PLANT BREEDING AND GENETICS NEWSLETTER

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TO THE READER

Dear Reader,

This is the second 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 Systems (NARS) 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. The newsletter is also 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.

Miroslaw Maluszynski

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B. FORTHCOMING EVENTS

Second Project Co-ordination Meeting of the regional project AFRA III-18 on "Development of improved crop varieties" (RAF/5/042), 22-26 February 1999, Bamako, Mali.	Third and final Research Co-ordination Meeting of a FAO/IAEA Coordinated Research Project on Improvement of new and traditional industrial crops by induced mutations and related biotechnology, 2-6 August, 1999, Corvallis, Oregon, USA.	Third and final Research Co-ordination Meeting for FAO/IAEA Co-ordinated Research Project on Radio-actively Labeled DNA-Probes for Crop Improvement, 12-16 July 1999, Frankfurt, Germany.

Seminar on Mutation techniques and biotechnology for tropical and subtropical plant improvement in Asia and the Pacific Region, October 1999, Los Baños, The Philippines.

The main objective of this seminar is to discuss with scientists of the region the current status of mutation techniques and related molecular genetic approaches. The seminar will focus on the application of these technologies in the areas of plant research and crop improvement. The latest developments in genetic engineering present a number of possibilities for the improvement of crops not previously in the main stream of research. This especially applies to the crop species of importance for tropical and subtropical regions. Molecular genetics, in combination with mutation techniques, provides very powerful tools to enhance the production of local and traditional crops in stress prone areas and, in this way, to improve farm sustainability.

C. PAST EVENTS

Regional (AFRA) Training Course on Selection Methods for Drought Tolerance in Cereals and Legumes, Pretoria, South Africa, 20-25 April 1998.

The training course held in Pretoria, South Africa from 20-25 April 1998 was the second training activity under the component 2 of the regional project AFRA III-18 on "Development of improved crop varieties". It was jointly organised by FAO/IAEA and the Roodeplaat Vegetable and Ornamental Plant Institute of the Agricultural Research Council, Pretoria, South Africa. 11 plant breeders and/or agronomists from Algeria, Egypt, Kenya, Morocco, South Africa, Sudan and Tanzania were trained in various techniques for selection of drought tolerant cereals and legumes under field, greenhouse and laboratory conditions. This training was organised to enable research teams participating in the project to select more efficiently for traits related to drought tolerance under various drought conditions in segregating populations derived from mutation induction or cross breeding. In addition to lectures given by staff from various institutes in South Africa (Mr. J. Caetano, Ms. K. de Ronde, Dr. G. Krüger, Mr. A. Leucuona, Dr. F. Rossouw, Ms. R. Slabbert, Ms. M. Spreeth, Dr. M. Steyn, Ms. A. van der Mescht, Mr. W. de Clerq, Mr. J. van den Bergh), lectures were given by Dr. A. Blum (Israel) and Dr. B.B. Singh (IITA, Nigeria). Special thanks to these persons for the time and effort they invested to make this course a success.

Third and final RCM on "*In Vitro* Techniques for Selection of Radiation Induced Mutants Adapted to Adverse Environmental Conditions". 17-21 August 1998, Shanghai, China.

This RCM was organized by the Horticultural Research Institute, Shanghai Academy of Agricultural Sciences, and held at the Hotel Jin Sha. The President Prof. Pan Ying Jie and the Vice President Mr. Lu Nanxing of the Academy inaugurated the meeting, and thanked the Agency for holding it in China P.R. All 10 participants from Bangladesh, China, Colombia, Egypt, Ghana, India, Netherlands, Pakistan, Peru and the USA participated. From this meeting conclusions and recommendations were formulated covering the general aspects of mutation techniques in the target crops, and specific recommendations for each crop such as, potato, alfalfa, sweet potato, garlic, sugarcane, and pineapple. Appropriate *in vitro* culture protocols were developed, and optimal doses for mutagnesis in all crops determined. Advanced material with tolerance to adverse environmental conditions were developed in potato and alfalfa where some salt tolerant mutants were found, and in sweet potato where heat stress and drought tolerance was induced. A technical document on the outcome of this project will be prepared and published in 1999.

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

The meeting was held at the National Rice Research Centre (NRRC) of USDA-ARS, Stuttgart, Arkansas. More than 20 scientists from 14 countries took part in the RCM. Twenty scientific papers were presented summarizing results achieved under each Research Contract or Agreement.

On the basis of presented reports by contract and agreement holders the implementation of the CRP brought significant results of both scientific and economic value. Among the scientific results the most important are:

Methodologies: development of a very efficient method for production of doubled haploids through microspore culture in barley, improving systems for symetric and asymetric fusions in rice, application of doubled haploids for fixation of mutants heterotic performance in barley, identification of heterosis in crosses of somaclones in rice, improvement of systems for assessing aluminum tolerance in cereals, development of multilocus marker system for application in rice and barley, applications of GISH (geneomic in situ hybridization) for diagnosis of genomes in rice somatic hybrids.

Genetics and molecular analysis: description, genetic mapping and tagging of mutated genes for high tolerance to salinity in barley, genetic mapping and tagging of genes responsible for vernalization requirement and stress tolerance in *Triticeae*, tagging of two genes with resistance to head blight in wheat, establishment of homology aluminum tolerance genes of rye and wheat, description of pleiotropic effect of mutated anthocyanin biosynthesis genes on stress tolerance.

Induced diversity for breeding: new genes for aluminum tolerance in wheat and barley (Brazil, Poland), salt tolerant variety of rice (Costa Rica), high yielding - improved drought tolerant wheat mutant lines (Pakistan), improved doubled haploid mutant lines in rice (China P.R.), high yielding salt tolerant somaclones of rice (Burundi), high yielding mutant line of tartary buckwheat (China P.R.).

Regional (AFRA) Training Workshop to Review Mutant Germplasm Evaluation Experiments, Cairo, Egypt, 5-10 September 1998

The Regional Workshop held in Cairo, Egypt, was a review activity under component 1 of the Project AFRA III-18 on "Development of improved crop varieties". It was jointly organized by the IAEA and the Plant Research Department of the Atomic Energy Authority in Cairo. The objective of the workshop was to review the mutant evaluation trials in each of the participating countries, to discuss the work plans for 1999/2000 and the regional mutant germplasm exchange. The meeting was attended by plant breeders from Egypt (6), Ghana (2), Libya (1) and Nigeria (1), the Project Scientific Consultant and the Technical Officer from the Joint FAO/IAEA Division. Results of the evaluation trials of sesame, safflower, cassava, cocoa, barley, kenaf and sorghum were presented and reviewed, the work plans for 1999/2000 activities were discussed and revised and recommendations for further project implementation including regional mutant germplasm exchange were agreed upon.

The cassava mutant from Ghana - developed under previous Agency's support - was released in Ghana in 1997 and will be propagated *in vitro* for evaluation in other AFRA Member States. Three sesame mutants are under National Variety Testing in Egypt and were grown on six locations. All of them had increased seed yield in comparison to the standard variety and it can be expected that after another season of evaluation at least one mutant will be released as an improved sesame variety. The other crops need one or two more years of evaluation before they can be submitted for National Variety Testing. Revised work plans for the continuation of mutant evaluation of sesame, safflower, cassava, cocoa, barley, kenaf and sorghum were prepared. The regional exchange of promising sesame, safflower, barley, kenaf, sorghum, cassava, and cocoa mutants and procedures for its implementation was agreed. All officially released mutants will be included in the FAO/IAEA Mutant Variety Database.

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

The final FAO/IAEA RCM was held at the National Agricultural University La Molina, Lima, Peru. More than 16 scientists from 8 countries took part in the RCM. Nine scientific papers were presented summarizing results achieved under each Research Contract.

On the basis of presented papers it is possible to state that mutation induction has become an often used tool to generate biodiversity in Costa Rica, Cuba, Brazil, Guatemala, Peru and Uruguay. Leading institutes from these countries moved already to the application of mutation techniques in connection with modern biotechnology. This indicates

that the main objective of the CRP was fully met. The CRP also brought significant results in development of new technologies/approaches and in the generation of the desired diversity for breeding programmes.

New methodologies/approaches:

a) Induced mutation was very effective for obtaining germplasm carrying different single resistance genes effective against the different genetic families (lineages) of the blast pathogen (*Pyricularia grisea*) found in Colombia. Combination and accumulation of these resistance genes, leading to the development of resistant varieties, can be obtained by crossing those mutants carrying the different resistance genes. In addition, induced mutation produced mutant lines exhibiting intermediate levels (less disease than irradiated parent) or partial resistance to blast but of high importance in the event of occurrence of a breakdown of those genes conferring a complete incompatible interaction. Mutant lines exhibiting a partial resistance to other rice pathogens such as rice hoja blanca virus and/or its vector (*Tagosodes oryzicolus*) in Colombia and Cuba, and stem rot in Uruguay were also reported. These mutants can also be used effectively in crosses for the development of rice cultivars with resistance to these pathogens which have no known resistance genes.

b) A method to induce mutations in citrus trees by irradiating cuttings was successfully modified and applied to development desired new germplasm with agronomically important characters such as seedless, reduced plant growth or thin skin.

c) The phenomenon of high level mutant heterosis was confirmed on a large scale experiment with gamma ray induced wheat mutants in Chile. Heterotic effects were also observed in some experiments with barley and rice in other countries.

Induced diversity for breeding:

The CRP stimulated plant breeders from the Region to organize mutant collections of valuable mutants for conservation and as a valuable source of markers for genomic research:

Uruguay - 218 rice mutants from two varieties (characters: dwarfness, earliness, grain quality, disease resistance);

Cuba - more than 100 mutants from 5 rice varieties (dwarfness, earliness, grain quality, salinity tolerance, blast resistance);

Guatemala - 15 rice mutant lines with resistance to particular blast lineages;

Colombia (CIAT) - 15 rice mutant lines with resistance to particular blast lineages;

Brazil - 25 wheat mutants (aluminum tolerance, earliness, dwarfness), 20 rice mutants (morphological characters);

Peru - 155 barley mutants from three varieties, 300 quinoa mutant lines.

The successful implementation of induced mutation technologies in the Region, documented by already released mutant varieties or promising mutant lines has generated a demand to FAO and IAEA for establishing projects in new areas of application of induced mutations. The following projects were suggested, after long discussion, for consideration by the FAO/IAEA, as specially economically important for the Region:

1. The characterization of genetic structure and virulence diversity of rice pathogens in Latin America. Expected participating countries: Argentina, Brazil, Colombia, Costa Rica, Cuba, Guatemala, Peru, Uruguay, USA, Venezuela. Contributing organizations: FLAR and CIAT.

⇒ Objective: Development of durable resistance to rice pathogens for increasing rice productivity, reducing production costs and protecting the environment through molecular and virulence characterization of pathogen populations

⇒ Duration: 5 years

2. Fixation of F_1 heterotic performance through double haploids in cereals. Expected participating countries: Bolivia, Brazil, Colombia, Costa Rica, Cuba, Guatemala, Mexico, Peru, Uruguay.
   ⇒ Objective: produce rice, wheat and barley doubled haploid varieties with significantly increased yield potential and good grain quality through exploring phenomenon of mutant heterosis.
   ⇒ Duration: 6 years
3. Induced mutations for abiotic stress tolerance in Latin American Region. Expected participating countries: Bolivia, Brazil, Cuba, Mexico, Peru.
   ⇒ Objectives:
      * to increase productivity of highland crops through development of tolerance to frost and soil stresses mutant varieties
      * to develop mutant varieties with tolerance to drought, aluminum, salinity, alkaline soils in important crops for the Region.
   ⇒ Duration: 5 years
4. Induced mutations for conservation of biodiversity of native crops. Expected participating countries: Bolivia, Mexico, Peru.
   ⇒ Objective: to avoid genetic erosion of native crop species through germplasm collections and induced biodiversity in characters which increase their attractiveness for international market and local consumption by mutagenic agents.
   ⇒ Duration: 5 years
5. Evaluation of induced biodiversity in crop species in the Region through molecular markers Expected participating countries: Argentina, Bolivia, Brazil, Colombia, Costa Rica, Cuba, Guatemala, Peru, Uruguay, Venezuela.
   ⇒ Objective: To develop capabilities in countries of the Region to characterize induced biodiversity in crop species through molecular markers.
   ⇒ Duration: 3 years
6. Induced mutations for herbicide resistance of various crops. Expected participating countries: Argentina, Brazil, Colombia, Costa Rica, Cuba, Guatemala, Peru, Uruguay, USA, Venezuela.
   ⇒ Objective: Develop varieties of major crops with herbicide resistance through induced mutations. Reduction of the use of herbicides. Risk assessment on the use of herbicide resistant mutant varieties.
   ⇒ Duration: 5 years

FAO/IAEA Regional Training Course on Basic Mutation and In Vitro Culture Techniques for the Improvement of Vegetatively Propagated Crops for Sustainable Crop Production in Africa, Pretoria, South Africa, 16-27 November 1998.

This course was organized with the Roodeplaat Vegetable and Ornamental Plant Institute of the Agricultural Research Council, Pretoria, South Africa. The programme

covered basic mutation- and *in vitro*-techniques for the improvement of vegetatively propagated crops such as cassava, sweet potato, yam, banana, plantain, tropical fruit trees and ornamentals. Some 18 plant breeders from Algeria, Cameroon, Egypt, Ethiopia, Gabon, Ghana, Kenya, Libya, Mauritius, Niger, Sudan, Tunisia, Tanzania and South Africa participated.

First RCM on "Application of Biotechnology and Mutation Techniques for the Improvement of Local Food Crops in LIFDCs". 7-11 December 1998. Vienna, Austria.

The meeting was held at the Vienna International Centre. 15 participants from Bolivia, Costa Rica, Ecuador, France, Ghana, India, Indonesia, Italy, Mexico, Slovakia, South Africa and Thailand took part in the meeting. All presented their current activities in crops such as amaranth, bambara groundnut, bitter potato, cocoyam, grass pea, naranjilla, okra, quinoa, and yam. Specific work plans for the future were discussed and determined for implementation in the coming years

D. STATUS OF EXISTING CO-ORDINATED RESEARCH PROJECTS

Radio-actively Labeled DNA-probes for Crop Improvement

This project, which started in 1995, has at the moment 8 participants from Costa Rica, Germany, UK and the USA. 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. The third and final Research Coordination Meeting within this project is planned for 12-16 July 1999 in Frankfurt, Germany.
(see also: http://www.iaea.or.at/programmes/d2/radprobe.htm)

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

This CRP started in 1995 and emphasizes the application of induced mutations and related biotechnologies to oil crop and fiber plant improvement programmes. At present there are 16 participating institutes from Bangladesh, Brazil, Canada, China, Germany, Greece, Hungary, India, Pakistan, Spain, Turkey and USA. The third and final Research Co-ordination Meeting (RCM) will be held in August 1999 in Corvallis, Oregon, USA. (See also 'Forthcoming Events')

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

This CRP was initiated in 1994 with the general goal 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. Since 1996, Belgium has become an important contributor to this CRP. The next Research Coordination

Meeting is planned for October 1999 in Douala, Cameroon.

E. NEW CO-ORDINATED RESEARCH PROJECTS

Mutational Analysis of Root Characters in Annual Food Crops related to Plant Performance

Background:

Agriculture in developing countries faces a number of serious challenges. These result largely from population increase, climatic changes and the decrease in available land for agricultural production. The loss of land will become most critical over the next thirty years. Therefore the maintenance of agricultural output and food supply demands the development of more efficient crops in terms of water and nutrient use. Roots with their associated soil flora are the organs where these processes occur and the manipulation of root architecture, function and root/microbe interaction will provide a means for improving crop plants in the future. This question has been indirectly addressed in the past, but a new and more targeted strategy is required. A genetic approach will supply us with (1) novel phenotypes, (2) a broader genetic base of root traits and (3) tools for molecular breeding.

An important area of root function relates to the development of specific associations with the soil microflora in the rhizosphere. While these include pathogenic relationships more mutualistic associations and close symbioses such as that between legumes and Rhizobium. The general occurrence of mycorrhizal associations among crop species offers a special opportunity to assess the impact of altered root features of agronomic significance.

Historically, the genetic analysis of root traits has been neglected largely because of the difficulty in accessing this below ground organ. Consequently, few root mutants of crop plants have been described to date. The paucity of root mutants has resulted in the inability to evaluate specific root traits in breeding programmes. However, some changes in attitude are developing and root associated QTLs have recently been identified for wheat and rice.

The achievements in the model plant *Arabidopsis thaliana* demonstrate the power of employing a genetic approach for the identification of key factors determining root characters. At least 25 root mutants displaying changes in morphology, architecture and nutrient uptake have been described in the literature to date. Subsequent cloning of the affected genes has revealed a number of functions such as transcription factors, ion carriers etc. that are involved in root development and function. It is therefore timely that a similar mutational analysis should be applied to crop plant root systems. The generation of a population of root mutants will provide a pool of genetically variable material. It is anticipated that a variety of characteristics of root function will be altered in these new genetic stocks. These lines will include mutants with changes in branching pattern; root hair frequency, size and distribution; root diameter; fraction and distribution of various root types. It is anticipated that these mutants will exhibit functional changes in water and nutrient (N, P, K and Fe) uptake and mycorrhizal development. This material will therefore be of particular relevance for the modification of the plants' response to a variety of environmental conditions in developing countries.

Plant traits that are important for agricultural production, include adaptation to environmental stresses such as drought, salinity, soil compaction, nutrient imbalances and water logging. Each of them mediated through the root system and which all have drastic effects on plant development and decrease yield. Also, while coarse roots senesce slowly, fine roots have an increased rate of turnover. Distinction between root types is especially important for plants forming mycorrhizae as functional symbiosis is established over a period of approximately 6 weeks. Roots and mycorrhizae are the organs where water and nutrient uptake occurs. Their manipulation would be of particular importance to improve the acquisition and utilisation of water and nutrients. Furthermore, genetic analysis is an essential component for the identification of the inheritance of structural variation such as branching, shallow versus deep rooting and overall root architecture.

It is anticipated that this CRP will utilise a number of methodologies. Efficient mutagenesis procedures and screens are of crucial importance. While these have been successfully established in Arabidopsis and in part in maize for early periods of root development, they still must be adapted for a wider range of crop plants. Populations of young seedlings can be screened for morphological or functional traits considering root type specific properties. In addition more mature plants will be screened for functional and for mycorrhizae associated phenotypes.

For the assessment of mutants, methodologies for observation, description and evaluation of larger and more mature root system architecture using minirhizotrons, aeroponics, hydroponics, and root boxes will be required. Novel non-destructive and long term root observation systems should be developed. Physiological traits such as nutrient and water uptake will be characterized and used for the evaluation of promising mutants.

Overall Objective:

To generate and utilize mutants for the identification of root properties and genes related to crop productivity and sustainability

Specific Objectives:

To make mutational analysis of some of the essential characters related to root formation and function.

To assess the impact of specific root traits on yield potential under filed conditions leading to the exploitation of these traits in future breeding programmes.

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.

Molecular characterization of mutated genes controlling important traits for seed crop improvement

Background

Genetic improvement of crop plant species has been a very important approach for achieving higher yields and more stable crop production. Future advances in crop production will become more dependent on genetic manipulation of crop genomes because other resources and inputs (e.g. high quality land, water, fertilizer, chemicals) have reached their sustainable limits in some regions of the world. Also, more severe biotic and

abiotic stress will be encountered as crop production expands and intensifies in developing regions of the world. Historically, genetic improvement of crops has been a rather haphazard and mysterious process because the underlying genetic factors and mechanisms were largely unknown and undescribed in meaningful biological terms in relation to the phenotypes undergoing artificial selection. Also, genetic gain was limited by the genetic resources (the types of genes) and the extent of allelic variation in the primary and secondary gene pools.

Several recent developments have changed the way scientists conduct investigations in plant biology and genetics. First, *Arabidopsis thaliana* and *Oryza sativa* have emerged as model systems for studying many aspects of plant biology. Concurrently, a series of technical revolutions in DNA sequencing, large-scale analysis of patterns of gene expression (e.g. production of mRNA and protein) with DNA chips and microarrays, insertional mutagenesis, comparative genetic and physical mapping, transformation and computational biology have enabled gene isolation and the dissection and analysis of entire genomes such as yeast and Arabidopsis. Collectively, these technical advancements are often referred to as structural and functional 'genomics'. The final development is the growing awareness of the redundancy and unity that exists at the molecular level among a wide range of seemingly unrelated species (e.g. wheat and Arabidopsis) and sexually isolated species (wheat and rice, maize and sorghum): most of the genes found in the model systems are also found in other species, including crops.

Such infrastructure and methods have enabled the isolation and characterization of mutant genes for important phenotypes in model species and crop plants. For example, the genes for blast resistance in rice, rust resistance in wheat and maize, flowering in sorghum, plant height in maize and wheat and oil quality in soybean and brassica have been isolated through structural and functional genomics applied in opportunistic and flexible ways: sometimes one begins with the phenotype to isolate the DNA sequence and gene while in other situations, one starts with the DNA sequence and matches them with the phenotypes. These activities are defining the genetic components of crop productivity and enabling novel approaches to plant breeding and crop improvement.

Various species are characterised by molecular redundancy and unity. Therefore, many of the approaches and much of the information may be applied to parallel efforts in underfunded and underutilized orphan crops without experiencing the great expenditures related to infrastructure development for genomics. Thus, much of the information about genes isolated and studied in the model species (e.g. rice, maize and arabidopsis) may be transferred or used to isolate similar genes in other species. Many of those genes will have functions of great relevance to crop improvement. The information from the model species will often enable and expedite those steps provided that there is an adequate array of allelic variation and mutant phenotypes in the model and target species. Eventually, the technical edge provided by the model systems and the allelic richness of the target species will provide the synergism necessary to understand many aspects of molecular variation and its relationship to phenotypic variation. Understanding this relationship is fundamental to future genetic improvements of crops. The key steps will be isolating the genes and defining their functions in the context of the crop plant's biology and productivity.

These new developments complement previous achievements such as the generation and phenotypic characterization of mutants in various crops (e.g. barley, maize and tomato), doubled haploid lines, isogenic lines, deletion stocks and germplasm collections.

These genetic stocks are proving to be of immense utility because they enable connections between the molecules and DNA sequences with their biological functions revealed by the mutant phenotypes. Thus, it is important to utilize myriad approaches in the characterization and isolation of genes for crop improvement. The ability to match the molecules with the mutants is essential. The rapid development of genomics has exposed serious gaps and a shallowness in collections of mutations: at the moment, there are too many molecules to match with the repertoire of specific mutations (the 'phenotype gap'). This dearth of mutations may inhibit our ability to assign biological function to the genes and their molecules.

Description of the Problem:

The overall problem is the lack of understanding of the genetic and biological basis of phenotypes important for crop plant productivity. Contemporary genetic research is expensive - the development of physical and human resources, their maintenance, collection and distillation of the fundamental information and material and the development of raw material and information into new and improved varieties. Thus, it is not reasonable or feasible to create the necessary infrastructure for each crop species.

Other problems:

1) lack of basic genetic and biological information for all crops;
2) gap in technology and information for developing countries, 'orphan' and local crops (e.g. tropical maize, African rice);
3) limited resources for conducting comprehensive genetic research in such crops and regions;
4) lack of mutant phenotypes and alleles for key traits in all crops;
5) poor linkages among research communities (between developing and developed countries and among developing countries);
6) inadequate training and awareness of the opportunities for transferring information and material from model species to crop species.

This CRP will meet the need for characterization of induced genetic variation necessary to exploit the full power of structural and functional genomics for gene isolation directed at the improvement of orphan crops and allied species. The project will be developed by creating linkages, information and material capable of enhancing and exploiting the power of model species for the genetic improvement of orphan and local crops.

Criteria used for selection of crops:

1. evidence of local breeding activities;
2. limitations of crop production resolvable through genetic improvement of the crop plant;
3. potential synteny and/or gene conservation between the target crop species and the model systems;

Potential crops to be included:

Orphans (suggested examples):

1. Maize (tropical)
2. Sorghum

3. Teff
4. Millets
5. Wheat
6. Solanaceous spp.
7. Alfalfa or other forage legumes
8. Oats

Models for molecular research:
1. Rice
2. Maize
3. Tomato
4. *Medicago trunculata*

Criteria used for selection of phenotypes:
1. importance to the breeding and productivity of the orphan crop;
2. the existence of candidate loci and mutants (orthologues or paralogues) in one or more model species;
3. feasibility of evaluating the phenotype in the orphan crop;
4. existence of candidate loci, mutants and phenotypes in the orphan crop;
5. feasibility of transferring and utilizing the information in the orphan crop and the model species.

Phenotypes and genes to be included:
1. resistance to biotic stress
2. resistance to abiotic stress
3. plant stature
4. nutritional composition
5. photoperiod response
6. root development.

Overall objective:
To assist Member States in the application of the molecular genetics of mutated genes to improve production in both major cereals and related under-utilised crops.

Specific objective:
-To collectively develop, characterise and establish a data-base on mutant collections of key crops for application by CRP members and the world's scientific community.
- To molecularly characterise new or existing mutants affecting key agronomic traits in major crops and using comparative approaches in under-utilised crops with a view to their eventual isolation.

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

F. ACTIVITIES AT THE PLANT BREEDING UNIT, SEIBERSDORF

Increase the efficiency of callus production and green plant regeneration in rice anther culture by identifying morphological markers

This activity aims at the development of an efficient method for anther culture in rice for its application in induced mutation studies; to identify morphological markers associated with the pollen stage in which callus induction response could be maximised; and to find out if correlations exist among the markers to be studied such as distance between flag and subtending leaf, panicle length, spikelet positions in the panicle, spikelet colour, anther positions and anther colour.

After plating 27,327 anthers of the japonica variety Taipei 309, we obtained 8,282 calli. 1798 calli produced 2973 single or multiple green plants. We can conclude that:

- Callus induction was high when pollen was at the uninucleate stage, when the distances between the flag and subtending leaf ranged from 8-12 cm, when the panicles had a length of 21 to 29 cm, when the spikelet colours were yellow to yellow green and when the anther colour was whitish yellow or yellow.
- Plant regeneration was increased 8 to 12 % when calli were pre-treated with 10mg/l Abscisic Acid (ABA) for two weeks or were dehydrated for 24 hours.
- The best plant regeneration response was obtained when basic Murashige and Skoog (1962) medium supplemented with 1 mg/l BAP, 0.5 mg/l NAA, 100 mg/l inositol, 30 g/l sucrose and 4.5 g/l agarose was used.

Development of salt susceptible isogenic rice varieties from salt tolerant lines by using gamma irradiation and anther culture to characterize responsible genes

We aim to identify salt tolerant mutant(s) using mutation induction, anther culture and a rapid screening method. To achieve this objective we carried out radio-sensitivity test in six indica rice varieties and the established a salinity screening technique for rice seedlings.

⇒ Radio-sensitivity test in six indica rice varieties

The objective of the present study was to determine the optimum dose of ^{60}Co gamma irradiation of different salt susceptible and tolerant varieties for mutation. The varieties were IR29, IR51500-AC11-1 (Bicol), IR63731-1-1-4-3-2, IR58430-68-14-1-2, Nona Bokra and Pokkali. The doses used were 100 Gy, 200 Gy, 300 Gy, 400 Gy , 500 Gy and the control. Different parameters were used (seedling height, survival rate, seed setting) to determine the optimum dose for mutation induction.

The results of this study are summarized as follows:

- The optimum doses for mutation induction is variety specific. Based on seedling height reduction the dose ranges for the following rice varieties varied from:

  200-300 Gy for IR29
  300-400 Gy for IR51500
  300-400 Gy for IR63731
  400-500 Gy for IR58430
  300-400 Gy for Pokkali and
  Above 500 Gy for Nona Bokra

- The results of response of salt susceptible and tolerant varieties to irradiation suggest that there is a correlation between the stress caused by irradiation and stress caused by salinity (the more salt

tolerant the less susceptible the variety was to irradiation).
- Percentage of seed setting was seriously affected by irradiation.
- To find out the optimum dose measurement of seedling height should be used, when the seedling height reduction reaches at plateau.

⇒ Establishment of a salinity screening technique:
The research covered the establishment of a screening technique to assess the salinity tolerance of rice seedlings for its application in mutation induction studies and to find out the adequate salinity level and correct time for scoring (results of four experiments).
Until now we found that IR 29 was an adequate susceptible check; Pokkali and Nona-Bokra were both adequate tolerant checks; the best salinity level for screening tolerant lines to salinity stress was 10 dS/m.; and the best time for scoring was 12 days after salinization.

Natural light as an alternative light source for the in vitro culture of banana (*Musa acuminata* cv. 'Grande Naine')

We are developing cost effective tissue culture protocols for chimera dissociation using micro-propagation with the use of alternative inputs. The concept of using sunlight for micro-propagation systems is proposed as a way of reducing tissue culture costs.

Shoot tips of *Musa acuminata* cultivar 'Grande Naine' were cultured in a non-controlled natural light environment during summertime.
- Significantly more shoots were produced by plantlets cultivated in a sunlit room with photosynthetic photon flux densities (PPFD) fluctuating up to 570 µmol m-2 s-1, temperatures between 23 and 30°C and photoperiods of 12 to 16 hours, than by plantlets under artificial light in a growth chamber providing controlled conditions of a constant PPFD of 65 µmol m-2 s-1, temperatures ranging from 23 to 29°C and a 16 hour photoperiod.
- Highest multiplication rates were achieved in a greenhouse with PPFD reaching 860 µmol m-2 s-1 and temperatures of 18 to 43 °C, but browning of leaves and loss of turgor occurred. Nevertheless, rooted plantlets showed 100 % survival during acclimatisation and normal development.
- Photoperiods of 12 to 16 hours did not affect the multiplication rates.

Moreover, external windows in *in vitro* culture rooms can be a satisfactory alternative to artificial lighting. The ideal set up would be a sunlit room with high standards of hygiene similar to those of a growth chamber. A decision pro or contra will depend on the crop, the *in vitro* technique used and the climatic conditions. The main advantages are: 1) no costs for lighting, 2) simplified installations thus lower construction costs, 3) reduced need of maintenance and spare parts, and 4) lower stress for plants during acclimatisation. Especially laboratories with a poor infrastructure and limited local resources will benefit from this system.

Improved methods to increase diversity in Musa using mutation and tissue culture techniques.

Banana and plantain (*Musa* sp.) are two of the world's major crops. Many inhabitants from the tropics make their living directly or indirectly from *Musa* by using these crops as a source of food or export earnings. The world production is considerable and reaches approximately 75-80 million tons

per year. Approximately 90 % of the total production is used as a food for domestic consumption. In some African countries daily consumption may exceed 3 bananas per person per day, whereas in North America and Western Europe is about one banana a week per person in average.

Cross breeding consisting of genetic recombination to generate variability is difficult due to the extremely complicated genetic system of Musa: different genomic constitutions, heterozygosity, polyploidy and the sterility of the edible varieties. The complexity of Musa genetics emphasizes the need for an additional system to support conventional breeding programmes. The use of *in-vitro* techniques such as shoot-tip cultures couple with mutation techniques has offered several advantages over the conventional in vivo techniques such as the possibility to irradiate a greater number of healthy plants. Nevertheless, *in vitro* induced mutation methods applied to Musa improvement have still some constraints:

- Several Researchers have irradiated shoot-tips with gamma rays to induce mutations in vitro. However a complete study on the whole Musa genus and a clear cut idea on the suitable dose/s to be used is still necessary. It is thus essential to determine the useful dosage for the genotypes and the characters of importance, and to standardize the methodology.

- The appearance of chimeras after irradiation of tissues necessitates subculture of regenerated plantlets to dissociate chimeras and this slows down the selection of mutants.

Comparison of the mutation induction efficiency of several types of Musa genotypes concerning their reaction to gamma irradiation:

Excised shoot-tips from seven clones representing the different genomic constitutions of the genus Musa: Calcutta-4 (AA); Tani (BB); Grande-Naine (AAA); Williams (AAA); Three Hand Planty (AAB) and Cachaco (ABB) were treated with 60Co gamma irradiation doses ranging from 10 to 100 Gy.

Four parameters were assessed after irradiation treatment:

· Survival rate · Shoot height
· Fresh weight · Multiplication rate

Of the four parameters studied we suggest to use the following doses:
10-20 Gy of gamma irradiation for diploid clones Calcutta-4 and Tani (BB).
30-40 Gy of gamma irradiation for the triploids Grande Naine (AAA), Williams (AAA) and Three Hand Planty (AAB).
40-50 Gy of gamma irradiation for the triploid Cachaco (ABB).

Overcome and/or minimize chimeras:

It is known that mutagenic agents (physical or chemical) on shoot-tips results in a high degree of chimeras. This is a serious obstacle to mutation techniques since it is not yet possible to distinguish mutated cells from non-mutated cells. It is important to know if mutants can be recovered and after how many subcultures. Knowing that colchicine is a mutagenic agent which doubles the chromosome number and that rapid ploidy screening can be accomplished on large scale by flow cytometric analysis we can study how to overcome or at least minimize chimeras. Flow cytometry was used to monitor cytochimeras and their dissociation through *in vitro* shoot-tip culture after treatment with colchicine. 684 shoot-tips of the triploid banana variety Grande Naine (AAA) were treated with 5.0

mM colchicine for 48 hours.

10% of the shoot-tips survived thirty days after treatment from which 81 % shoots proved to be mixoploids; of these 10 mixoploid shoots were selected and their descendants screened after each subculture. The percentage of mixoploidy decreased from 100% to 67% after one subculture, to 48% after the second and to 39% after the third. The process is being monitored in the subsequent subculture until complete mixoploidy dissociation is obtained. Our preliminary results suggest that three subcultures, as is currently applied by *in vitro* mutation technology for vegetatively propagated crops, are not sufficient to dissociate chimeras after mutagenic treatments of shoot-tips.

The occurrence of mixoploidy regenerates after colchicine treatment indicates the multicellular origin of shoot-tips. By screening the progeny of colchicine treated explants such as adventitious buds, embryogenic cell suspensions or protoplasts would allow us to verify their cellular origin and thus speeding-up the mutation breeding process by reducing or even elimination the transfers to dissociate chimeras.

Future activities:

· Development of a fast propagation system such as adventitious buds, embryogenic cell suspensions or protoplasts culture.
· To validate the use of toxins as early mass screening agent for selecting Musa genotypes resistant to black Sigatoka.

These activities are being carried out in collaboration with:
The laboratory of Tropical Crop Improvement, Katholiek University of Leuven (KUL), Belgium.
The Plant Pathology Unit, Faculte des Sciences Agronomiques de Gembloux (FUSAGx), Belgium
The International network for the Improvement of Banana and Plantain (INIBAP), Montpellier, France.
These activities are in support of the Coordinated Research Project funded jointly by IAEA and the Belgian Administration for Development Cooperation (BADC).

Activation of retrotransposable elements in rice and induction of insertional mutagenesis as a tool for cloning genes of interest

Mutagenesis by insertion of known DNA-sequences into the genome is a powerful tool for identification and isolation of genes. One possibility to achieve insertional mutagenesis is to take advantage of natural existing mobile genetic elements. Retrotansposons belong to a group of these elements (class I elements) which move via an RNA intermediate. In most cases retrotransposons are not active under normal growth conditions. Certain stress conditions however can lead to transcription of some retrotransposons and in the end to a stable integration of new copies into any site of the genome.

The retrotransposon Tos 17 is one of a few elements in rice which were shown to be activated by protoplast culture. In order to avoid time consuming protoplast culture with all its uncontrollable effects the induction of the transposon by irradiation of seeds would be of considerable advantage.

In combination with a good selection system for a desired trait, mutants will be investigated for insertion of the transposed element by molecular and cytogenetic means. A

gene tagged by this way could be isolated by probing a genomic library with the cloned transposon.

Our research regarding this project is focused on the following objectives:

- To investigate the influence of mutagenic treatment on activation of the Tos 17 retrotransposon in rice by molecular and cytogenetic means. The main questions are:
  - Is the transcription and transposition of the retro-element inducable by γ-irradiation?
  - Does the activation follow a dose/effect relationship?
  - How many new copies of the element can be expected to be integrated into the genome after treatment?
- To use retrotransposon induction for tagging genes coding for salinity tolerance or other important traits.
- To isolate and clone tagged genes or tightly linked sequences to these genes.

Preliminary results show an increase in the number of Tos 17 integrations in some Taipei 309-plants (*japonica* rice) derived from a radiosensitivity test. In gametoclonal variants of the *indica* variety 'Pokkali' a reduction of DNA-bands in Southern blots probed with a Tos 17 sequence was detected. This result was shown to be due to DNA-methylation. A Southern blot analysis of Taipei 309 M_3-plants selected for salinity tolerance will now be pursued.

G. PUBLICATIONS

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