IAEA

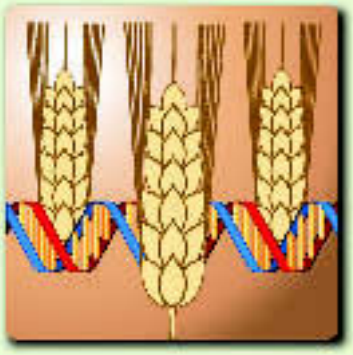
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf

Plant Breeding & Genetics Newsletter

No. 14 December 2004

http://www.iaea.org/programmes/nafa
http://www.fao.org/WAICENT/Agricul.htm

ISSN 1564-2569



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Control (top left): Red purple flower colour – Mutant (top right): Variegated red flower colour
Control (bottom left): Red flower colour – Mutant (bottom right): Red grey flower colour
TC project INS/5/031

To Our Readers

These last six months, the Plant Breeding and Genetics (PBG) Section of the Joint FAO/IAEA Division (NAFA/AGE) implemented five Research Coordination Meetings (RCMs) and one Consultants Meeting for a new Coordinated Research Project (CRP) on "Molecular tools for quality improvement in vegetatively propagated crops including banana and cassava" (8–11 November 2004, Vienna). Other salient points were the training courses we implemented this semester in the framework of different Technical Cooperation (TC) projects. You will find details about these activities inside this Newsletter. A highlight of these activities, as every year since 2001, was the Interregional Training Course on "Mutant Germplasm Characterization using Molecular Markers" at the Seibersdorf Laboratories (Austria, 27 September–22 October 2004). The 20 participants from 20 Member States gave us much pleasure by their commitment during these four weeks of intense interactions. This Fourth Training Course was again

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masterminded by Dr. Stephan NIELEN, and this year co-directed by Dr. Chikelu MBA (Head, Plant Breeding Unit).

I take this opportunity to thank Stephan for his dedication to Sub-programme affairs, shouldering more than once in excess of his share of the professional burden. However, the Agency's rules are such, that at this moment in time we have to say "Goodbye". Stephan has served with enthusiasm his full term of seven years with the Agency, first at the Plant Breeding Unit in Seibersdorf, and the last four years at the Agency's Headquarters in Vienna. I thank Stephan for he high level of professionalism, knowledge and know-how that he brought to our activities and wish him every success at EMBRAPA-CENARGEN where he will continue his career as a Molecular Cytogeneticist. This year, we successfully organized an FAO/IAEA/RCA Strategic Meeting on "Nuclear Techniques for Rice Improvement in Asia", coordinated by our colleague Dr. Qingyao SHU under the umbrella of the World Rice Research Conference (WRRC), held in Tokyo and Tsukuba, Japan, 4-7 November 2004. An overwhelming positive response rewarded us for all the efforts spent to materialize this event. We wish to express our gratitude for the innumerable people outside and inside the Agency who assisted us in this endeavour and we want to especially thank the organizers of WRRC and our Japanese counterparts and resource persons for their efficiency and cooperation.

Last, but not least, I would like to inform you that by the time you read this Newsletter, Ms. Kathleen WEINDL, Senior Office Clerk at the PBG Section will have retired. Kathy has served the Joint Division with undaunted and unbent loyalty for more than twenty-three years, often going well beyond the call of duty, and to many of you she was a faithful and knowledgeable contact. Kathy will retire and will be able to devote more time to her family and garden. We will lose both the soul and the institutional memory of our Section. Her high standards have set a goal for all of us, reminding us by her efficiency and her professional, yet cheerful and humane attitude what the real meaning of international civil service is. It is an honour to have been able to work with Kathy, she enriched my professional life, and it will be a pleasure to stay in private contact with her, occasionally swapping seeds from and for our private gardens.

Dr. Stephan Nielen

Ms. Kathleen Weindl

Pierre J.L. Lagoda

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Forthcoming Events

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

Drought and salinity are major constraints on crop production and food security, and have adverse impact especially on socio-economic aspects in developing countries, so the development of crops with tolerance is a priority. A major constraint to improved tolerance is the lack of understanding of its complex genetic basis and the difficulty in efficiently combining favourable alleles into an optimal genotype, which has led to the limited success of previous efforts at improvement using conventional techniques. This Coordinated Research Project (CRP) will address the problems associated with screening natural and mutated germplasm, and identifying and pyramiding genes contributing to abiotic stress tolerance, and will use marker-assisted methods and induced mutations to accelerate improvement. It will focus on those cereals and grain legumes, which are important for food security at least at the local level. There are 14 research contract holders from India, China, Tunisia, Pakistan, Cuba, Turkey, Thailand, Vietnam, Ghana, Egypt and Indonesia. The six research agreement holders are from USA, Italy and Israel.

First Research Coordination Meeting on "Molecular Tools for Quality Improvement in Vegetatively Propagated Crops Including Banana and Cassava", Vienna, Austria, 6–10 June 2005

Technical Officer: C. Mba

This 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 agro-ecosystems. 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

In order to find information on how to participate in CRPs, as well as the necessary forms to fill out, please consult the following web page: http://www-crp.iaea.org.

Second Research Coordination Meeting on "Effects of Mutagenic Agents on the DNA Sequence in Plants", to be scheduled in South Korea or South Africa – tentatively second half of 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. *in prep.*). 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 retroelements, 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.

Second Research Coordination Meeting on "Physical Mapping Technologies for the Identification and Characterization of Mutated Genes Contributing to Crop Quality), Reykjavik, Iceland, 5–9 September 2005

Technical Officer: P.J.L. Lagoda

The CRP is based on the development and use of cutting-edge technologies, including DNA microarrays, preparation and screening high density colony arrays and fluorescence *in situ* hybridization (FISH) on mitotic and meiotic chromosomes and DNA fibres. Their application relies on sophisticated instrumentation and experience with advanced methods of plant molecular biology and genomics. In order to facilitate the access to these technologies it was recommended, during the first RCM, to strengthen existing links and establish new links between participants. In particular, participants working on related species and objectives are going to share and complement techniques. The participants agreed to share DNA clones, probes and vectors where appropriate.

This second RCM, in addition to project steering and progress evaluation, is bound to focus on discussing high-technology physical mapping, adding to the data obtained by the participants, and centering on the newly developed material. For example, extra low-copy probes where technology was not available in the home laboratory, or fiber DNA *in situ* hybridization to increase the resolution of physical mapping, or microarray construction to examine deletions in mutated material, are tentative discussion items.

Regional Training Course on "Molecular Marker Techniques for Mutant Characterization" RAS/5/040, Suwon, Republic of Korea, 13–24 June 2005

Technical Officer: Q.Y Shu

The training course will be open to scientists working on project RAS/5/040, for genetic characterization of induced mutants. The main objective is to train young scientists in molecular marker techniques for the identification of mutated trait. The training will be comprised of theory lectures on molecular marker technologies, genotyping and gene tagging using molecular markers, and their applications in plant breeding, particularly the characterization of induced mutants. Practical excercises will be included from DNA extraction; PCR based molecular markers and gene mapping.

The CRP is directed towards accelerating crop-breeding programmes through the application of physical mapping and complementary genomic approaches and the characterization and utilization of induced mutants for improvement of crop quality. Among the traits that have been reported to be assessed for improved quality within the CRP are bread making (wheat/ *Leymus*), fruit colour and carotinoide (tomato and pepper), aroma and waxes (rice), fibres (cotton), oils and fatty acids (*Brassica*), and secondary metabolites, in particular medicinal alkaloids (poppy). A wide range of material and resources, such as genetic aneuploid stocks, chromosomal translocations and deletions, wild germplasm, mutant lines, genetic maps and BAC libraries are available within the CRP.

Past Events

Coordination Meeting of the Regional Project on the "Field Evaluation of Bayoud Resistant Date Palm Mutants" RAF/5/049, Sfax, Tunisia, 7–11 June 2004

Technical Officer: S.M. Jain

The first date palm project (RAF/5/035) began in 1995 with the main objective to isolate Bayoud disease resistant date palm mutants. Initially, date palm tissue culture technology has been via somatic embryogenesis and organogenesis for plant regeneration. Each participating country presented their report, which was followed by discussion. Each participant presented a progress report. Reports were informative and highlighted the results on Bayoud disease, including the screening technique and national needs to upgrade the available facilities. Considerable time was spent to address the supply of Bayoud toxin, isolated from Bayoud disease causal fungus *Fusarium oxysporum* sp. *albedinis* (FOA), for the selection of Bayoud disease tolerant mutants. Overall, the project has made substantial progress by isolating putative Bayoud disease resistant date palm mutants that will be finally evaluated in the field.

National Training Course on "*In Vitro* Large-Scale Plant Production and Cryopreservation of Mutants" MAL/5/024, Bangi, Malaysia, 12–16 July 2004

Technical Officer: S.M. Jain

The purpose of the course was to train plant breeders/biotechnologists in large-scale plant production and multiplication, long-term storage of mutant lines, hardening of *in vitro* plants, low cost of plant production, and greenhouse technology. The training was comprised of theory lectures on bioreactor technology and their applications in plant multiplication, secondary metabolite production; cryopreservation; low temperature storage, hardening of *in vitro* plants, greenhouse types and maintenance; cost reduction of plant production; practical demonstration in bioreactor and cryopreservation. There were 26 participants from five different institutes/universities (two from Indonesia). The invited lecturers were: Dr. Bart Panis, Belgium, and Dr. P.K. Saxena, Canada. Participants were provided with lecture notes.

Regional (AFRA) Training Course on "Screening Techniques for Drought Tolerance and Salinity" RAF/5/050, Tangiers, Morocco, 12–16 July 2004

Technical Officer: S. Nielen

The purpose of the course was to share experience with other collaborating scientists from the region. Drought screening techniques discussed and taught focused on those traits that have been identified at the Lusaka meeting (Zambia, November 2003) as being most relevant as measurement to determine tolerance to drought stress. Drought screening guidelines had been drafted in the previous workshop in Lusaka, as a preliminary working document. This Training Course was attended by plant breeders/agronomists from institutes included in the project RAF/5/050 and engaged research teams involved in the development of mutant germplasm of major crops with improved drought tolerance. Practical exercises on appropriate screening techniques for drought and salinity tolerance addressed specific technical problems as defined in the Lusaka meeting. This course was also the occasion to introduce these techniques to participants without previous experience. A field visit to the Moroccan Research Station completed the more technical modules. Presentations delivered by the participants and lectures provided by international experts are bound to become the basis for continuous improvement of the tentative screening guidelines previously drafted as a working basis.

Second Interregional Training Course on "Application of Induced Mutations and Biotechnology for Crop Salt Tolerance Improvement" INT/5/147, Beijing, China, 2–6 August 2004

Technical Officer: S.M. Jain

This course was organized to train plant breeders in mutagenesis, molecular markers, and biotechnology including doubled haploid technique and in vitro plant regeneration for accelerating the breeding cycle for developing salt tolerant lines. It was held at the Institute of Crop Science, Beijing China. This was the second Training Course organized under INT/5/147 project. Eight participants came from China, Cuba, Iran, Morocco, Pakistan, Thailand, and Tunisia and they shared a common goal to develop salt and drought tolerant varieties. During the field trip to Changping Experimental Research Station, all participants had an opportunity to see breeding programs of sorghum, wheat, maize and rice. Participants were provided with CD-rom disks containing all course lectures. The invited lecturers were: Dr. M. Foolad, USA, and Dr. R.K. Sangwan, France.

National Training Course on "Molecular Markers and Screening Techniques for Biotic Stress Tolerance in Black Pepper" SRL/5/034, Matale, Sri Lanka, 30 August–3 September 2004

Technical Officer: S.M. Jain

The purpose of the training course was to train plant breeders/biotechnologists in molecular markers for the identification of trait specific markers and other applications and to screen techniques for biotic stress tolerance in order to select and maintain drought/heat tolerant black pepper mutants. The major topics of the Training Course were: Molecular markers in plant improvement, and covered different types of molecular markers such as RFLP, AFLP, SSRs etc.; applications of markers; identification of trait specific markers; molecular marker-assisted selection and breeding. The other component of this course was screening techniques for biotic stress tolerance, e.g. drought, salinity etc., in the greenhouse and the field. It covered biochemical, radioisotopes, and both physiological and molecular aspects. There were 16 participants with academic background in the areas of plant physiology, agronomy, plant breeding, molecular biology and plant pathology. Computer demonstrations were given on publically available (and widely used) software 'MAPMAKER' for linkage analysis and for localisation of genes/QTLs associated with traits in crop plants. The invited lecturers were: Dr. R.K. Agarwal, India, and Dr. R. Yadav, UK.

Regional Training Course on "Induced Mutations for Crop Quality Improvement" RAS/5/040, Beijing, China, 30 August–8 September 2004

Technical Officer: Q.Y. Shu

Fourteen plant breeders from eight countries (China, India, Indonesia, Korea, Pakistan, Sri Lanka, Thailand and Vietnam) participated in the 10–day RTC. The lectures covered the following aspects (1) mutation techniques and induction of quality mutants in food, legume and oil crops; (2) introduction of different quality characters including consumer preference, processing property, and nutrition value; (3) the genetics, genomics and biochemistry of important quality characters; (4) methodologies, protocols and national and international standards for quality evaluation. The training course was enriched by practical exercises/demonstrations of advanced equipment, facility and new techniques for mutation induction and selection; quality character improvement and research using induced mutants.

The Training Course started with the participants' presentation of projects or research at their home institutes. A general discussion was organized, and all participants presented a proposal for future activities based on the new knowledge, skill and ideas they learned during the training.

Fourth Research Coordination Meeting on the "Molecular Characterization of Mutated Genes Controlling Important Traits for Seed Crop Improvement", Faro, Portugal, 6–10 September 2004

Technical Officer: P.J.L. Lagoda

The genetic improvement of crops remains a vital discipline in the early 21st century. That crop agriculture has benefited hugely from mutagenesis programmes is beyond any dispute. Although yield remains the top priority for breeders of most crops, there is increasing pressure to breed varieties with improved quality, biotic and abiotic resistances, and environmentally "greener" characteristics, that will allow reduced usage of pesticides and other agrochemicals, and that will also address human dietary health issues. In addition, food safety and security are assuming ever-increasing importance by governments of Member States, and the improvement of human health through enhanced nutrition is a long-term goal.

It is becoming particularly evident that existing genetic variation is insufficient to achieve the major shifts in plant breeding required for these key traits. Further advances will rely heavily on increased exploitation of germplasm resources (e.g. allele mining and alien introgression), as well as mutated genes arising from mutagenesis programmes or gene 'engineering' endeavours, as significant genetic gains will require extensive novel genetic variation. Future advances will require genomic resources, such as EST databases, to identify candidate genes and large insert DNA libraries for map-based isolation of 'trait' genes, and the application of molecular markers to crop plant improvement. Single nucleotide polymorphism (SNPs) and other mutation events in DNA sequences will play an increasingly important role in allele identification and marker development.

The specific research objectives of this CRP were as follows:

1 To collectively develop characterized and database mutant collections of key crops for application by CRP members and the world scientific community.
2. To molecularly characterise new or existing mutants affecting key agronomic traits in major crops and using comparative approaches in under-utilized crops with a view to their eventual isolation.

This CRP has achieved and, indeed, exceeded its original objectives, as set out by the Plant Breeding and Genetics Section (Reference: 312.D2.02.5, dated 1999–01–26) and undoubtedly contributed to bridging the 'phenotype gap'. A major achievement has been the establishment of mutant plant populations for wheat (2), rice (5), barley (4), maize (1), pearl millet (1), pea (2), soybean (1), and flax

(1). Moreover progress was made in developing germplasm that can be used for mutagenesis in recalcitrant outbreeding crop species (i.e. potato). These populations have been extensively characterized phenotypically and evaluation data for some (e.g. rice) have been entered into publicly accessible databases. Populations have been deposited into the Mutant Germplasm Repository (MGR). Improved rice varieties have been developed from mutated germplasm, and further varietal development is in progress in rice and other crops.

This CRP has proved to be an extremely efficient knowledge transfer vehicle. Considerable progress has been made in the deployment of more advanced marker technologies within the lifetime of this CRP. As a direct result of the high level of communication and knowledge sharing between members of the CRP, there has been a shift in marker usage away from less robust and relatively inefficient molecular marker technologies (e.g. RAPD) towards more advanced and efficient marker methodologies (i.e. SSRs, AFLPs, SCARs, SNPs etc) in their programmes. Genome based analytical approaches have been supplemented by methods which aim to characterise gene products by, for example, transcriptional profiling (e.g. cDNA-AFLP, differential display, cDNA-RDA), proteomics, and metabolomics (LCMS, GCMS).

The use of robust SNP-based marker types, AFLPs, microsatellites and SCARs, provide accurate and efficient tools for mapping and cloning strategies making the pace of achieving results much faster when compared to the results obtained by participants prior to this CRP. The participants have developed efficient screening methods (hydroponics, aeroponics) for mutations in particular plant structures and pathways for abiotic (drought, flooding, acidic soils including aluminium and organic acids, cold, herbicide) and biotic (disease and insect resistance) stress tolerance. More functional markers are needed (i.e. with complete linkage to the trait). Decisions come down to cost, efficiency and reliability of markers, which preferably should be located in genes themselves. Thus, SNPs are becoming the most sought-after marker type. With recent developments, SNP genotyping is developing into a cost-effective marker technology. Additionally, Cel1 and denaturing HPLC assays are allowing SNP and 'InDel' mutant screening in mutagenised populations (e.g. TILLING) to progress at a faster pace.

There are many good examples of the genetic characterisation of agronomically important mutated genes within this CRP. These mutant genes include those for resistance to biotic and abiotic stresses, photoperiod sensitivity, grain quality, root characters, morphological traits, nitrogen fixation, and herbicide resistance. In most cases the genes have been mapped to chromosomal locations, with concomitant development of linked markers that have potential for use in marker-assisted-breeding and gene pyramiding. Markers have been transferred to breeders and are being deployed in breeding programmes. In some cases the genes corresponding to the mutated trait alleles have been isolated within this CRP and several other candidate genes have been identified in:

- flax - high oleic acid and seed colour;
- barley - hairless root;
- maize - photoperiod sensitive, opaque endosperm and Asian corn borer resistance;
- soybean - supernodulation;
- rice - semidwarf, male sterile, low phytic acid, photoperiod sensitive male sterile, cold tolerance, organic acid tolerance, bacterial leaf blight, rice blast and gall midge resistance;
- wheat - yellow rust, powdery mildew and leaf rust resistance;
- pea - powdery mildew resistance;
- potato - late blight and potato cyst nematode resistance;
- foxtail millet - sethoxydim resistance.

The use of advanced bioinformatics resources has benefited this activity. Several partners are using map-based cloning approaches to isolate target genes. BAC libraries have been developed for rice (two libraries), potato (two libraries), millet (one library) and expertise in making such libraries has been developed in partner laboratories.

Many students have been trained in the use of mutations for genetic analysis and improvement of seed crops as a result of this CRP.

In addition, opportunities were provided for network formation as a platform for sharing information, knowledge and germplasm arising from other collaborative research projects related to agronomically important traits.

First Research Coordination Meeting on the "Pyramiding of Mutated Genes Contributing to Crop Quality and Resistance to Stress Affecting Quality", Vienna, Austria, 13–17 September 2004

Technical Officer: Q.Y. Shu

Seventeen participants from 16 countries (Australia, Bulgaria, China, Cuba, France, India, Indonesia, Iran, Japan, Korea, Macedonia, Pakistan, Poland, Thailand, UK, and CIAT) participated in this RCM. Each participant made two presentations; one on the status of research in relevant field of their project, and the other on the workplan of each group. After discussion and consultation, individual workplans were further improved and group activities decided on.

This CRP will work on eight crops (barley, cotton, groundnut, okra, potato, rice, sorghum, and wheat) and various quality characters, e.g. traits relating to consumer preference, nutritional quality, processing quality, and tolerance to stresses affecting quality. Both mutation and conventional germplasm will be used for identification of

genes contributing to the above quality characters. Desirable genes will be pyramided into elite breeding lines using molecular marker techniques. Collaborative links were established among some participants during the RCM and more will be sought. It was recommended that second RCM be held in China in 2006.

Third and Final Research Coordination Meeting on the "Mutational Analysis Of Root Characters in Annual Food Plants Related to Plant Performance", Antalya, Turkey, 11–15 October 2004

Technical Officer: Q.Y. Shu

In this RCM, participants from 12 countries (Argentina, Australia, Belgium, Brazil, China, Germany, India, Israel, Poland, South Africa, Turkey and UK) participated. Each participant gave a 30-minute oral presentation followed by 10 minutes of discussion. This RCM was successfully concluded with a final document that included achievements, conclusions and recommendations. Major achievements are:

- Mutational analysis has shown that changes in root architecture are correlated with crop plant response to stress.
- New mutant germplasm and populations in various crops have been developed and genetic analysis performed.
- Mutants for root traits have been used in production of new cultivars suitable for stressed environments.
- New specific root mutations provided a means to identify genes important for root traits.
- New methods in phenotyping root architecture have been developed.
- New research collaborations have been formed and a consortium, "Crops Root Research", established (http://www.crop-roots.org).

Fourth Interregional Training Course on "Mutant Germplasm Characterization using Molecular Markers", Seibersdorf, Austria, 27 September–22 October 2004

Technical Officers: S. Nielen & C. Mba

The Plant Breeding and Genetics Section together with the Plant Breeding Unit organized the first Interregional Training Course on Mutant Germplasm Characterization in 2001. The fourth course was held this year at the Agencies Laboratories in Seibersdorf, Austria. So far 80 trainees from 44 different countries have been trained over the last four years. For the 2004 course 20 participants from 20 countries were selected from 90 applicants to participate in the four-week course. The selected participants came from Brazil, Bulgaria, China, Costa Rica, Cuba, Egypt, Gabon, Ghana, Honduras, India, Indonesia, Iran, Kenya, Lithuania, Pakistan, Philippines, Poland, Syria, Uzbekistan and Vietnam. The 2004 course included various changes compared to the previous cluded various changes compared to the previous courses as regards the organization as well as the programme and course content. This Training Course was organized in cooperation with the International Plant Genetic Resources Institute (IPGRI) through the Global Programme for *Musa* Improvement (PRO*MUSA*) of the International Network for the Improvement of Banana & Plantain (INIBAP) in the framework of the Challenge Programme. IPGRI/INIBAP advertised the course through their channels, preselected five participants who applied through INIBAP, and provided financial support to the course. The course programme contained lectures and practical exercises on mutation induction using gamma rays (Co_{60}) while focusing on some of the most important marker techniques such as Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSRs), Inter Simple Sequence Repeats (ISSRs), retrotransposon based marker technologies (REMAP and IRAP), and chromosomal analysis using fluorescence *in situ* hybridization. Whereas previous courses still had a strong focus on basic techniques in plant molecular biology this year's course required a certain degree of knowledge and experience in this field. The reason for this was the introduction of new modules, which should bring the participants in touch with latest technologies in germplasm characterization and genomics research. This was on the one hand a three-day workshop on **T**argeting **I**nduced **L**ocal **L**esions **I**n **G**enomes (TILLING), a technology for high throughput screening for gene mutations in a mutagenized population. The Workshop contained a detailed introduction to the technique, exercises and data analysis. It was generously supported by the company LI-COR Biosiciences, USA, who provided their DNA Analyser and full technical backing. On the other hand and last but not least as a reaction on recommendations expressed by participants of previous courses, a new module on bioinformatics was introduced. During three days the participants were familiarized with the principals on which bioinformatic tools such as homology search are based and on the use of these tools. Apart from lectures the training contained practical exercises using bioinformatic resources available in the Internet. Four invited and two internal scientists participated in teaching the different aspects of molecular markers: Dr. Uri Lavi, ARO, Volcani Centre, ISRAEL; Dr. Bradley Till, Seattle TILLING Project, University of Washington, USA; Dr. John S. (Pat) Heslop-Harrison, University of Leicester, UK; Dr. Klaus Mayer, GSF Research Center for Environment and Health (Munich Information Center for Protein Sequences), Germany, and Dr. Pierre J.L. Lagoda and Dr. Chikelu Mba, both of the Joint FAO/IAEA Division. Dr. Stephan Nielen, Plant Breeding & Genetics Section, directed the course. Staff members of the Plant Breeding Unit gave full support in organization and implementation of the laboratory work, in particular Dr. Rownak Afza, Ms. Mirta Matijevic, Mr. Franz Zwiletitsch, Mr.

Andreas Draganitsch, Mr. Günter Berthold, and Ms. Sharon Soliban. Ms. Kathy Weindl, Ms. Katty Allaf and Ms. Ruby Cueto as the Course Secretary on site handled all administrative matters. The IT support of Mr. Herbert Sommerer is acknowledged. The group of participants met the required scientific and technical background, and was highly motivated. The results of the experiments and the level of interaction with the lecturers indicated that the training was very successful, thus might have a positive impact on their future work in this field. Evaluation forms were completed at the end of the course. The response to the various questions was to a large extent very positive and useful comments were made, which will help to further improve the training programme. Asked for an overall assessment of the course 10 participants ticked excellent, 9 good, 0 average, 0 limited and 0 weak. As an indication for the sustainability of the training, 19 participants answered the question if they would have the opportunity to apply the increased knowledge in their home institutes with "to a great extent" or "to a sufficient extent". Only one person answered this question with "to a small extent', 0 with "not at all". Although it was noted that for example in the case of TILLING an automated DNA analyser is not available in most of the institutes, the prevailing opinion was that it was very useful to learn the principals of this technology, which is going to play a more and more important role in genomic research in the future. In addition the participants learned about possible collaborators for joint projects that could include TILLING as well as all other topics of the course.

Third Research Coordination Meeting on the "Improvement of Tropical and Sub-tropical Fruit Trees through Induced Mutations and Biotechnology", Nelspruit, South Africa, 4–8 October 2004

Technical Officer: S.M. Jain

Participants from 12 countries (China, India, Indonesia, Iran, Israel, Malaysia, Pakistan, Philippines, Thailand, South Africa, UK, and USA) participated in this RCM. Each participant gave a 45-minute oral presentation. This RCM was successfully concluded with a final document that included achievements, conclusions and recommendations. Each participating group reported on general and specific achievements and recommendations, list of publications, and human resource development. The outputs of this CRP are 1) new varieties; 2) material to go into evaluation and breeding programmes; 3) protocol development; 4) technology and biological understanding of genetics and breeding. It was strongly recommended that the CRP be extended for a further period because the experimental crops in the CRP are long-lived perennials (and the starting point of limited previous research). In particular, this would ensure that most of the crops covered have progressed to advanced field evaluation, and more will reach varietal release and grant of plant breeder's rights.

A number of important links have been identified between laboratories using complementary molecular, breeding, tissue culture and selection methods. These would be appropriate for technical cooperation support and training grants and therefore recommendation was given that IAEA consider such applications (included through the TCP and Training Grant schemes).

It is important that national agencies and institute managements recognize the importance of the achievements of this CRP, and continue to support these necessarily long-term research and breeding projects.

It was recommend that evaluation of the materials developed is extended; protocols and knowledge gained is published primarily in a format available to the whole world through book and internet formats; data from specific programmes is published in refereed journals appropriate to the crops and developments reported.

It is strongly recommended that the results of this CRP be published as a specialized book on mutagenesis and biotechnology in tropical and sub-tropical fruit crop improvement. International scientists should be invited to contribute chapters in order to include important aspects of fruit breeding and nutrition, which would certainly improve the quality of this book. This will be of great benefit to the Member States interested in improving fruit crop species.

National Training course on "Application of *In Vitro* Culture, Mutations and Molecular Markers in Horticultural Crop Improvement" PHI/5/029, Manila, Philippines, 25–29 October 2004

Technical Officer: S.M. Jain

The Training Course was jointly organized by the International Atomic Energy Agency and the Government of the Philippines through the Philippine Nuclear Research Institute (PNRI) under PHI/5/029 project. Nineteen participants attended the course, representing various research institutes. Its purpose was to train plant breeders in mutation techniques, molecular markers, and biotechnol-

ogy including somatic embryogenesis, doubled haploid techniques and *in vitro* plant regeneration for accelerating the breeding cycle by applying appropriate techniques. Trait specific molecular markers will help in expediting molecular-assisted breeding of crops, and hopefully shorten the breeding cycle in a cost effective manner. The invited lecturers were: Dr. S. Ochatt, France; and Dr. Alan Schulman, Finland. Dr. Perry Gustafson, USA, was the invited expert.

IAEA/RCA Project Progress Review Meeting on "Mutant Mutli-location Trials and Mutation Enhancement of Genetic Diversity", Suwon and Seoul, Republic of Korea, 29 October–3 November 2004

Technical Officer: Q.Y. Shu

Sixteen Scientists from eleven RCA Member States participated in the meeting. Dr. Seong Hee Lee, Director General of the National Institute of Crop Science (NICS), Rural Development Administration (RDA), Republic of Korea, and Dr. Prinath Dias, RCA Coordinator opened the meeting. The NICS of RDA acted as host.

The participating scientists reported on their research activities and achievements during the last year and had intense discussions. Nine Korean scientists were invited to introduce their researches through seminars. The participants also visited the National Institute of Crop Sciences of Rural Development of Korea, Suwon and Seoul National University.

Participating institutes during the past year has made substantial progress. Through regional mutant multi-location trials (RMMTs), some mutant lines have proven of direct or indirect use in countries other than donor country, and field demonstration will be set up in 2005. New mutant lines resistant (tolerant) to biotic/abiotic stresses (e.g. drought tolerant wheat and mungbean yellow mosaic virus resistant mungbean) have been developed.

During the meeting, it is recommended that:

- The project should be extended to 2006.
- India is to be the leader Country Coordinator, with assistance from China, Pakistan and Indonesia.
- Each participating country should identify a National Coordinator of the project and establish a research team with clear responsibilities. A workplan for 2005 and 2006 should be submitted.
- The participants recommended that the RTC on Molecular Marker Techniques for Mutant Characterization" be held in Korea during the second and third week of June, and the Progress Review and Coordinating Meeting in Indonesia during the third week of November 2005. It was also decided that the RTC on "Target-selected Mutagensis in Plants" be held in China and the final review meeting in India during 2006.
- A book on Mutation Breeding in Asia be prepared, if possible and necessary in cooperation with FNCA.
- The participants continue exchange of mutant germplasm for direct introduction and breeding.

FAO/IAEA/RCA Strategic Meeting on "Nuclear Techniques for Rice Improvement in Asia", Tokyo and Tsukuba, Japan, 4–7 November 2004

Technical Officer: Q.Y. Shu

This strategic meeting was organized in conjunction with the World Rice Research Conference (WRRC) held in Tokyo and Japan 4–7 November 2004. Twenty rice breeders/geneticists participated in the Meeting. Among them, 11 were sponsored by the RCA project (RAS/5/040), five by national TC projects, and the others are from Japan. The Institute of Radiation Breeding (IRB) of National Institute of Agrobiological Science (NIAB) of Japan hosted this meeting. All participants presented a country report on the achievements and prospects of infuced mutation in rice improvement.

An FAO/IAEA/RCA "Workshop on Nuclear Techniques for Rice Improvement in Asia" was held during the WRRC, 6 November 2004. It was one of the six workshops in this WRRC. Nine invited participants presented their work in the Workshop, which attracted more than 80 people. The presentations covered topics from rice mutation breeding in Member States, new mutation techniques, biological basis of induced mutations, novel mutants for rice improvement and function genomic researches, etc.

Apart from attending the Workshop and some WRRC program, the participants also visited the Genebank the IRB of NIAB. The Gamma Field in IRB is now the biggest operational gamma field for plant breeding in the world.

Consultants Meeting on "Vegetatively Propagated Crops", Vienna, Austria, 8–11 November 2004

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

Five Consultants, Drs. Jaroslav Dolezel (IEB, Czech Republic), Nicolas Roux (INIBAP, France), J.S. (Pat) Heslop-Harrison (University of Leicester, United Kingdom), Martin Fregene (CIAT, Columbia), NeBambi Lutaladio (FAO, Italy), were invited to attend this Consultants Meeting at the Vienna International Center in Austria for recommending and consolidating a new CRP proposal on "Molecular tools for quality improvement in vegetatively propagated crops including banana and cassava". The finalized project will be submitted by Dr. Chikelu Mba (Unit Head, Plant Breeding, Seibersdorf Laboratories) to the Research Contracts Committee (RCC) for approval. If the response of the RCC is posi-

tive, the first Research Coordination Meeting (RCM) for this CRP could be organized for mid 2005 (June).

On the global scale, seed propagated crops provide about 70% of the diet for the human population. However, the nutrition of a billion people relies on vegetatively propagated crops. Vegetatively propagated crops are critical for food security in the tropical and subtropical regions of Africa, Asia and Latin America. Increased and stable yields in these crops are a must to meet the calorie needs of the rapidly growing populations in these regions. The challenges are particularly urgent in sub-Saharan Africa, where rapid population growth and an alarming rate of climate change are making vegetatively propagated crops even more vital for achieving food security. The production of improved varieties that are nutritionally acceptable, minimize post-harvest losses, show tolerance to abiotic and biotic stresses, and satisfy the diverse preferences and agro-ecologies across the tropics and subtropics is a daunting task given the biological constraints of vegetatively propagated crops. However, improvement of these crops can be greatly facilitated by new tools of genomics-assisted breeding, induced mutants, and cell culture techniques.

As sources of nutrition, cassava and banana play important roles as staple foods. They give food security in much of the developing world, contributing calories, nutrients, and limited protein, to the diet. When traded in local markets they provide income and employment to rural populations. As an export commodity, banana is a contributor to the economies of many low-income, food-deficit countries. Most cassava and banana is grown by small-holders for self-consumption and sale in local markets, while some 15% of both cassava and bananas are sold in the world market. Both banana and cassava are large herbaceous crop plants, restricted to the tropics and subtropics, growing on a production cycle of around 12 months, and usually propagated through planting substantial plant segments. Genetic improvement of these important crops will help directly to meet the UN Millennium Development Goals, particularly those related to health and nutrition, and the reduction of poverty and hunger. Both species have challenges and opportunities requiring improved yield, starches or dry matter, micronutrients, and resistances to abiotic and biotic stresses (with particular challenges related to virus transmission), while the environmental impact of the crops can be reduced by genetic improvement. Because of these similarities and their complementary importance as crops for food security, a focus on cassava and banana is recommended. This Coordinated Research Project (CRP) aims to give a rational basis for providing technical solutions to the issues raised by sustainable intensification of production, based on application of induced mutations and genetic resources, supported by technical tools of modern genomics, and underpinned by global capacity building and dissemination so the solutions become available and applicable in all Member States.

This CRP will make every effort to ensure that the existing tools of genomics for banana and cassava are brought to bear on efforts to produce varieties of both crops. It will achieve this goal by the development of gene discovery grids, building upon the experience of the IAEA in the use of induced mutations for the genetic improvement in both crops, facilitating access to genomics resources held by advanced labs, and the development of doubled haploids and gene mapping populations. The above tools will be aggressively applied to improve the efficiency of improving quality traits, and related abiotic stress constraints in banana and cassava in active cassava and banana breeding programs in Member States. The results of these activities will also be documented to provide a proof of concept on the use of genomics and induced mutation to dissect complex genetic traits and their application in crop improvement.

Regional Training Course on "Cost Effective Up Scale *In Vitro* Plant Production and Long-Term Storage of Mutant Plant Material" RAF/5/049, Sfax, Tunisia, 29 November–3 December 2004

Technical Officer: S.M. Jain

The purpose of the training course was to impart training to plant breeders/biotechnologists in large-scale plant production and multiplication, long-term storage of mutant lines, hardening of *in vitro* plants, low cost of plant production, and greenhouse technology. The major topics of the training course were liquid and solid cultures for plant propagation. These topics covered different methods, e.g. bioreactors, liquid medium, use of solid medium, photoautotrophic etc.; conservation of cultures: cryopreservation and cold storage; hardening of *in vitro* plants: plants grown in liquid and solid cultures; cost reduction in plant production; and greenhouse technology. Practical demonstrations were given for bioreactor and cold storage, to the 15 participants from Alegria, Morocco, and Tunisia. The invited lecturers were Dr. Bart Panis, Belgium, and Dr. Hervé Etienne, CIRAD, France.

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 tentatively planned to be held in Reykjavik, Iceland, 5–9 September 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 lated 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 will be held in Vienna, Austria, 14–18 March 2005.

(For details, please refer to FORTHCOMING 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 Research Coordination Meeting on “Effects of Mutagenic Agents on the DNA Sequence in Plants” is tentatively scheduled to be held either in South Korea or South Africa during the second half of 2005.

(For details, please refer to FORTHCOMING EVENTS)

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

Technical Officer: S.M. Jain

This CRP was initiated in 2000. The last RCM was held in Nelspruit, South Africa, 4–8 October 2004.

(For details, please refer to PAST EVENTS)

Technical Cooperation Projects

Currently Active Projects

Project Number	Title	Technical Officer
GHA/5/030	Improved Cocoa Productivity through Control of Cocoa Swollen Shoot Virus Disease	S.M. Jain
GHA/5/031	Enhancing Cassava Production through Supplementary Nutrient Application	S.M. Jain
INS/5/030	Sustainable Agriculture Development in Yogyakarta	S.M. Jain
INS/5/031	Mutation Breeding of Horticultural Crops	S.M. Jain
INT/5/147	Developing Salt-Tolerant Crops for Sustainable Food and Feed Production in Saline Lands	S.M. Jain
IRQ/5/015	Induction of Mutations in Crops through *In Vitro* Culture	P.J.L. Lagoda
KEN/5/024	Crop Improvement and Management through Application of Nuclear and Biotechnology Techniques	Q.Y. Shu
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 Mutations to Improve Salt-Tolerance in Non-Aromatic Rice Varieties	P.J.L. Lagoda
PER/5/024	Introduction of Barley and other Native Crop Mutant Cultivars	P.J.L. Lagoda
PHI/5/029	Enhancing Agricultural Productivity through Radiation Technology in Mindanao	S.M. Jain
RAF/5/049	Field Evaluation of Bayoud-Resistant Date Palm Mutants	S.M. Jain
RAF/5/050	Increasing Production of Nutritious Food through Mutation Breeding and Biotechnology (AFRA III-3)	Q.Y. Shu
RAS/5/040	Enhancement of Genetic Diversity in Food, Pulses and Oil Crops and Establishment of Mutant Germplasm Network (RCA)	Q.Y. Shu
RAS/7/014	Monitoring of Food Fortification Programmes Using Nuclear Techniques	P.J.L. Lagoda
ROK/5/033	Quality Improvement of Major Crops and Integrated Plant Nutrition Management in the Low-Input Agricultural System	P.J.L. Lagoda
SRL/5/034	Radiation-Induced Mutations for Black Pepper Improvement	S.M. Jain
SRL/5/036	Virus Screening of Improved Banana Mutants for Large-Scale Dissemination	S.M. Jain
SUD/5/026	Improvement of the Productivity and Sustainability of Industrial Crops	Q.Y. Shu
VIE/5/014	Rice Mutant Varieties for Saline Land, Phase II	Q.Y. Shu
YEM/5/003	Applying Nuclear Techniques for Improvement of Crop Yield	S.M. Jain
ZAI/6/009	Mutation Techniques for Improving Medicinal Plants with a Curative Effect on Human Diseases	S.M. Jain
ZAM/5/022	Crop Improvement through *In Vitro* Mutation Techniques	Q.Y. Shu

Development of drought tolerant tomato – TC project CUB/5/016
A mutant line R15 (left) developed using γ rays from variety AMA (right) showed enhanced drought tolerance
(Instituto Nacional de Ciencias Agricolas, La Habana, Cuba)

Ongoing Activities at the Plant Breeding Unit, Seibersdorf

Banana

Field evaluation of mutants

Four hundred *in vitro* plantlets that were derived from Calcutta-4 banana apical meristems that had been irradiated at different doses of gamma rays are being field-tested in Uganda under a collaborative project with the Kawanda Agricultural Research Institute, Kampala, Uganda. Part of the aims of this project is to establish baseline data on the fertility of induced banana mutants in the field. The generation of induced mutants in banana and their incorporation into genetic improvement programs is important in order to mitigate the effects of the narrow genetic base of banana germplasm available to the breeder for genetic improvement. Because of the peculiar parthenocarpic nature of banana, the edible bananas being triploids and hence sterile, some major hybridization programs therefore are carried out at the diploid level. Establishment of the fertility status of the induced mutants is thus important.

From the 400 plantlets, 272 have been successfully weaned from the hardening nursery and shall be transplanted to the field with the onset of rains by March 2005.

Banana genomics

A two-pronged approach has been adopted for the development of simple sequence repeat (SSR) markers for the banana genome in order to contribute to the on-going global initiative for the development of banana genomics resources, and in the framework of the Challenge Program, that will be employed amongst other things in a systemic characterization of the banana genome. Forty-one SSR continuing segments of the banana genome have been isolated *in silico* from databases and the corresponding primer pairs have been designed. The primers are being synthesized and their fidelity will be tested on a panel of 48 banana genotypes being used by the Musa Genomics consortium.

Parallel to this effort is the construction of a banana whole genomic small insert library that was enriched for SSR motifs. The screening of this library is on going. The SSR markers that will be derived from this library will be more informative as they are expected to be randomly distributed across the entire genome as against the markers derived *in silico*.

Musa genetic resources

The replanting of the *in vivo* Musa accessions in PBU's greenhouse and the sub-culturing of the *in vitro* collections were carried out during this period under review. These materials are made up of wild type and mutant *Musa* accessions across varying ploidy levels.

Rice

Improvement of Malagasy rice lines

Climatic conditions, especially a cold period during the months of June and July, affect the rice production in Madagascar especially in the highlands. There is an on going collaborative effort with the Université d' Antananarivo Madagascar to introgress cold tolerance into well adapted rice varieties in order to mitigate this effect. Complementary to this is the induction of doubled haploids in three putative rice mutants that have shown significant tolerance to cold in the highlands of Madagascar. The doubled haploids will be homozygous lines that would carry the mutated region of the genome conferring

cold tolerance in a homozygous state that would greatly enhance the efficiency for selecting for stable induced mutations for this trait.

Molecular genetic fingerprinting of Malagasy rice lines using SSR markers and AFLP is also on going with the aim of delimiting heterotic groups that would be used in hybridisation for fixing the desirable alleles of the gene(s) controlling cold tolerance and other desirable traits in Malagasy rice varieties. Discriminating alleles will also be useful as molecular tags for traits of interest.

Rice varieties for harsh environments

Part of the 2004-2005 activities of the Sustainable Intensification of Crop Production Systems (SICPS) is the development of crop production packages that remove production constraints attributable to harsh environments. In collaboration with our colleagues in the Soils Unit, preliminary activities are being initiated with the aim of generating diversified genetic base for selected crop germplasm that would fit into these activities. We are therefore building on our experiences on development of rice mutants that have shown tolerance to saline environments. The Crop, Water and Soil Sciences Program of the International Rice Research Institute (IRRI), The Philippines have declared a keen interest in utilising the mutants to be generated as genetic resources in an on-going Coordinated Research Project (CRP) that addresses this theme. Some putative salt tolerant mutants have been obtained based on the assessment of phenotypic damage to the seedlings using a salinized hydroponics assay system. Further screenings for salt tolerance at different stages of growth as well as molecular marker characterization of the putative mutants are ongoing.

Cassava

We are initiating a program that will feed resources into ongoing initiatives at the international and national levels for the genetic improvement of Cassava, *Manihot esculenta Crantz (Euphorbiaceae)*, one of the most important staple crops in the tropics and subtropics. Currently our efforts have been geared at the optimization of strategies for the efficient production and evaluation of cassava mutants.

Nodal cultures of different cassava varieties received from the International Center for Tropical Agriculture (CIAT, its Spanish acronym), Cali, Colombia and the Kenya Agricultural Research Institute (KARI), Njoro, Kenya have been used in determining the optimal doses for gamma irradiation of cassava. The induced mutants are being propagated *in vitro* with the aim of dissolving chimerism, an intrinsic problem in induced mutation. After developing a sizable population of these mutants, they will be shipped to collaborating Member States' cassava improvement programs for field testing for desirable attributes. The adaptation and optimization of protocols for somatic embryogenesis from leaf lobes of these cassava varieties has also been successful with plantlets having been regenerated. The optimization of the technique is ongoing for these varieties and will be critical for the production of homozygous lines for the mutated segments of the genome. This is also important, as we shall be playing pivotal roles in the proposed CRP, "Molecular tools for quality improvement in vegetatively propagated crops including banana and cassava" through the production and distribution of mutated germplasm of cassava.

Intravarietal differences among Kenyan cassava varieties have also been delineated using molecular markers. The outputs will help elucidate the genetic relationships amongst cassava varieties and land races in Kenya as well as contribute information to the selection of varieties for genetic improvement of cassava in Kenya.

Irradiation Services

A total of 141 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	6
Number of species	6
Number of varieties	65
Number of treatments	141
Number of requesting Member States	5

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 300 DNA fragments were sequenced while DNA fragment separation was carried out on about 4000 samples.

Fellowship Training

The following Fellows were interned in the Unit during the period under review:

Name	Country	Subject Area	Period	Supervisor
OKWARO, Henry Otieno	Kenya	Induced mutations in cassava, molecular genetics and *in vitro* techniques	2004-04-01 to 2004-12-15	AFZA, Rownak
RAKOTOARISOA Noronirina Victorine	Madagascar	Induced mutations in rice, molecular genetics and *in vitro* techniques	2004-04-01 to 2004-11-30	AFZA, Rownak

Publications

1. Nandwani D., S.M. Jain and K. Ramavat, 2004. Micropropagation of woody plants. In: Tree Improvement and Biotechnology. P. Shanmughavel and S. Igancimuthu (Eds.). Pointer Publishers, Jaipur, India, Pp 16-52.

2. Jain, S.M. and M. Maluszynski, 2004. Induced mutations and biotechnology on improving crops. In: *In vitro* applications in crop improvement:Recent Progress. A. Mujib, M. Cho, S. Predieri, S. Banerjee (ed.). IBH-Oxford, India. Pp 169-202.

PLEASE COMPLETE THIS REGISTRATION FORM AND SEND IT TO THE PLANT BREEDING AND GENETICS SECTION AT THE FOLLOWING ADDRESS:

Wagramerstrasse 5, P.O. Box 100, A-1400 Vienna, Austria
Telefax: (+43-1) 26007, Telephone: (+43-1) 2600

New Crop Variety Developed through Mutation Induction or by Crossing with Induced Mutants

A. Latin name of species:

____

English name: ____________________

B. Name of new variety (cultivar):

____

C. Year of release from breeder: ________

D. Place and Date of official approval: ______________________________

E. Parent variety(ies) - if new variety results from a cross with mutant, indicate which is the mutant:

		mutant
1.	____________________	**yes / no**
2.	____________________	**yes / no**
3.	____________________	**yes / no**

F. Main improved characters of variety (indicate if character is derived from mutation or not):

		mutation derived
1.	____________________	**yes / no**
2.	____________________	**yes / no**
3.	____________________	**yes / no**

G. Kind(s) of mutagenic treatment: ____________________

H. Doses(s) and/or concentration(s): ____________________

I. Year of mutagenic treatment: ________

J. How was the variety bred:

____

K. Name(s) of breeder(s) and institute(s):

____

____

Address: ____________________

____

____

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!

Plant Breeding and Genetics Newsletter No. 14

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.

International Atomic Energy Agency
Wagramer Strasse 5, P.O. Box 5,
A-1400 Vienna, Austria

Printed by the IAEA in Austria,
December 2004

04-46541