/IAEA Co-ordinated Research Programme was held in Giessen, Germany, 30 June - 4 July, 1997. It was attended by 17 participants, from Bangladesh, Brazil, Canada, China, Germany, Greece, Hungary, India, Pakistan and USA, and a representative of FAO who acted as a scientific co-secretary of the meeting. The excellent local arrangements organized by Prof. Wolfgang Friedt and his team are greatly acknowledged.

Recommendations made during the meeting regarded cotton, jute, soybean, sunflower, groundnut, oilseed *Brassica*, *Cuphea*, meadowfoam, flax and false flax. All agreed on the need for germplasm exchange and to follow international safety guidelines. Mutation induction protocols were optimized for different species; improved germplasm was developed by induced mutations, intra- and inter-specific hybridization and genetic transformation; molecular markers for marker-assisted selection were developed; genes were cloned (e.g. for fatty acid biosynthesis).

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

This CRP was established in 1994, following the initiative of the World Bank on the Banana Improvement Project (BIP), to join efforts on the improvement of one of the most important crops in many developing countries. The general objective of the project is to integrate radiation induced mutations, *in vitro* culture and molecular genetics methods into the conventional breeding of banana for generation of desired variation such as disease resistance, dwarfism and earliness. In January 1996, Belgium, which has been

an important contributor to the improvement of banana and plantain research in developing countries, joined this CRP through two unique institutes: the Laboratory of Tropical Crop Improvement K.U. Leuven which concentrates on banana genetic manipulation and the Plant Pathology Unit, Faculty of Agronomy, Gembloux (F.S.A.Gx.) which deals more with banana diseases. The research activities proposed in this CRP are in line with the research priorities established by the Belgian Administration for

Development Corporation (BADAC).

The following countries in addition to Belgium, are participating in the project: Brazil, Cameroon, Czech Republic, Colombia, Cuba, Ecuador, France, Germany, Guyana, Honduras, Israel, Malaysia, Philippines, South Africa,

Tanzania, Uganda, USA, Vietnam and Zaire

*International Organizations:*

IITA (Nigeria), INIBAP (International Network for Improvement of Banana (France).

## **E. NEW CO-ORDINATED RESEARCH PROJECTS**

### **Application of Biotechnology and Mutation Techniques for the Improvement of Local Food Crops in LIFDCs.**

#### *Background:*

Food production per capita in 'Low Income Food Deficit Countries' (LIFDCs) is on the decline mainly as a result of population increase. Production of export oriented commodity crops such as tea,

coffee, cocoa and palm oil, while important to the economy, has not helped populations at large to secure the minimal calories required to avoid malnutrition. Increased production and an assured food supply can be achieved by improving crop yields on

small land holdings and family-oriented, labour intensive agriculture. The strategy for increasing production should consist in maximizing stability of the farming systems by increasing yield per unit area without over-exploiting land resources. There are many traditional food crops adapted to the agroclimatic and biotic stresses which are used by local communities as the primary source of carbohydrates, proteins, minerals, vitamins and other micro-nutrients. Some of the crops are grown only in specific regions to supplement basic dietary needs but are not known to the outside world. It is essential that the local cultivars and land races are improved in yield, quality and tolerance to stress. Other food plants are completely neglected or "orphan" crops having received little or no attention with respect to developing improved varieties compatible with modern farming. The importance of improved crop varieties in enhancing food production and security is well recognized. In many LIFDCs, existing varieties of traditional crops are heterogeneous mixtures of land races, and often represent simply the adapted natural germplasm which needs to be improved through genetic manipulation.

There are several reasons for using mutation breeding techniques for improving food crops. Improvement of crop plants can be speeded up by combining *in vitro* techniques with mutation induction and mutant isolation, particularly in vegetatively propagated plants. The use of mutation techniques in haploids to recover homozygous mutants is much faster than conventional sexual recombination. These technologies are well advanced and are being used extensively to complement conventional plant breeding approaches in developed countries. These techniques, however, are either not available or are in their infancy in LIFDCs. The access to *in vitro* culture techniques and their application can thus play a significant role

in the improvement of traditional and neglected crops of these countries.

*Objective:*

Improved basic and neglected food crops in LIFDCs through application of biotechnology and mutation techniques to:

- a. Improve local varieties of basic food crops for yield and quality, early maturity, and tolerance to biotic and abiotic stresses.
- b. Initiate mutation induction in the local germplasm of neglected crops and promote their collection.
- c. Establish protocols for various *in vitro* techniques (such as micropropagation, somatic embryogenesis, haploid production) of basic and neglected food crops.
- d. Evaluate performance of mutants and parent varieties for nutritional value and quality traits (protein content, starch, cooking quality, shelf-life).

*Expected outputs:*

This CRP proposes to increase the food production potential by developing improved and better yielding breeding lines of local food crops through mutation induction and related biotechnologies, which could contribute to food security and income generation. It will, furthermore, enhance collaboration between the participants of the CRP in exchange of technologies and germplasm, with various NARS and with the relevant International Agricultural Research Centers.

*Action Plan:*

- Develop appropriate methodologies for the application of mutation techniques and related *in vitro* and molecular techniques in under-utilized local food crops.



- Induce mutations for desired traits in the different crops of interest.
- Broaden the base of breeders' populations and enhance their diversity.
- Select desired useful mutants and mutant lines for further evaluation.
- Incorporate promising mutant lines in existing crop improvement and cross breeding programmes.

*Expected participants:*

This project will involve participants from agricultural research institutions and universities from the LIFDCs. Twelve research contracts are expected to be awarded. In addition, it is foreseen that 3 technical contracts will be awarded for the development of specific mutation techniques and biotechnologies related to some of these neglected crops, and 4 research agreements will be made with researchers from non-LIFDC countries which have either active research programmes on related food crops or are engaged in developing technologies related to their improvement.

**Mutational Analysis of Root Characters Related to Drought Tolerance to Sustain Crop Production in Arid and Semi-Arid Zones**

*Background:*

Most of the world's hungry live in countries that are classified as low-income and food-deficit (LIFDCs). More than 80 countries are considered in this category and over half of them are in Africa. Most of the LIFDCs are located in areas where drought is a major factor limiting crop production. Critical evaluation of progress in plant breeding over past decades has demonstrated genetic improvements in yield under both favourable and stress conditions. The yield improvement under drought stress resulted partly from genetic improvement of yield potential and partly from improvement of stress resistance or

It is foreseen that the support for specific research needs such as mutation induction, *in vitro* and molecular techniques related to the project will be carried out by the Plant Breeding Unit of the FAO/IAEA Agriculture and Biotechnology Laboratory in Seibersdorf.

It is planned to start this CRP in 1998, the final selection of crops, objectives and approaches will be made after the Proposals are evaluated.

To apply for a contract/agreement in this programme, please complete a Proposal for Research Agreement or Research Contract Proposal and return it to the IAEA's Research Contracts Section.

growth duration. Genetic and plant physiological investigations have indicated that several mechanisms lead to an increase of drought tolerance in cereals. Among them are: maintenance of high leaf water potential under stress, deeper root growth, higher root density, higher root mass, stomatal control over transpiration, osmotic adjustment, proline accumulation, high epicuticular wax load and cellular membrane stability. All these mechanisms are usually polygenically inherited and as such are difficult to apply as selection criteria. It is also considered that the use of yield as a selection index under stress is inefficient and usually limited to a

particular drought condition. It is therefore recommended to develop trait-based selection procedures for improvement of drought tolerance.

There is no doubt that drought tolerance depends on desired characters of the stem and root system. Although many investigations were carried out on various stem characters in relation to abiotic stress tolerance, investigations on root system characters are relatively limited. The simple fact that roots grow in soil and as such are invisible is the greatest inhibitor to advancement of knowledge on root systems and particularly about their genetics. For the same reason there is very limited knowledge about the level of genetic diversity in root systems (only about 30 plant genera have been investigated for the evaluation of genetic variation of roots). Recent emphasis on the genetic diversity of root system has concentrated on the need for increased drought, salt, acid soil tolerance or nutrient uptake. Unfortunately, the great environmental component of root system variability is an additional factor complicating a clear genetic conclusion from such investigations. In contrast to the plant shoot where many morphological characters such as shape and colour of flower, leaf or culm were well described and genetically investigated long ago, in roots, there are only a few known characters which could be used as genetic markers facilitating genetic analysis of agronomically important physiological traits. In addition, where variation was discovered, it is often polygenic in nature.

Against this background geneticists and plant breeders are now using mutation techniques to generate monogenically inherited root morphological markers. This is now more realistic as some nondestructive methods of root investigation, including hydro- and aeroponics and computer image analysis, have been developed. Also very promising

results have been obtained on the model plant species - *Arabidopsis*. These studies have shown that numerous important root characters can mutate after treatment of seeds with physical and chemical mutagens or as a result of insertional mutagenesis, and that large numbers of already developed root mutants are monogenically inherited. Similarly, preliminary results obtained in barley and maize indicate a high frequency of root system mutants in mutated generations. It is therefore now possible to induce and develop a germplasm collection of root system mutants and to initiate mutational analysis of both morphological and physiological characters; thereby establishing suitable breeding programmes for increased tolerance to drought or other stresses, with an improved root system as the main component. The latest developments in biotechnology will facilitate implementation of such a programme through rapid homozygotization of mutants and molecular marker assisted selection of desired genotypes followed by characterization, isolation and cloning of the most important mutated genes.

*Objective:*

To make mutational analysis of some of the essential characters related to root formation and functioning, their contribution to crops' tolerance to drought, and the effects of the relevant mutated root system characters including their various physiological manifestations and linkages.

*Expected outputs:*

This project will lead to development of mutant lines with desired root types and soil penetration dynamics. Furthermore, these mutant lines will be genetically characterized and a strategy will be developed for the use of root system mutants in cross breeding programmes for drought tolerance.

*Action plan:*

- Induce mutants with altered root system characters
- Select and evaluate promising mutants with desired root types and altered soil penetration habits.
- Conduct genetic analysis of the selected mutant lines.
- Incorporate root system mutants in existing crop improvement and cross breeding programmes in the specific plants.

If you are interested in applying for a contract/agreement in this programme, please complete a Proposal for Research Agreement or Research Contract Proposal and return to the IAEA's Research Contracts Section. The final selection of the crops, objectives and approaches will be made after the proposals are evaluated.

## F. ACTIVITIES AT THE PLANT BREEDING UNIT, SEIBERSDORF

The Plant Breeding Unit of the FAO/IAEA Agriculture and Biotechnology Laboratories at Seibersdorf provides technical support to the Joint FAO/IAEA Division's programme in the area of plant breeding and genetics. This support involves three major components;

- *research and development* in advanced breeding techniques using nuclear and biotechnological methods in crops such as

banana, plantain and rice;

- *training* of individual fellows and groups of scientists from developing Member States;
- providing mutation induction *services* and technical advice to scientists in Member States.

The current activities cover the following:

### **Induction and verification of autotetraploids in diploid (*Musa acuminata*) by *in vitro* techniques.**

Flow cytometry and stomata characteristics were used for screening ploidy levels in a large population of *in vitro* induced autopolyploids of the *Musa acuminata* breeding clone SH-3362. Culturing shoot tips in liquid medium supplemented with 5.0 mM colchicine for 48 hours or 30  $\mu$ M oryzalin (3,5-dinitro-N4,N-dipropylsulphate) for seven days, both in combination with 2% (v/v) DMSO, resulted in a high (23 % and 29 %) frequency of non-chimeric tetraploids in the fourth vegetative generation.

Although mixoploidy persisted in subsequent cycles of vegetative propagation, tetraploids as identified by flow cytometry remained solid non-chimeric during two more cycles. These autotetraploids were propagated for field testing. A rough pre-selection of regenerated V<sub>4</sub> plants based on their stomata characteristics resulted in a population in which only 56 % of the plants were solid tetraploids.

## **Histology of somatic embryo initiation and organogenesis from rhizome explants of *Musa* spp.**

Bananas and plantains are important staple food crops for people living in tropical and sub-tropical countries. Declining yields due to the spread of virulent diseases such as Black Sigatoka, Fusarium wilt and Banana Bunchy Top Virus, has resulted in increased efforts to genetically improve this crop. However, conventional breeding of *Musa* cultivars remains a difficult endeavor because of high sterility and polyploidy.

Plant tissue culture techniques can potentially overcome some of the factors limiting traditional approaches to banana and plantain improvement. These techniques enable plants to be regenerated from normal and genetically modified cells and tissues in an efficient way under sterile conditions. The generation of genetic variation by induced mutations or genetic transformation is a single cell event. Therefore treatment of multi-cellular cell initials leads to the formation of chimeras. Genetically altered and non-altered cell-lineages compete during the proliferation and growth of multi-cellular initials and lead to the formation of mericlinal or sectorial chimeras. These are not manifested phenotypically and require repeated subculture to rescue genetically modified homohistont tissue prior to mass-screening and/or selection for desirable traits. The genetic improvement of *Musa* by *in vitro* mutation breeding or genetic engineering requires the efficient regeneration of genetically modified single cells into complete plants.

The origin of somatic embryos derived from rhizome explants of triploid *Musa* c.v. Grand Naine, was the subject of histological studies during different phases of ontogenetic development. Our histological observations revealed that the embryogenic mass and somatic embryos developed in most instances from several morphologically competent adjacent cells while the occurrence of single cell origin in vascular tissues of rhizome explants of *Musa* was less frequent. The majority of somatic embryos showed normal root formation and consisted of highly vacuolated cells in the poorly structured shoot apex.

## **Preliminary studies on the use of mutation induction and anther culture in the improvement of *Eragrostis tef***

Tef is the single dominant crop grown widely amongst the indigenous and introduced field crops of Ethiopia. It is considered to be well adapted to drought and waterlogged conditions. Lack of adequate genetic improvement of the present cultivars and agronomic problems such as lodging and leaf rust are the major causes for low yield. Of the more than 2000 germplasm materials collected so far, none is resistant to lodging. Therefore, induced mutation breeding has been considered as one of the alternatives.

### *Assessment of optimal gamma ray and EMS doses*

Radiosensitivity of seeds to gamma rays was genotype specific. Suitable concentrations for treating tef seeds with ethylmethane-sulphonate (EMS) were between 0.6% and 0.9% with the applied chemomutagen treatment regime.

### *Anther culture*

Significant genotypic differences were noted in the callus formation in anther culture. A very high proportion (88-97 %) of the spikelets cultured produced calli. No significant difference was obtained among the four genotypes used and the response was found to be high both from gamma ray treated and untreated spikelets. The young spikelets of tef could be used as an excellent source of explant to induce callus for various tissue culture purposes. Studies still need

to be done to determine if spikelets are a possible source of explant for the production of doubled haploid plants.

### **Regeneration of *Ensete ventricosum* through somatic embryogenesis and adventitious buds**

In southern and south-western Ethiopia, *Ensete ventricosum* is grown as an important starchy, staple food crop, supporting the diet of a quarter of the Ethiopian population. Due to difficulty in germinating seed and the long vegetative period, breeding ensete is extremely difficult. Adventitious buds and somatic embryos were induced from callus derived from corm tissue

and cultured on Murashige and Skoog's (MS) basal medium supplemented with benzylaminopurine (BAP) or 2iP. Elongation of somatic embryos was achieved on the same medium and rooting was induced on half strength MS basal medium supplemented with IBA. No phenotypic variation was observed among more than 200 potted regenerants.

### **Assessment of genetic diversity in three African populations of malabar spinach (*Basella* spp.)**

Malabar spinach (*Basella* spp.) is an important leaf vegetable in the human diet of West Africa. The nuclear genome size, the ploidy state, the degree of genetic chromosome counting, flow cytometric techniques and RAPDs (Random Amplified Polymorphic DNA) analysis.

The sexually propagated samples, i.e. the two populations named Sri Lanka and Congo domesticated, showed the same ploidy level ( $2n=36$ ). These two populations also gave similar results with a total DNA content of 6.83 pg (2C). The third population, vegetatively propagated, showed a lower DNA content of 5.73 pg. In the vegetatively propagated plants complex mixoploidy was observed with chromosome numbers ranging from 36 to 48.

Fragment profiles obtained with the RAPD primers were scored manually as present (1) and absent (0). This method of scoring elucidated an average of 11.9 loci per primer.

variation present in the three African populations of Malabar spinach (Congo domesticated, Sri Lanka, vegetatively propagated) were investigated using From a total of 296 amplifications products scored, 31% were found polymorphic. Of the 94 primers, 35 detected no polymorphism although they successfully amplified a range of monomorphic bands. The average number of RAPDs detected per polymorphic primer was 3.7

The low level of polymorphism detected among the three African populations of Malabar spinach, may reflect the tendency of inbreeding crop species to develop towards homozygosity when compared to an obligate outbreeder. It can also be symptomatic of a narrow genetic base or a loss of diversity when introduced from Asia during the early colonial times in Congo, where plants probably derived from the few seeds or cuttings.

### Supporting services for application of mutation techniques in Member States

A radiation treatment service is provided to FAO and IAEA Member States to support national crop improvement programmes. Seed and vegetative plant structures were received and treated with <sup>60</sup>Co gamma rays and fast neutrons.

#### RADIATION SERVICE STATISTICS (1996-1997)

No. of treated samples	675
No. of treated species	36
No. of treated cultivars	159
No. of recipient Member States	30
Seed samples	674
Vegetatively propagated samples	1
No. of <sup>60</sup> Co gamma ray treatments	515
No. of fast neutron treatments	160

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