



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

Plant Breeding & Genetics Newsletter

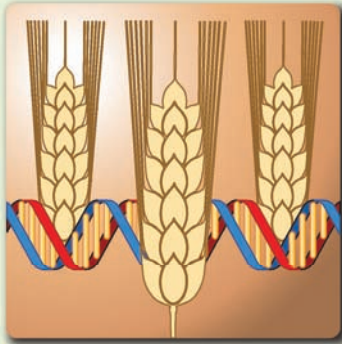
No. 15

July 2005

<http://www.iaea.org/programmes/nafa>

ISSN 1564-2569

<http://www.fao.org/WAICENT/Agricul.htm>



Zhejiang University prepares the soccer field for Olympics 2008 in China using mutant Tef (yellow)

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To Our Readers

In this issue I want to point out two items which will accompany us as increasingly important foci of the Agency's Subprogramme on "Sustainable Intensification of Crop Production Systems" into the next biennia: hardy crops in harsh environments and hidden hunger. You might refer to the First Research Coordination Meeting (RCM) on "Identification and Pyramiding of Mutated Genes: Novel Approaches for Improving Crop Tolerance to Salinity and Drought" (refer to PAST EVENTS), which was held in Vienna, Austria this March and to the Technical Cooperation (TC) Regional Project RAS/7/014 "Monitoring of Food Fortification Programmes Using Nuclear Techniques" (refer to PAST EVENTS), for which the First Project Coordination Meeting was held this February in Bali, Indonesia in conjunction with the Second Reporting Meeting on "Selection Efficiency for Low Phytate (LP) Mutants in Rice and Nutrient Bioavailability from Fortified Foods". Related to the latter, I want also to direct



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your attention to the establishment of a Research Consortium on Low Phytate Crops in China (refer to NEWS SPARKS).

The last semester was rather tough on us, and I wish here to express my appreciation to my colleagues for having graciously taken the burden of supplementary work put upon us by temporary understaffing due to staff turnover.

Shri Mohan Jain (Ph.D.) retired from the Agency at the end of April. As a technical officer, Dr. Jain performed his duties conscientiously and willingly shared his



knowledge and scientific interests in his fields of competence with his colleagues and counterparts, putting a great deal of creativity and effort into proper publishing of results from CRPs and reports from TCPs as well as technical and scientific documentation. More than 35 literature references, including 15 edited books as well as research and review

papers, are a credit to his workforce and a lasting testimony for his stay with the Agency. As a colleague, Mohan leaves tender memories of profound and thoughtful humanism with us, and many a laugh. Indeed, we wish our colleague a very active and fulfilling "retirement" at the University of Helsinki (Finland). I am sure that we will meet him at one or the other scientific Workshop or Symposium around the world.

Whereas it is always difficult to let go and say good-bye, these "departures" are on the other hand also opportunities to welcome new "arrivals". It is my privilege and pleasure to introduce four new colleagues to you:

Barbara Anne Ventrello, originally from New York, NY, migrated to Vienna in the 1980's. She has worked for the



IAEA, and UNICEF and the UN in NY in administration and secretarial areas. Of Italian heritage, she enjoys traveling to Italy and improving her knowledge of Italian and Italian cuisine. Barbara enjoys working with international colleagues and is a true advocate of the goals and work of the United Nations. For all of you colleagues, counterparts and experts

reaching out to us, it will be highly probable that your first contact will be through Barbara.

Marie Madeleine Spencer, Senegalese by heart, and Cape Verdean by birth graduated with a "Thèse de Doctorat d'Etat ès Sciences" (Ph.D.) from the University Cheikh Anta Diop (Dakar, Senegal). With a big love for teaching, she stayed on at the University and dedicated herself to the whole *cursus* from sixth to twelfth grade. But the chance, and opportunity, was that at a University one can also do research, which became her second passion,

eventually leading her to the Soybean Breeding Programme at the University of Tennessee (Knoxville,



USA). Madeleine has been working in Plant Science for the last 20 years with a focus on Plant Biotechnologies: plant physiology using radioisotopes, plant tissue culture (morphogenesis, micropropagation a.o.), plant molecular biology on super-nodulating soybean mutants, introgression of RR[®] genes into elite

Tennessean soybean lines, and more recently, identification of RFLP and SSR markers for high stearic acid, low palmitic and low linolenic acid content soybean lines. A mother of three, Vanessa, Roselyne and Nilton, she loves socialising, going places and good food (cooking plus eating).

Manoela Pessoa de Miranda (Ph.D.) is a plant molecular biologist from Brazil whose studies and profession have



taken her across a few continents (Germany, Japan, Canada). She joined the Plant Breeding and Genetics Section in January. Among her duties, she is now taking up the challenge of coordinating the mutation induction research programme on banana, which is carried out jointly by the Section and the Plant Breed-

ing Unit. Manoela has a passion for outdoor activities, including sailing, hiking, scuba diving and, of course, traveling. She joined the Agency with the belief that each of us can be an instrument of goodwill in helping others to achieve a more dignifying life.

And last but not least, we welcome

Zephaniah Dhlamini from Zimbabwe, who has joined us, as a consultant to help with our duty chores related to TCPs and CRPs. Zephaniah is an agricultural biotech-



nologist who has taught applied genetics and biotechnology and used molecular markers and metabolic engineering techniques for sorghum improvement at the University of Zimbabwe. He joins us after a 3-year stint as an associate biotechnology officer with the Research & Technology Development Service of FAO in Rome and looks forward to

sharpening his newly acquired research project management and impact assessment skills. Zephaniah is a wildlife enthusiast and loves cooking traditional dishes and likes experimenting with Italian gastronomy as well.

To all our new colleagues, I wish a fruitful stay with us and luck and success in all their endeavours.

Pierre J.L. Lagoda

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* Separated from the Agency on 29 April 2005

Forthcoming Events

First Research Coordination Meeting on “Molecular Tools for Quality Improvement in Vegetatively Propagated Crops including Banana and Cassava”, Vienna, Austria, 18–22 July 2005

Technical Officer: C. Mba

This Coordinated Research Project (CRP) emphasizes the application of induced mutations and new tools of functional genomics to solve long-standing and critical constraints of quality and related traits in banana and cassava as a way to secure their place as staple food security crops and increase their value in tropical agroecosystems. The goal is the development of well characterized mutants, advanced and pre-breeding lines, applicable data about genes, and a suite of genomics tools that can be combined with field-based breeding methods to increase the efficiency and reduce the time for the improvement of multiple quality and related traits in vegetatively propagated crops including banana and cassava. The results of these activities will be widely disseminated to provide a proof of concept on the use of genomics and induced mutations to dissect complex genetic traits and their application in crop improvement. In the long term, induced mutants, molecular tools, and knowledge developed under this project will increase the efficiency and productivity of quality improvement in banana and cassava breeding programmes in Member States that will ultimately lead to improved livelihoods and food security of the human populations that rely on these crops for sustenance.

The CRP will employ induced mutants and functional genomics, at the single gene level (conserved orthologous [COS] markers) and at the transcriptome level (a collection of ESTs), as well as appropriate genetic mapping populations, including doubled haploids, to identify molecular markers for marker-assisted selection (MAS) of quality and related traits.

To reach the above-mentioned objectives, inside this CRP, major tasks can be defined:

1. Development of induced mutation-derived gene discovery grids for quality traits in cassava and banana with an emphasis on an increased content of starch/sugar, pro-vitamin A, micro-nutrients, protein, and post-harvest loss
2. Development of doubled haploid (DH) protocols and their dissemination in participating countries for cassava and banana
3. Development of conserved orthologous sequence (COS) markers from candidate genes involved in tolerance to abiotic stresses including drought, poor soil fertility, and genes involved in biosynthetic pathways of starch/sugar, pro-vitamin A production

4. Development of low-cost (PCR-based) marker technology for marker assisted breeding
5. Development of new and distribution of existing genetic mapping populations for banana and cassava, segregating for target traits
6. Development of gene expression resources based on DNA array technologies
7. Development of bioinformatics platforms for the analysis of ESTs and gene expression data coming out of activities in the CRP
8. Creation of a guideline addressing intellectual property rights (IPR) issues related to the exchange of mutated germplasm
9. Capacity building

There are 12 research contract holders from Bangladesh, Brazil, China, Cuba, Ghana, India, Indonesia, Kenya, Mexico, Nigeria and the Philippines. The five research agreement holders are from Czech Republic, United Kingdom, (CIAT) Colombia and (INIBAP) France.

Fifth Interregional Training Course on “Mutant Germplasm Characterization using Molecular Markers”, Seibersdorf, Austria, 1–26 August 2005

Technical Officer: C. Mba

Capacity building is one of the main mechanisms for providing assistance to plant breeders from developing Member States of FAO and the Agency in the use of induced mutations and ancillary biotechnologies for developing superior crop varieties. In addition to the several regional training courses and fellowship programmes, the Joint FAO/IAEA Programme for Nuclear Techniques in Food and Agriculture annually organizes a 4-week training programme for 20 participants from 20 developing Member States in order to gain theoretical and hands-on experience in induced mutations; high throughput detection of mutation events; and the application of commonly used molecular genetic and cytogenetic markers for crop germplasm evaluation and genetic studies. The course participants are also exposed to the use of standard population genetics analytical tools for molecular genetic data management.

The 20 participants for this year's course scheduled for 1–26 August in Seibersdorf and Vienna, Austria are drawn from Angola, Ethiopia, Ghana, Nigeria, Tanzania, Uganda, Indonesia, Korea, Malaysia, P.R. China, Pakistan, Sri Lanka, Syria, Thailand, Vietnam, Romania, Brazil, Chile, Costa Rica and Cuba.

Second Research Coordination Meeting on “Physical Mapping Technologies for the Identification and Characterization of Mutated Genes Contributing to Crop Quality”, Reykjavik, Iceland, 22–26 August 2005

Technical Officer: P.J.L. Lagoda

The rapid development of genomics has exposed serious gaps and shallowness in collections of mutations: at the moment, there are too many molecules to match 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. Thus, the ongoing CRP is focusing on the utilization of various approaches in the characterization and isolation of genes for crop improvement. Especially molecular cytogenetics and physical mapping techniques are being explored for their utility as breeding tools. Eight contract holders and five agreement holders from 12 countries (Argentina, Bulgaria, People’s Republic of China, Czech Republic, Germany, Iceland, Pakistan, Poland, United Kingdom, Ukraine, United States of America, Vietnam) work together on staple length in cotton, carotenoid contents in tomato, development of chromosome and chromosome-arm specific markers in three *Chenopodium* species and *Brassica campestris*, establishment of TUNEL and comet assay protocols for *Hordeum vulgare* (barley), chromosomal identification in banana, cytogenetic characterization of *Capsicum* species, screening for modified fatty acid composition in *Brassica napus* and *B. juncea* mutant lines, and on the model crop rice (interspecific crosses, search for species specific repeats, analysis of the alkali spreading value and other agronomic traits such as semidwarfness). All tasks in the ongoing CRP have been implemented as planned and the project has made significant progress, particularly the development of new mutant generations and the biochemical, molecular and cytogenetic characterization of advanced mutant lines. The second RCM in Reykjavik (Iceland) will be the opportunity to compare notes and decide on the future developments of this interesting CRP.

Second Research Coordination Meeting on “Effects of Mutagenic Agents on the DNA Sequence in Plants”, Seoul, Republic of Korea, 14–18 November 2005

Technical Officer: P.J.L. Lagoda

Modern plant breeders and farmers can exploit a wealth of natural biodiversity, which may be widely broadened through the application of mutation induction techniques. The impact of induced mutation on crop improvement is reflected in the more than 2300 officially registered varieties <http://www-mvd.iaea.org/MVD/default.htm>; IAEA's database on officially registered mutant varieties, MVD) carrying novel induced variation. Moreover, about

three-quarters of these are direct mutant varieties derived from treatment with gamma rays, thus highlighting the importance of physical mutagens. All this translates into a tremendous economic impact on agriculture and food production that is currently valued in billions of dollars and millions of cultivated hectares (Ahloowalia *et al.* 2004). However, while the agronomic potential of induced mutation is well understood, the precise effects of different mutagenic agents on the DNA sequence in plants have never been described. Furthermore, in recent years novel reverse genetics and gene discovery technologies have spurred renewed interest in induced mutation. For these new applications it is necessary to understand more clearly the types of mutations generated by the different classes of mutagens, and to measure their frequency and distribution along the genome. Today, and for the first time, the technologies are in place to undertake the experiments necessary to gain this understanding.

Mutagenic agents can be classified into three categories: physical (e.g. gamma rays), chemical (e.g. ethyl methane sulphonate) and transposable elements (e.g. transposons, retrotransposons, T-DNA, retroviruses). At present, limited data are available on the scope of genetic effects induced at the molecular level in plants and on the specificity and relative efficiency of these different categories of agents. These effects involve DNA damage, which results in base pair changes (single/simple nucleotide polymorphisms, SNPs), small insertions and deletions (indels) and chromosomal rearrangements. Even less is known about how induced mutations interact with epigenetic processes, such as methylation, activation of retro-elements, and perturbation of higher order DNA structure.

While breeders have been using mutation induction to broaden the genetic base of germplasm, and have used the mutant lines directly as new varieties or as sources of new variation in cross-breeding programmes, knowledge of the precise nature of the induced mutations was not necessary. Intuitively a conservative level of small base pair rearrangement and deletion was considered to be ideal. Nowadays, the use of mutation techniques has expanded beyond applications in breeding to gene discovery and reverse genetics. These new high-throughput applications require specific classes of mutations that are induced with high efficiency over entire crop plant genomes, and consequently knowledge of the precise nature of induced mutation is becoming an issue.

High-throughput gene discovery methods depend heavily on insertional ‘knockout’ lines, the now classical ‘gene machines’, and deletion ‘knockout’ libraries. Insertional mutagenesis involves inducing increased activity of transposition of known transposable elements (e.g. retrotransposons which tend to transpose into active genes) to produce series of lines in which, in theory, every gene in

the genome will have been inactivated by the transposon insertion. These lines can be used to identify genes that cause particular phenotypes or, conversely, can be used to identify gene function by searching for a phenotype associated with the inactivation of a particular known gene. However, insertional mutants have a tendency to be unstable (i.e. excision of the transposon tag, e.g. Ac/Ds binary system, in the next generation might cause the phenotype to revert to the original parent type, or activation of retrotransposon tags through different stresses might multiply insertion events, e.g. during micropropagation). In comparison to insertional mutagenesis, conventional mutation induction (i.e. using physical or chemical agents) provides the advantage of stable mutations.

In theory, the production of deletion libraries involves inducing moderately large deletions, ideally spanning 1kb to 100kb in size, in each of a series of lines. These deletions should encompass segments of every gene in the genetic repertoire and should be represented at least by one line in the deletion library. These deletion lines can, when used together with whole genome gene arrays, be used to identify genes responsible for particular phenotypes or to confirm the association of known genes with particular phenotypes.

A novel and important reverse genetics approach is 'targeting induced local lesions in genomes' (TILLING). Here, large numbers of small changes, either DNA base pair substitutions or small deletions spanning no more than a few base pairs, are induced in a series of lines. In these lines gene function can be ascertained by associating a phenotype with changes in a particular gene and novel alleles of known genes can be generated.

Over the coming years, new technologies such as these will have increasing impact in practical plant breeding. However, they will require different types of mutations induced at specific frequencies. In order to tailor the mutation process, there will be a need to understand how specific classes of mutations are generated and distributed over genomes. In the past, this has not been possible because of lack of analytical tools and an inadequate

knowledge of both the process of DNA damage and the architecture of plant genomes. In addition, only a restricted number of plant genes were sequenced. Today, high-throughput DNA sequencing methods coupled with bioinformatics and functional genomic approaches provide extensive knowledge on genome architecture. The complete genomic DNA sequence of a model dicotyledonous plant, *Arabidopsis*, and a model monocotyledon, rice has become available recently. Also scientists find themselves now with an array of methods, mostly developed as molecular marker technologies that can be adapted to quantify changes in DNA sequence. All in all, the stage is set to transfer the science of DNA damage induced by physical and chemical mutagens from human genetics to plant systems. A range of technologies can now be used to quantify both the underlying base rate, over numbers of generations, of spontaneous mutation and the instantaneous effects of mutation agents. Thus scientists now finally find themselves in a position to undertake experiments that can unravel the sorts of mutations induced by different mutagens so that future users of induced mutation may use the technology in a fully informed manner.

This Coordinated Research Project aims to understand the mechanism of mutation induction in plants and to quantify the types (base pair changes or deletions), incidence (frequencies and rates of change relative to mutagen dose) and patterns (heterogeneities in the induction of changes in the genome) of mutation induced at the DNA level by a range of physical and chemical agents. Molecular marker, DNA array, and novel reverse genetic methodologies are being used in a unique approach to analyze and survey the induction of mutations elicited in a number of crop plant species of agronomic importance. These results will be used to provide protocols and guidelines important for plant biology.

The second RCM will provide the opportunity to assess progress made in the different participating laboratories and institutes for efficiently steering the future workplans of the CRP towards the projected objectives.

Past Events

First Project Coordination Meeting in conjunction with the Second Reporting Meeting on “Selection Efficiency for Low Phytate (LP) Mutants in Rice and Nutrient Bioavailability from Fortified Foods” of the TC Regional Project RAS/7/014 “Monitoring of Food Fortification Programmes Using Nuclear Techniques”, Bali, Indonesia, 21–25 February 2005

Technical Officers: P.J.L. Lagoda & N. Mokhtar (NAHU)

The RAS/7/014 project was initiated to evaluate interventions aimed at addressing micronutrient (e.g. iron and vitamin A) deficiencies in Asia. The Project Formulation Meeting was organized in 2002 in Thailand with a Working Material prepared. The project was approved for implementation in a five-year duration (2003-2007). The objectives of the project are twofold: i) to evaluate and monitor the food fortification intervention programmes in five participating Member States (China, Indonesia, Pakistan, Thailand and Vietnam); and ii) to develop rice mutants with low phytic acid (PA) from the country's high-yield rice varieties. The implementation started in 2003, and two project teams were established in each country to undertake the activities for respective components.

The Project Coordination Meeting (PCM) was organized to review project progress and develop a strategy and work plan for 2005-2007, with the emphasis to strengthen coordination between the two project components. The PCM was organized in conjunction with Second Reporting Meeting on Selection Efficiency for LP Mutant in Rice and Nutrient Bioavailability from Fortified Foods. A study visit was organized for the participants to, *inter alia*, observe agricultural practices, rice cultivation and irrigation systems in Bali.

The meetings were participated by 13 official representatives from the five participating countries. The Indonesian Government, through BATAN, provided necessary logistical arrangements and secretariat support to the meetings.

Two separate Reporting Meetings (RM) were organized on: Selection Efficiency for LP Mutants in Rice, and Nutrient Bioavailability from Fortified Foods, which the respective project counterparts participated in.

Country reports were presented, reporting on the project status, progress implementation, problems and constraints faced by each country. Due reference was made to the project work plan and activities as outlined in the Working Document, which had been developed during the project formulation meeting in 2002.

Concerning the breeding part, the programme is right on track. All project milestones for 2004 have been reached. All in all, the breeding counterparts over-performed in relation to the scheduled and planned milestones, and the initial difficulties and delays (related to the international political situation developing since 2001, and recurrent regional epidemiological concerns since 2003) in the implementation of the work programmes are being overcome by all participants. Advanced lines for stable mutations putatively representing at least three different genes of the biosynthesis pathway of phytic acid are available (maximum reduction in PA of 65%). Both *indica* and *japonica* varieties have been successfully mutated for LP production. At least one of the LP stable mutants also shows improved quality characters (low amylose content). Several stable mutants show an enhanced concentration and a shift in compartmental distribution of minerals (Fe, Zn and Ca). Molecular work has been initiated and one tightly linked SSR marker has tagged the *lpa1-1* locus. All participating countries have observed a relatively high incidence of lethal mutations. Heterozygotes of this category are available for upstream scientific activities (reverse genetics and genomics).

The deliberations included, among others, the aspects and issues that are specifically faced by each individual country. Views were exchanged as to the possible improvement of the project implementation and the strengthening of regional cooperation for greater impacts.

Based on the technical discussions, the meeting identified and recommended priority activities for 2005-2007, and developed a sectoral workplan and strategy for further deliberation at the plenary session.

Protocols for further studies for measurement of micronutrient bioavailability and for standardization and harmonization of nuclear technique-based protocols for induced mutation enhanced breeding in each country were developed. Priority needs and additional assistance to strengthen national capacity were identified. A protocol to measure the impact of LP rice on iron bioavailability was discussed and developed. The study will be made in China, who has the most appropriate facilities and have developed advanced LP mutant lines. It is proposed that LP rice be fed to women consuming typical Chinese meals of low, medium and high iron bioavailability.

The Project Coordination Meeting (PCM) was conducted following the RMs to allow active interaction and discussion between the two study groups (nutrition and rice breeding). The meeting was held in plenary session and attended by all participants.

The group reporters summarized the outcomes of the RMs, emphasizing the progress implementation and the

proposed workplan for 2005-2007. The meeting deliberated on the coordinating strategy and necessary mechanisms to strengthen coordination between the two groups, especially to ensure that the output produced would be supplemented to each other.

It was found that the project implementation is satisfactorily progressing in all countries, although in a different level of progress and achievement. Capacity building on application of isotopic techniques has been established.

Under the nutrition component, programme development and evaluation, through application of stable isotope technique, will continue for micronutrient bioavailability. The rice breeding component will continue the iterative and recurrent process to produce genetic resources for mutational analysis of the phytic acid biosynthesis pathways. The produced mutant rice will be tested and evaluated for its micronutrients availability and body absorption.

The meeting deliberated in greater details the possibility and strategy to build up collaboration with other institutions, financially and technically.

The project has generated valuable resources (enhanced mutant germplasm, effective food fortification strategies) and results, and built up technical capacities both in nutrition and breeding aspects. With these preliminary results and success of the project, the meeting recommended discussing and planning further extension on different levels.



First Research Coordination Meeting on “Identification and Pyramiding of Mutated Genes: Novel Approaches for Improving Crop Tolerance to Salinity and Drought”, Vienna, Austria, 14–18 March 2005

Technical Officer: S.M. Jain

A major constraint to food production is the lack of crop varieties with enhanced tolerance to environmental stress. Salinity and drought most severely limit crop production. A minimal understanding of the complex genetic basis for crop tolerance, and the difficulty in efficiently combining favorable loci or alleles into a local adapted genotype, and achieving adequate yield stability, has led to the limited success of previous efforts at improvement using conventional breeding techniques. This CRP will address the problems associated with screening natural and mutated germplasm, and identifying and pyramiding genes that contribute to abiotic stress tolerance and yield stability. The goal is to develop local crop varieties with enhanced yield capacity in drought and salt stress environments. Classical breeding techniques that capture natural genetic variation will be used in combination with induced mutations, marker-assisted methods and biotechnology to introgress genetic loci that will enhance drought and salt. Field evaluations will be the core foundation to establish whether sufficient yield stability can be achieved in developing new tolerant crop varieties. The combination of biotechnological and participatory aspects in this CRP will be at the vanguard of research implementation to enhanced crop tolerance to drought and salinity stress.

Conclusions and Recommendations

Crops included in the CRP: cereals (wheat, barley, maize, rice, and sorghum), legumes (soybean, chickpea, and peanut), and others (mustard and tomato). The diversity in crops is because of local priorities and much of the technology is common for the development of each crop. Coordination at the level of sharing technologies is imperative.

Crop yield constraints: Drought and salinity stress are major constraints to crop production, and increased tolerance to these stresses is the focus for crops in this project. The principal goal is to increase yield and yield stability of breeding lines.

General Recommendations:

Publications and dissemination: All participants will make available project results in published form, e.g. peer reviewed journals, reviews, and book chapters, and present their findings at scientific meetings and symposia and other public forums. All publications resulting from the CRP should acknowledge full or partial support of IAEA. Copies of all publications should be forwarded to

the IAEA. The period of the CRP can be extended if sufficient documentation of accomplishment (e.g. publications or abstracts) is provided.

Exchange of genetic material: The participants should have free access to all genetic resources and reagents used and developed in this project. Public availability of all outputs is a requirement of the IAEA. If there are concerns about this requirement, a clear statement of the necessity of a waiver may be requested. Requests should be submitted to the IAEA, who will facilitate in the development and implementation of a formal mechanism of resource exchange.

Collaborative linkages: Interaction between groups is very necessary for synergistic progress of the entire project. Rapid exchange of experimental results is essential to reduce duplication of efforts and provide a current knowledge base of information that will facilitate successful design and implementation of experimentation. Such interchange will be greatly streamlined by the establishment of an information network possibly facilitated by the IAEA.

Training/Technology transfer: Sponsoring of outreach courses and necessary visits to participant laboratories or IAEA to enhance technology training is recommended.

Collaboration with animal nutritionist for analysing straw quality as animal feed: The participants recognize the long-term need for such expertise. It is up to the individual participant to pursue this additional component.

General Technologies: The approaches are to develop and identify genetic variation for enhanced yield stability in drought and saline stress environments, facilitate the introgression and pyramiding of identified genetic determinants of tolerance and yield stability into crop progenitor lines, and develop breeding lines with increased yield stability. The specific technical strategies and tactics that will be the focal approaches of research in this CRP are: a) Mutation-assisted breeding; b) Candidate gene approach for improving tolerance to drought and salt tolerance of plants, c) Biotechnology, d) Plant cell and tissue culture techniques for identification and pyramiding of mutated genes for improving crop tolerance to drought and salinity, e) Application of DNA markers, f) High through-put mutation detection technology (TILLING), and g) Salt and drought tolerance and yield stability screening and selection methods.

Fourteen research contract holders from China, Cuba, Egypt, Ghana, India, Indonesia, Pakistan, Thailand, Tunisia, Turkey and Vietnam and three research agreement holders from Israel, Italy and the United States of America attended the RCM in Vienna.



Consultants Panel on “Material Transfer Agreement (MTA) Related to Mutant Germplasm”, 21–23 March 2005

Technical Officers: P.J.L. Lagoda & C. Mba

The Joint FAO/IAEA Division asked for advice on the development of its *ex situ* genebank of mutant lines developed from mutant germplasm (the “Mutant Germplasm Repository”, or MGR). This is of particular importance as this is an *ex situ* collection held directly by the Joint Division and as the CGRFA has established an International Network of *Ex Situ* Collections Under the Auspices of FAO, in order to provide a framework in which international organizations may hold plant germplasm in trust for the international community, thereby resolving problems regarding the ownership of their collections. The International Treaty on Plant Genetic Resources for Food and Agriculture also provides for the IARCs and other International Organizations to bring their collections under the International Treaty.

In due course, it is proposed that the MGR be brought under the International Treaty, in accordance with Article 15.5.¹

Consultants were Cary Fowler,² Bert Visser³ and Clive Stannard (FAO, Senior Liaison Officer, CGRFA/AGD).

¹ The IT is at <ftp://ext-ftp.fao.org/ag/cgrfa/it/ITPGRe.pdf>.

² Professor / Director of Research, Department of International Environment & Development Studies (NORAGRIC) and Norwegian University of Life Sciences.

³ Director, Centre for Genetic Resources, Wageningen University and Research Centre.

Regional Training Course on “Evaluation of Breeding of Neglected Crops” RAF/5/050, De l’I.S.R.A, Dakar, Senegal, 21–25 March 2005

Technical Officer: Q.Y. Shu

Course Director: M.T. Diouf

The objectives of this RTC were to (1) introduce new methodologies and breeding strategies for neglected crop improvement; (2) train participants with techniques and methods recently developed for neglected crop breeding through seminars, case studies and practical demonstrations by invited lecturers; (3) improve/adjust the methodology and strategy adopted in participating institutes as appropriate, through consultations with experts and among participants.

Twenty-four researchers from 16 African countries (Algeria, Cameroon, Central African Republic, Ethiopia, Ghana, Kenya, Madagascar, Mauritius, Morocco, Niger, Senegal, Sierra Leone, South Africa, Sudan, Tunisia, Zambia) participated in the 5–day RTC. Both seed and vegetatively propagated crops considered as neglected and underutilized globally were discussed within the context of the development and exploitation of mutation breeding and biotechnological approaches, followed by practical demonstrations on both tissue culture manipulations and molecular (PCR) analyses and case studies on neglected crop improvement. Discussions were held with the experts on specific aspects of each participants’ presentations of the work done in their home institute, and suggestions and advice were given for future research.

Status of Coordinated Research Projects

Physical Mapping Technologies for the Identification and Characterization of Mutated Genes Contributing to Crop Quality

Technical Officer: P.J.L. Lagoda

This CRP was initiated in 2002. The first RCM was held in Vienna, Austria, 31 March–4 April 2003, followed by a three-day Workshop on fluorescence *in situ* hybridization (FISH) at the Plant Breeding Unit, Seibersdorf, Austria. The second RCM is planned to be held in Reykjavik, Iceland, 22–26 August 2005.

(For details, please refer to FORTHCOMING EVENTS)

Pyramiding of Mutated Genes Contributing to Crop Quality and Resistance to Stress Affecting Quality

Technical Officer: Q.Y. Shu

This CRP was initiated in 2004. The first RCM was held in Vienna, Austria, 13–17 September 2004.

The second RCM is planned for 2006; exact date and location to be announced at a later date.

(For details, please refer to PAST EVENTS)

Identification and Pyramiding of Mutated Genes: Novel Approaches for Improving Crop Tolerance to Salinity and Drought

Technical Officer: S.M. Jain

This CRP was initiated in 2004. The first RCM was held in Vienna, Austria, 14–18 March 2005.

The second RCM is planned to be held in Accra, Ghana in November 2006.

(For details, please refer to PAST EVENTS)

Effects of Mutagenic Agents on the DNA Sequence in Plants

Technical Officer: P.J.L. Lagoda

This CRP was initiated in 2003. The first RCM was held in Vienna on 1–5 March 2004. The second RCM is planned to be held in Seoul, South Korea, 14–18 November 2005.

(For details, please refer to FORTHCOMING EVENTS)

Molecular Tools for Quality Improvement in Vegetatively Propagated crops including Banana and Cassava

Technical Officer: S.M. Jain

This CRP was initiated in 2004. The First RCM is planned to be held in Vienna, Austria, 18–22 July 2005.

(For details, please refer to FORTHCOMING EVENTS)

Technical Cooperation Projects

Currently Active Projects

| Project Number | Title | Technical Officer |
|----------------|--------------------------------------------------------------------------------------------------------------------|-----------------------------|
| COS/5/021 | Plant Disease Diagnostics | P.J.L. Lagoda |
| COS/5/025 | Development of Induced Mutations and Biotechnology for Improved Productivity and Competitiveness | C. Mba |
| GHA/5/031 | Enhancing Cassava Production through Supplementary Nutrient Application | R. Serraj, C. Mba |
| GHA/5/032 | Enhancing Production and Use of Cassava | C. Mba |
| INS/5/030 | Sustainable Agricultural Development in Yogyakarta | M.M. Spencer |
| INS/5/031 | Mutation Breeding of Horticultural Crops | M.M. Spencer, M. Miranda |
| INT/5/147 | Developing Salt-tolerant Crops for Sustainable Food and Feed Production in Saline Lands | M.M. Spencer, M. Miranda |
| IRQ/5/015 | Induction of Mutations in Crops through <i>In Vitro</i> Culture | P.J.L. Lagoda |
| JOR/5/008 | Establishment of <i>In Vitro</i> Mutagenesis Laboratory | P.J.L. Lagoda |
| KEN/5/024 | Crop Improvement and Management through Application of Nuclear and Biotechnology Techniques | Q.Y. Shu |
| MAG/5/008 | Mutation Techniques and Biotechnology for Rice and Cassava | P.J.L. Lagoda |
| MAK/5/004 | Mutation and Doubled Haploid Techniques to Improve Wheat | P.J.L. Lagoda |
| MAL/5/024 | <i>In Vitro</i> Mutagenesis for Horticultural Crop Plants (Phase I) | M.M. Spencer |
| MYA/5/010 | Development of Improved Rice with Tolerance to Drought and Soil Salinity | Q.Y. Shu |
| NIR/5/031 | Radiation-Induced Mutations for the Development of Cowpea Varieties | P.J.L. Lagoda |
| PAK/5/040 | Improvement of Heat-Tolerant Semi-Dwarf Bread Wheat through Radiation Induced Mutations | P.J.L. Lagoda |
| PAK/5/042 | Induced Mutation to Improve Salt-tolerance in Non-aromatic Rice Varieties | P.J.L. Lagoda |
| PAK/5/044 | Improvement of Drought Tolerance in Chickpea through Induced Mutations | P.J.L. Lagoda |
| PER/5/024 | Introduction of Barley and other Native Crop Mutant Cultivars | P.J.L. Lagoda |
| PER/5/028 | Use of Nuclear Techniques to Improve Cotton Production | C. Mba |
| PHI/5/029 | Enhancing Agricultural Productivity through Radiation Technology in Mindanao | M.M. Spencer |
| RAF/5/049 | Field Evaluation of Bayoud-Resistant Date Palm Mutants | M.M. Spencer, P.J.L. Lagoda |
| RAF/5/050 | Increasing Production of Nutritious Food through Mutation Breeding and Biotechnology (AFRA III-3) | Q.Y. Shu, M.M. Spencer |
| RAS/7/014 | Monitoring of Food Fortification Programmes Using Nuclear Techniques | P.J.L. Lagoda |
| RAS/5/040 | Enhancement of Genetic Diversity in Food, Pulses and Oil Crops and Establishment of Mutant Germplasm Network (RCA) | Q.Y. Shu |
| ROK/5/033 | Quality Improvement of Major Crops and Integrated Plant Nutrition Management in the Low-Input Agricultural System | P.J.L. Lagoda |

| Project Number | Title | Technical Officer |
|----------------|---------------------------------------------------------------------------------------------------------|----------------------------|
| SAF/5/008 | Mutant Amaranth, Bambara Groundnut and Cowpea with Enhanced Abiotic Stress Tolerance | Q.Y. Shu |
| SIL/5/007 | Development of High-yielding Rice Varieties for Low-input Agriculture Systems using Mutation Techniques | Q.Y. Shu |
| SRL/5/034 | Radiation-Induced Mutations for Black Pepper Improvement | P.J.L. Lagoda |
| SRL/5/036 | Virus Screening of Improved Banana Mutants for Large-Scale Dissemination | P.J.L. Lagoda |
| SUD/5/026 | Improvement of the Productivity and Sustainability of Industrial Crops | Q.Y. Shu |
| TUN/5/023 | Radiation-Induced Mutations for Improvement of Cactus | M. Miranda |
| TUR/5/023 | Application of Nuclear and Gene-Based Biotechnology in Agriculture | P.J.L. Lagoda / M. Miranda |
| URT/5/020 | Improving Productivity of Basic Food Crops | Q.Y. Shu |
| URT/5/023 | Enhancing Crop Productivity through Radiation Technology | Q.Y. Shu |
| VIE/5/015 | Enhancement of Quality and Yield of Rice Mutants using Nuclear and Related Techniques, Phase II | Q.Y. Shu |
| YEM/5/003 | Applying Nuclear Techniques for Improvement of Crop Yield | P.J.L. Lagoda |
| YEM/5/007 | Use of Induced Mutations and <i>In Vitro</i> Culture for Improving Crops | P.J.L. Lagoda |
| ZAI/6/009 | Mutation Techniques for Improving Medicinal Plants with a Curative Effect on Human Diseases | M. Miranda, M.M. Spencer |
| ZAM/5/022 | Crop Improvement through <i>In Vitro</i> Mutation Technique | Q.Y. Shu |

TC Project Highlights

Nuclear technology for the improvement of cactus, TUN/5/023

Cactus is a cash crop for the production of fruits (human consumption) and pads (animal consumption) in dry areas. Due to an extremely resilient character, cactus plants grow well under severe drought and heat conditions, where other crops are not viable. Thus, sustainable cactus cropping plays a key role in food security for the arid and semi-arid areas of the world. Despite its great social-economical importance, cactus biodiversity and breeding potential have been neglected to a great extent.

Losses of material due to frosts and/or excessive irregular rainfall are one of the major obstacles hampering increases in the production of cactus in Tunisia and other countries. In addition to that, cactus usage is limited because, when fed alone to small ruminants, farmers need

to supplement animal diet with a nitrogen source, increasing the costs significantly. The Technical Cooperation Project TUN/5/023 “Radiation-induced mutations for genetic improvement of cactus for food and feed”, which has been recently implemented at the National Institute for Agricultural Research of Tunisia (INRAT), aims at addressing these questions through the use of nuclear technology for the production of improved mutant varieties that exhibit cold hardiness, higher nitrogen content and sweeter fruits with lesser seeds.



Ongoing Activities at the Plant Breeding Unit, Seibersdorf

The activities of the Plant Breeding Unit, Agency Laboratories, Vienna and Seibersdorf, continue to be focused on providing the laboratory platform for assisting the Member States (MS) in the use of induced mutations and ancillary biotechnologies for the development of superior crop varieties. It has therefore been the traditional role of the Unit to provide training in these areas as well as services (in irradiation of plant propagules; ploidy determination; and molecular genetic fingerprinting of mutants) to young scientists from MSs of both the FAO and IAEA. At the Unit also, the adaptation of crop improvement technologies to suit the needs of Member States has also continued through the induced mutations work on banana, cassava and rice.

Rice

Our main interest in rice has remained the development of saline tolerant rice mutant variants for integration into the breeding schemes of MSs. Several putative mutants have been developed from our laboratories in collaboration with the International Rice Research Institute (IRRI), Manila, Philippines and are at varying stages of use in the rice breeding schemes of many rice National Agricultural Research Systems especially in South Eastern Asia. We have continued to develop and evaluate others in recognition of the wide agroecological zones that will be targeted by the varieties. Our starting materials have been salt tolerant and susceptible varieties IR 51500, Pokkali, Nona-bokra, Bicol and IR 29, a modern glutinous rice variety, developed at IRRI that is highly susceptible to salinity. A highly efficient hydroponics evaluation system has been instrumental in the high rate of success with the generation of the mutants. Parallel to this effort is the generation of molecular tags for the mutated segments of the genome influencing the tolerance to high concentrations of salt.

Cassava

As part of the new efforts to contribute to the enhancement of cassava productivity through the development and deployment of cassava varieties with added value, the PBU has continued with the massive production of cassava mutants. These mutants are for shipment to counterpart NARS cassava improvement programmes in the tropics and neotropics. The development of the mutants has been facilitated by efficient *in vitro* methodologies for both the induction of mutations and the rapid multi-

plication of the mutants in order to dissociate chimeras and also to produce disease-free planting materials.

Banana

Field evaluation of mutants

Three hundred *in vitro* plantlets of the banana variety Grande Naine at the third cycle of vegetative regeneration (M_1V_3) are being evaluated for disease resistance and other agronomic traits in Kenya in collaboration with the Kenya Agriculture Research Institute (KARI) Njoro, Kenya. Also ongoing is the field evaluation of over five hundred mutant banana plants in Uganda in collaboration with the Kawanda Agricultural Research Institute (KARI), Kampala, Uganda. The main objectives include the identification of disease tolerant variants the development of linked molecular markers linked to disease tolerance. The marker fidelity of the over 50 simple sequence repeat (SSR) markers that we recently developed for the *Musa* genome is being validated with a panel of 48 *Musa* accessions being used by the members of the *Musa* Genomics Consortium.

Irradiation Services

A total of 93 of irradiation treatments were carried out in support of the activities of Member States services during the period June to November 2004 and are broken down thus:

| | |
|------------------------------------|----|
| Number of requests | 13 |
| Number of species | 17 |
| Number of varieties | 45 |
| Number of treatments | 93 |
| Number of requesting Member States | 8 |

Molecular genetic fingerprinting services

Most of our activities in this regard have been confined to the use of our high throughput facilities for in-house research and development activities. During the period under review, about 1000 DNA fragments were sequenced while DNA fragment separation was carried out on about 4000 samples.

Visiting Scientists

| Name | Country | Institute | Period |
|--------------------------|------------|-------------------------------------------------|---------------|
| SIDDIQUA KHANOM, O.N. | Bangladesh | Ministry of Science and Technology | February 2005 |
| LEBECKA, R. | Poland | Plant Breeding and Acclimatization Institute | May 2005 |
| SLIWKA, J. | Poland | Plant Breeding and Acclimatization Institute | May 2005 |

News Sparks

First Mutant Varieties Released in Iran

The Department of Nuclear Agriculture, Nuclear Research Center for Agriculture and Medicine (NRCAM), Atomic Energy Organization of Karaj, Iran, recently announced the release of two excellent rice varieties, Tabesh and Pooyaa. These two varieties, developed jointly with Rice Research Institute of the Ministry of Jihad Agriculture using gamma rays irradiation, are the first mutant crop varieties ever released in Iran. The two new varieties were registered in our Mutant Variety Database (<http://www-mvd.iaea.org/MVD/default.htm>) with ID 2349-2350.

First Batches of Mutant Groundnut Varieties developed in Bangladesh

The Bangladesh Institute of Nuclear Agriculture has recently developed three new groundnut varieties, the first batches of mutant groundnut varieties in Bangladesh. These varieties, released with the names of BINAchabadam-1, BINAchabadam-2, and BINAchabadam-3, were developed using gamma irradiation techniques. These varieties showed to be higher in yield, more tolerant to cercosporia leafspot and rust disease, and

better in quality than the control variety Dhaka-1. The three new varieties were registered in our Mutant Variety Database (<http://www-mvd.iaea.org/MVD/default.htm>) with ID 2346-2348.

Research Consortium on Low Phytate Crops in China

A research consortium on low phytate crops (LPCC) was recently established in China. Phytic acid, or inositol hexakisphosphate, categorized as a potential antinutritional component since its discovery, is the major phosphorus compound in cereals and legumes. Mutant lines, with phytic acid phosphorus reduction up to 90%, have been developed using chemical and physical mutagenesis in major food crops. Under the support of IAEA regional technical cooperation projects RAS7014 and RAS5040, a couple of low phytate mutant lines, of rice, soybean, maize, wheat and barley, have been developed in China. For accelerating and expanding the breeding and nutritional evaluation efforts, the IAEA-Zhejiang University Collaborating Centre for Mutant Germplasm Development and Exploitation in Plants established the LPCC, which attracted 15 research groups from 13 provinces of China. Dr. Dianxing Wu of Zhejiang University and Dr. Qingyao Shu of the Agency coordinate the LPCC.

Publications

Protocols for Somatic Embryogenesis in Woody Plants

Jain S.M. and P.K. Gupta. Springer, Dordrecht, The Netherlands.

(2005) ISBN 1 4020 2984.5

Major Mutation-Assisted Plant Breeding Programs Supported by FAO/IAEA

Jain S.M. Plant Cell, Tissue and Organ Culture, 82: 113-123.

(2005)

Protocols for Somatic Embryogenesis in Woody Plants Slash pine (*Pinus elliottii* Engelm.)

Newton R.J., W. Tang and S.M. Jain

Jain S.M. and P.K. Gupta (Eds.) Springer, Dordrecht, The Netherlands pp 1-10

(2005)

Global Impact of Mutation Derived Varieties

Ahloowalia B.S., M. Maluszynski and K. Nichterlein. Euphytica 135:187-204.

(2004)

Genetic Improvement of Under-utilized and Neglected Crops in Low Income Food Deficit Countries Through Irradiation and Related Techniques

IAEA TECDOC Series No. 1426

This publication contains the results of an FAO/IAEA Coordinated Research Project (CRP) on “Genetic Improvement of Under-utilized and Neglected Crops in LIFDCs through Irradiation and Related Techniques”. It highlights the integration of radiation induced mutations *in vitro* culture and molecular genetics methods into the conventional

breeding of neglected and under-utilized crops. The successful results included are: plant regeneration strategies in *Dioscorea* spp., grass pea and bambara ground nut, root rot disease tolerant putative mutants of cocoyam, and a genetic diversity bank of bambara ground nut, quinoa, naranjilla, okra, *Amaranthus tricolor*, and *A. cruentus*. This publication would be of immense benefit for enhancing the genetic improvement of neglected and under-utilized crops and for further advancing international programmes for improving food security, nutrition, socio-economic aspects, employment generation and sustainable food production.

(2004) ISBN 92-0-113604-8

IAEA-TECDOC-1426 €15.00

Changes of morphological and physiological traits of *Zea mays* plants and male gametophyte grown on condition in low dose gamma and β radiation

Tentiu A., F. Zapata-Arias, R. Afza, A. Kodym and A. Pavlenko. Universitatea Agrara de Stat din Moldova, Lucrari stiintific, vol. 13, Agronomy, pp 239-243.

L. Extent of acceptance by growers:

- **Commercial value:**

- **Hectares of cultivation:**

- **Other:**

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

Name of person contributing this information: _____

THANK YOU FOR YOUR COLLABORATION!



IAEA

International Atomic Energy Agency

Plant Breeding and Genetics Newsletter No. 15

July 2005

The PBG Newsletter is prepared twice a year by the Plant Breeding and Genetics Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf.

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Wagramer Strasse 5, P.O. Box 100,
A-1400 Wien, Austria

Printed by the IAEA in Austria,
June 2005

05-21191