PLANT BREEDING AND GENETICS NEWSLETTER

Joint FAO/IAEA Division
of Nuclear Techniques
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TO THE READER

This is the third issue of the Plant Breeding and Genetics Newsletter. Since publishing the last issue we have had significant staff changes in the Plant Breeding and Genetics Section. Dr. Leo van Zanten completed his contract with the Agency after 5 years of service as a Technical Officer. He was the person who also compiled the first two issues of the PBGN. Dr. Richard Litz, after almost three months of service, terminated his temporary assignment as a consultant on the application of induced mutations in connection with *in vitro* culture for vegetatively propagated crop improvement. The good news is that Dr. Mohan Jain joined the Section on 2 May, filling in the post previously occupied by Dr. Beant Ahloowalia. He will be responsible, among other duties, for our activity related to the improvement of vegetatively propagated crops by induced mutations and related biotechnology. He is now project officer of the FAO/IAEA/BARC Co-ordinated Research Project on "Cellular biology and biotechnology including mutation techniques for creation of new useful banana genotypes."

Our work in these six months has concentrated on planning two new CRPs which will be initiated this year. The evaluations of the research contract and agreement proposals for the FAO/IAEA CRP on "Molecular characterization of mutated genes controlling important traits for seed crop improvement" have been completed and 19 participants were selected. The CRP will use induced mutations to exploit the full power of structural and functional genomics for gene isolation directed at the improvement of orphan crops and allied species. It will focus on molecular characterization of new or existing mutants affecting key

agronomic traits in model crops and using comparative approaches in under-utilised crops. We have already called for research contract and agreement proposals related to the CRP on "Mutational analysis of root characters in annual food crops related to plant performance". Through this CRP we are expecting to generate a population of root mutants. It is foreseen that a variety of characteristics of root function will be altered in these new genetic stocks including root hair frequency, size and distribution; branching pattern; root diameter, fraction and distribution of various root types and mycorrhizal development. These genetically characterized mutant lines will be of particular value for modifying plant responses to a variety of environmental conditions especially in marginal areas of developing countries.

In addition to the daily activity related to the organization of Research Co-ordination Meetings, evaluation of progress reports and technical backstopping of Technical Co-operation Projects, work on the establishment of a database on successful radiation doses for development of new mutant varieties in crop species was continued. Activities at the Plant Breeding Unit of the FAO/IAEA Agriculture and Biotechnology Laboratory at Seibersdorf concentrated on the development of low-cost *in vitro* culture technology for induced mutations, development an efficient technique for rice anther culture and on activation of retrotransposons in rice by irradiation with gamma rays.

Miroslaw Maluszynski

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B. FORTHCOMING EVENTS

Research Co-ordination Meetings (RCMs)

Third and final RCM on "Improvement of new and traditional industrial crops by induced mutations and related biotechnology", Corvallis, Oregon, USA, 2-6 August 1999.

Third and final RCM on "Radioactively labelled DNA probes for crop improvement", Vienna, 6-8 September 1999.

First RCM on "Molecular characterization of mutated genes controlling important traits for seed crop improvement", Vienna, Austria, 4-8 October 1999.

Third RCM on "Cellular biology and biotechnology including mutation techniques for creation of new useful banana genotypes" Colombo, Sri Lanka, 4-8 October 1999.

First RCM on "Mutational analysis of root characters in annual food plants related to plant performance" is planned for the end of 1999 or the beginning of 2000.

Regional (AFRA) Training Course on "Molecular characterisation of genetic biodiversity in traditional and neglected crops selected for improvement through mutation techniques", Pretoria, South Africa, 6-17 September 1999.

The purpose of this training course is to train breeders participating in the regional AFRA III-18 project (RAF/5/042) in various molecular techniques for characterisation of biodiversity in selected traditional neglected crops (RAPDs, RFLP, AFLP, micro satellites, comparative mapping).

Workshop (RAF/5/035) on "Hands-on Experience on Molecular and Mutation Techniques", Vienna, Austria, 13-24 September, 1999.

The main objective of this workshop is to train date palm breeders in the use of molecular techniques (RAPDs, AFLPs) and mutation techniques through practical skills and hands-on experience. The participants to this workshop are limited to only Algeria, Morocco, and Tunisia.

Seminar on "Mutation techniques and biotechnology for tropical and subtropical plant improvement in Asia and the Pacific Region", Los Baños, The Philippines, 11-15 October 1999.

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

stress prone areas and, in this way, to improve farm sustainability.

Project Formulation Meeting of the Regional TC Project RAS/5/037 on "Mutational enhancement of genetic diversity in rice", Bangkok, Thailand, 8-12 February 1999.

The aim of this meeting was to guide representatives of countries participating in the RAS/IAEA Project on "Mutational Enhancement of Genetic Diversity in Rice" in the preparation of a detailed programme of activity and other project documents. Thirteen rice breeders represented eleven countries of the Region: Bangladesh, China, India, Indonesia, Korea, Malaysia, Myanmar, Pakistan, The Philippines, Thailand and Viet Nam. The meeting was organized at the newly established (with Agency support) Gamma Irradiation Service and Nuclear Technology Research Centre, Kasetsart University, Bangkok. The Centre is very well organized and offers excellent facilities for regional training courses and meetings. The International Rice Research Institute (IRRI) was represented by senior scientist Dr. D.S. Brar.
On the basis of intensive discussions a detailed plan of project activities was formulated and necessary documents drafted. Also, an Agreement on Exchange of Rice Seed Material in the form of the IAEA Project RAS/5/037 was drawn up and adopted by all participating countries.

2nd Project Co-ordination Meeting of AFRA III-18 project on "Development of improved crop varieties", Bamako, Mali, 15-19 February 1999.

The meeting was jointly organised by the IAEA and the Institut Polytechnique Rural de Formation et de Recherche Appliquée

C. PAST EVENTS

de Katibougou (IPR/IFRA) in Koulikoro. The purpose of the meeting was to evaluate the 1997/98 programme and plan and to c-ordinate the 1999/2000 programme of the regional project consisting of three different project components. The meeting was attended by the Project Co-ordinators from Algeria, Egypt, Ethiopia, Ghana, Libya, Madagascar, Mali, Mauritius, Morocco, Niger and Tanzania, the IAEA Project Officer and a Technical Officer from the Joint FAO/IAEA Division. Representatives of FAO, the Ministries involved in agriculture, research, education and energy participated in the opening and closing sessions of the meeting. A breeder from ICRISAT, Mali, participated in parts of the meeting. Results of all project components and countries including evaluation trials of sesame, safflower, cassava, cocoa, barley, sorghum, lentil and durum wheat mutants were presented and evaluated as well as the breeding programmes to improve drought tolerance in cereals and legumes and to improve traditional and neglected crops. An analysis of strengths, weaknesses, opportunities and threats (SWOT) was conducted, the programme for 1999/2000 activities was discussed and revised and recommendations for further project implementation including regional mutant germplasm exchange were agreed upon.
One cassava mutant from Ghana and eight sorghum mutants from Mali - developed under previous Agency support - were officially released as improved varieties. The official release of the improved sesame mutant varieties is expected in Egypt by the end of 1999. Mutants of cocoa (Ghana), barley (Libya, Tanzania), lentil (Morocco), safflower (Egypt) need further evaluation in order to be considered for submission to the

National Variety Release Committees. Delays in implementation of mutant evaluation trials were mainly due to abiotic stress factors during the cropping seasons. The breeding programmes under component 2 (improvement of drought tolerance in cereals and legumes) and component 3 (improvement of traditional and neglected crops) are still in an initial phase of evaluation of germplasm collections and early mutant generations. Only programmes which have been initiated in the early 90's - under the previous Agency's TC support - such as barley in Algeria and durum wheat in Morocco have advanced mutant generations available.

Completion of the CRP on Use of novel DNA fingerprinting techniques for the detection and characterization of genetic variation in vegetatively propagated crops.

This CRP was completed with the publication of the proceedings of the final Research Co-ordination Meeting, Mumbai, India, 24-28 February in October 1998.
The activities and achievements of the CRP can be summarized as follows:

Various molecular marker technologies have been used to measure genetic diversities of vegetatively propagated crops as shown in the individual papers presented in this RCM. The building of this research network has permitted the transfer of technology and the enrichment of knowledge and plant genetic resources available to individual projects where technologies of DNA profiling [restriction fragment length polymorphism (RFLP); randomly amplified polymorphic DNA (RAPD); simple sequence repeats (SSR); amplified fragment length polymorphism (AFLP); DNA amplification fingerprinting (DAF); and randomly amplified microsatellite polymorphism (RAMPO)] have been evaluated. Cost, convenience, reliability and information content have been recognized as key criteria for selecting an appropriate profiling technology.
The association of markers and morphological traits led to the generation of maps in several species which allow the exploitation of alternative life cycles of vegetatively propagated plants. For example, in banana one important strategy is the identification of the diploid accessions most closely related to modern cultivars for improvement of desirable traits in these diploids by MAS, thus recreating polyploid, sterile and parthenocarpic cultivars. Another example is yam, *Dioscorea* spp., where mapping of the genome of *Dioscorea tokoro*, a dioecious diploid species closely related to the economically more important yam crops, allowed the tagging of important genes, fostering isolation of these genes for eventual transformation of yam for genetic improvement. In this fashion, where linkage analysis (detection of linkage disequilibrium) is possible, there are no technological barriers to extend these strategies to vegetatively propagated crops of interest, and to genetically engineer agronomically important genes.

Specific achievements of this CRP

The participants in this CRP have investigated various vegetatively propagated species with different molecular marker systems. The details of their progress are presented below.

Burg, Austria - The identification and cloning of minisatellite type repetitive elements of the oak genome supplies a new marker system for the analysis of the genetic diversity of oak resources.

Leal, Cuba - Molecular evidence of somaclonal variation has been found in sugarcane. There is possibly a role for several genes involved in the osmotolerance response (ATPase, delta-pyrroline-5-carboxylase reductase, osmotin and heat shock proteins).

Lagoda, France - A common basis was established in which breeders communicated their problems to molecular geneticists. Bridging the communication gap clarified the need for a molecular breeding programme on banana and plantain requiring the input of a mapping and a genetic diversity analysis programme. The development of additional markers was a prerequisite for applications outside the laboratory, especially PCR detection kits. This has been achieved and will be completed in the future by developing sequence tagged microsatellite sites (STMS) markers and sequence characterized amplified regions (SCARs) from AFLP.

Sangwan, France - In cassava, induced genetic variations have been characterized *in vitro*, Using PCR-based markers (RAPD and microsatellites). The RAPD analyses were reproducible and showed distinct polymorphic bands.

Kahl, Germany - For yam, an intense application of various DNA profiling techniques and sequence comparisons of chloroplast tRNA gene regions revealed genetic diversity in various *Dioscorea* species, various accessions of economically important yams and parents for crosses, e.g. *D. tokoro*. The same techniques were employed to clarify taxonomic problems in *D. bulbifera*, phylogenetic relationships between *D. cayensis* and *D. rotundata* (Guinea yam), and between these and their putative progenitors. For the first time, a defined cross of selected parents of *D. tokoro* permitted profiling progeny. For greater exploitation of the RAPD polymorphisms, a new hybridization-based method, RAMPO, was designed that expands the information content of a single gel several-fold.

Parida, India - Molecular markers were used for genetic characterization, species identification, establishing phylogenetic and species relationships in the Indian mangrove species. Markers have also been used to select priority areas for conservation and consolidation of genotypes with the capacity for tolerance to salinity.

Rao, India - Tissue cultures have been established from thirteen banana genotypes belonging to different genomic groups including wild diploids. *In vitro* multiple shoot cultures have been irradiated and in the early stages of field planting, regenerants have exhibited chlorophyll variations, earliness, changes in plant height and plant type and further observations of these will follow. Molecular studies for finger printing have been initiated with different banana accessions and variants isolated from irradiation experiments.

Lavi, Israel - Several kinds of DNA markers (including minisatellites, AFLP and SSRs) were applied to both mango and avocado. Achievements include: identification of individuals, study of genetic relationships, identification of linkage between some DNA markers and genes coding for agriculturally important traits in avocado, and the generation of a preliminary genetic map in avocado.

Low, Malaysia - Somaclonal variations and changes in DNA methylation were demonstrated for the first time in rubber trees, *Hevea brasiliensis*, by various DNA fingerprinting techniques. Gamma irradiation resulted in changes in DNA profiles, but these changes were complicated by, and indistinguishable from, somaclonal variations.

Mignouna, Nigeria - Genetic variation among cassava varieties was analysed with RAPD markers. The molecular taxonomy of cultivated and wild yams was established with RAPD and microsatellite markers.

Terauchi, Japan - High levels of genetic diversity have been found in natural populations of wild yam suggesting the importance of maintaining a small number of large populations to protect genetic diversity.

Iqbal, Pakistan - Local varieties of sugarcane and banana were compared with RAPDs to assess their genetic diversity.

Mansvelt, Republic of South Africa - RAPD markers were used to generate fingerprints for differentiation of deciduous fruit cultivars. Gene transfer technology was developed for deciduous fruit trees using *Agrobacterium* as a vector.

Gresshoff, USA - Arbitrary primer technology using DAF was optimized using mini-hairpin primers, secondary amplification of DAF products (ASAP), 7M urea-10% PAGE, a 55°C annealing temperature, and maintenance of high primer (3 μM) and low template (1-2 ng/ 20 μL reaction volume) concentrations. Molecular markers were valuable for anchoring yeast artificial chromosome (YAC) and bacterial artificial chromosome (BAC) clones on molecular linkage maps facilitating the next step of map-based cloning of genes induced by mutagenesis.

D. STATUS OF EXISTING CO-ORDINATED RESEARCH PROJECTS

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

This CRP was initiated in 1998 with the objective to overcome major constraints to increase productivity of neglected and underutilized crops by genetic improvement, in order to enhance the economic viability and sustain crop species diversity, and in future to benefit small farmers. Mutation techniques in combination with biotechnology are applied for the improvement of various vegetatively and seed propagated crops: quinoa (*Chenopodium quinoa*), cocoyams (*Colocasia esculenta*, *Xanthosoma* spp.), yams (*Dioscorea* spp.),. grain and vegetable amaranths (*Amaranthus* spp.) Bambara groundnut (*Vigna subterranea*), grasspea (*Lathyrus sativa*), okra (*Abelmoschus esculentus*), bitter potatoes (*Solanum jucepzukii*, *Solanum ajanhuiri*) and naranjilla (*Solanum quitoense*). The first Research Co-ordination Meeting was held in December 1998 in Vienna, Austria. At present there are 18 participating institutes from Bolivia, Costa Rica, Ecuador, France, Germany, Ghana, India, Indonesia, Mexico, Slovakia, South Africa, Syria and Thailand

including an agreement holder from IPGRI based at ICARDA.
Corvallis, Oregon, USA. (See also 'Forthcoming Events')

Radio-actively Labelled DNA-probes for Crop Improvement

This project, which started in 1995, has at the moment 8 participants from 4 countries. This project helps to foster international co-operation to transfer modern biotechnology to developing countries with the active participation of leading laboratories. An important activity is the promotion of probes and primers. The third and final RCM is planned for 6-8 September 1999 in Vienna, Austria.

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

This CRP started in 1995 and emphasizes the application of induced mutations and related biotechnologies to oil crop and fiber plant improvement programmes. The programmes mainly focus on genetic improvement of agronomic traits, resistance to biotic and abiotic stress factors and product quality in rapeseed, Indian mustard, soybean, sunflower, linseed, Cuphea, meadowfoam, evening primrose, cotton and jute. Mutation protocols were optimized for those crops. Improved germplasm lines were developed by induced mutations, intra- and interspecific hybridization and genetic transformation. Molecular markers for marker-assisted selection are available for some traits. Genes involved in fatty acid biosynthesis of Cuphea could be isolated from mutant germplasm. At present there are 11 participating institutes from 10 countries in this project.. The third and final RCM will be held in August 1999 in

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

This CRP was initiated in 1994 with the general aim to integrate radiation induced mutations *in vitro* culture and molecular genetics methods into the conventional breeding of banana to induce desirable variation such as disease resistance, dwarfism and earliness, and also to promote the development of methods for large-scale and rapid multiplication of the mutants/ segregants through micropropagation and somatic embryogenesis. Plants can readily be regenerated via somatic embryogenesis for large-scale plant production, which is ideal for *in vitro* mutagenesis to the selection of mutants with desirable agronomic traits. Flow cytometry analysis of nuclear DNA content in Musa showed that Musa A and B genomes differ in size. Since 1996, Belgium has become an important contributor to this CRP. Twenty institutions world wide are involved. The next RCMis planned for 4-8 October 1999 in Colombo, Sril Lanka.

Induced Mutations for Sesame Improvement

This CRP was concluded in 1998. The final proceedings are now being edited and will be available as IAEA technical publication

(TEC-DOC) (see issue no. 1, p. 10-11 for report from final meeting).

Induced Mutations and other Advanced Technology for Production of Crop Mutants Suitable for Environmentally Sustainable Agriculture

The CRP was concluded in 1998. The final proceedings are now being edited and will be available as a book summarizing the results (see issue no. 2, p. 4-5 for report from final meeting).

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

The CRP was concluded in 1998. The final proceedings are now being edited and will be
available as an IAEA technical document (TEC-DOC) (see issue no. 2, p. 5-7 for report from final meeting).

E. NEW CO-ORDINATED RESEARCH PROJECTS

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

Details of this CRP were published in the last issue of this Newsletter and in Plant Breeding News (February 1999). The 1st Research Co-ordination Meeting will be held from 4-8 October, 1999 in Vienna.

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

Details of this CRP were published in the last issue of this Newsletter and in Plant Breeding News (May 1999) The 1st RCM is planned for the end of 1999 or beginning of 2000, in Vienna.

F. TECHNICAL CO-OPERATION PROJECTS

Over the last three years, the Section has had technical responsibility for 43 Technical Co-operation projects. In each Newsletter we will highlight the activities and achievements in a few of our Technical Co-operation projects. The following projects are discussed in this issue:

Sorghum mutants released as improved varieties in Mali (MLI/5/014*)*

In Mali, sorghum is the second most important crop and is grown on some 650,00 ha, but yields only about 800 kg/ha. By irradiating locally grown well adapted material of the guinea types with 200-300 Gy gamma rays, followed by selection in the subsequent generations and field performance tests over several years, plant breeders at the Institut Polytechnique de Katibougou, Koulikoro, have released 8 mutants to suit various sorghum growing regions of Mali. These have been added to

the list of cultivated varieties and species of
the Department of Agriculture, Ministry of

Rural Development. These mutants have the potential to yield 1000 to 1500 kg/ha, are 3.5 to 4.2 m tall (local farmers prefer tall types, since the stems are used for feeding cattle, building grain storage and shading canopies) with long panicles, resistant to lodging, some are early ripening and others have improved resistance to drought. With names like 'Fambe', 'Tiedjan', 'Gnome', 'Sofin', 'Djeman', 'Djemanin', 'Gnoumanin', 'Sadje', true to the West African culture, these new varieties promise to help sustain food production in the sub-Sahel of Africa. In 1998, about 2000 kits of one kilo seed each of three varieties, 'Fambe', 'Tiedjan', and 'Gnome' were distributed to selected growers for on-farm performance validation. It is recognized that there is no better way to conserve genetic diversity than growing local land races and varieties on farms. Since the mutant varieties have been derived from native material, their release would also lead to conservation of the native sorghum germplasm as a bonus to the increased productivity. This research has been accomplished through the support provided by the Agency under various Technical Co-operation and CRPs during the past 15 years.

Rice mutant varieties released in the Mekong Delta of Vietnam (VIE/5/013)

Rice is the most important food and export crop in Vietnam. The Mekong Delta in southern Vietnam has the dominant role in rice production of the country with 46% of the total rice area. Much attention has been given in the past to increase rice production on alluvial soils under irrigation and high input conditions using high yielding varieties with low cooking quality. However, on saline and acid sulphate soils of coastal areas of the south only traditional varieties with good cooking quality but low yields can be grown. Therefore, the rice breeders at Cuu Long Delta Rice Research Institute in Oman, Cantho Province in southern Vietnam initiated a rice improvement programme to develop higher yielding varieties with good cooking quality for such stress-prone soils suitable for export. They were supported under a previous research contract to induce mutations using gamma treatment of rice seed of traditional varieties well adapted to saline and acid sulphate soils in order to increase production; improved mutant lines were selected. Under a TC project the institute is now evaluating the mutant lines in southern provinces. Two mutant lines have been selected for further evaluation and distribution to farmers. The mutant TNDB-100 was released in 1997. It was developed from the land race Tai Nguyen Dot Bien which is photoperiod sensitive, 1.3-1.5 m tall, maturing in 180 - 200 days with grain yields of 2 - 3 t/ha. The mutant TNDB-100 is a photoperiod insensitive variety, which matures in about 100 days. It can have grain yields of 4.5 -8 t/ha. It has quality characteristics (long grain, low chalkiness) that fit well in the export market. Because of TNDB-100's shorter growth cycle farmers can grow two to three crops per year in the Mekong Delta. It has a wide adaptation to different soil conditions and performs well even on poor soils. The rice growers and consumers like this variety and in the 1998/99 dry season more than 80,000 ha of it were grown in southern Vietnam. Another mutant THDB, originates from mutation treatment of the land race Tep Hanh Dot Bien. It is earlier maturing (125-135 days) than the land race (200-220 days), is shorter and yields 5 to 8 t/ha (land race 2 to 3 t/ha). In 1998 it was grown in the coastal area of the south as an autumn - winter crop - on 13,000 ha.

Current Operational Projects are:

BGD/5/019	Extension services to farmers on promising mutant varieties
COL/5/017	Mutation breeding of plantain and rice
COS/5/021	Radioactive probes for plant disease diagnosis
COS/5/023	Improved mutant varieties of rice and banana
CPR/5/010	Induced mutations for improvement of rice
CPR/5/011	Improvement of cotton and rapeseed through induced mutations
CPR/5/013	Induced mutations to improve rice quality
ECU/5/020	Resistance to disease in cacao and babaco
ELS/5/008	Improvement of potato through *in vitro* mutation breeding
ETH/5/011	Improvement of tef through mutation breeding
GHA/5/026	Improvement of cassava through mutation breeding
GUA/5/012	Mutations and biotechnology for crop improvement
INS/5/026	Mutation breeding of bananas
INS/5/027	Mutation breeding of ornamental plants
IRA/5/007	Mutation techniques for crop improvement
IRQ/5/011	Nuclear techniques in cereal production
IRQ/5/015	Induction of mutations in crops through *in vitro* culture
KEN/5/021	Improved drought resistance of crops by induced mutations
MAG/5/008	Mutation techniques and biotechnology for rice and cassava
MAK/5/004	Mutation and doubled haploid techniques to improve wheat
MAL/5/021	Mutation breeding and biotechnology for plant improvement
MLI/5/014	Field performance of selected mutants of sorghum and rice
MON/5/009	Nuclear techniques to improve production of wheat and legumes
MYA/5/008	Mutation breeding in grain legumes
PAK/5/033	Development of leaf curl tolerant varieties of cotton
PAK/5/035	Development of salt tolerant varieties of basmati rice
PAK/5/039	Pest resistant chickpea through induced mutation
PER/5/024	Introduction of barley and other native crop mutant cultivars
PHI/5/027	Mutation breeding of priority agricultural crops
RAF/5/029	Nuclear techniques in plant breeding and biotechnology (AFRA X)
RAF/5/035	Control of bayoud disease in date palm
RAF/5/042	Development of improved crop varieties (AFRA III-18)
RAS/5/037	Mutational enhancement for genetic diversity in rice (RCA)
RLA/5/035	Evaluation of cereal crop mutants (ARCAL XXIA)
SRL/5/030	Mutation breeding in bananas and plantains
SUD/5/023	Improving cotton and sugar cane crops (phase II)
THA/5/045	Radiation induced mutations for bean and chrysanthemum
URT/5/020	Improving productivity of basic food crops in Tanzania
VEN/5/018	Genetic improvement of fruits and pepper
VIE/5/013	Improvement of basic food crops through induced mutations
VIE/5/014	Rice mutant varieties for saline land
ZAM/5/020	Improvement of beans through mutation breeding

G. ACTIVITIES AT THE PLANT BREEDING UNIT, SEIBERSDORF

Development of low-cost tissue culture technology for induced mutations`

Mutation induction combined with *in vitro* techniques can provide useful tools to enhance genetic variability for the improvement of agronomic traits such as earliness in fruiting, pest and disease resistance, yield and quality. Tissue culture techniques such as shoot-tip culture facilitate the isolation of homohistonts in mutation induction studies and in the selection and multiplication process of improved genotypes.

- A study was conducted to reduce expenses in tissue culture systems by using sunlight instead of artificial light during culture incubation and readily available cheap sugars and gelling agents in the medium.
- *Musa* cv. 'Grande Naine', our model plant, was micropropagated under various conditions. The experiments were carried out during two experimental periods (during July to September 1997 and April 1998 to January 1999). Three different locations - a growth chamber, a sunlit room and a greenhouse were available for culture incubation. In the growth chamber, the cultures were kept at 25 ^{o}C with a photoperiod of 16 hours at 65 µmol m^{-2} s^{-1} provided by cool-white fluorescent tubes. In the sunlit room without air conditioning, the boxes were placed in front of a window facing west. Under natural light conditions plants were exposed to temperatures from 16 - 43 ^{o}C, PPFD (Photosynthetic Photon Flux Density) of up to 860 µmol m^{-2} s^{-1} and photoperiod ranged from 8 - 16 hours.
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- In both experimental years, the number of shoots produced was always highest in the greenhouse followed by the sunlit room and was lowest in the growth chamber. Plant quality was satisfactory in all three locations but under natural light conditions the plants were lighter in colour and had a bigger leaf size than in the growth chamber. During summer 1997 when average PPFD were around 200 µmol m^{-2} s^{-1}, browning of leaves and loss of turgor were observed in the greenhouse, but in any case acclimatisation was not affected. In summer 1998 additional shading covered the cultures, reducing average PPFD below 100 µmol m^{-2} s^{-1} . No browning occurred, thus showing that by managing light intensity problems with plant quality could be overcome.
- A wide selection of commercial sugars from different countries was tested. The sugars varied in their colour (white to dark brown) and were produced from sugar beet or sugar cane. All white and light brown sugars were found equal substitutes for tissue culture grade sucrose. Dark brown sugars were found to be of limited value, if electrical conductivity exceeded 400 µS/cm.
- Starch at concentrations that supported plant growth gave problems in handling. Satisfactory results were obtained with a mixture of corn or potato starch (6 %) combined with gelrite (0.05 %) or agar (0.1 %).
- Under our experimental conditions, the use of natural light, commercial sugar and starch/gelrite would be 90 % cheaper than the use of artificial lighting, tissue culture grade sucrose and gelrite.

Effect of spikelet position on rice anther culture efficiency

The potential of anthers from different parts of the panicle to induce callus was investigated with the *japonica* rice variety Taipei 309. Our results showed that the callusing abilities of anthers from different spikelet positions were significantly different. After plating 4483, 4496, 4348 anthers from the basal, middle and top parts, the percentage of anthers forming calli was 20% in the basal part, 12% in the middle part and 8% in the top part. The anthers of basal parts containing pollen at all uninucleate stages, including early, middle and late, showed higher callus induction frequency than those from middle and top parts. The green plantlet regeneration frequencies of top, middle and basal spikelets were around 18% in all three cases. From our results it would appear that anthers from the basal part of the panicle should be used in anther culture of rice in order to obtain higher efficiencies, and thereby optimise the usefulness of this technique in rice breeding programmes.

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

This investigation was focused on the effect of mutagenic treatment on retrotransposon activation in rice. The Tos 17 retrotransposon known to be activated by protoplast culture was isolated from rice and used as a DNA probe in Southern hybridizations. By analysing rice plants derived from radiosensitivity tests it was shown that the induction of new retrotransposon integrations was possible by γ-irradiation, but it was a relatively rare event. The number of new copies in the genome was very low compared to the copy number after protoplast culture. This is of great advantage for the aim of tagging a gene by insertional mutagenesis because the identification of an affected gene will be easier as with multiple new integrations. In a molecular analysis of rice gametoclonal *variants using* again Tos 17 as a probe, a restriction fragment length polimorphism (RFLP) was detected. One fragment that appeared in the controls did not appear in the gametoclones. The DNA methylation status of these plants was checked and it was proved that the missing band was not due to changes in the methylation pattern.

Development of *in vitro* culture protocols in tef (*Eragrostis tef* ZUCC.)

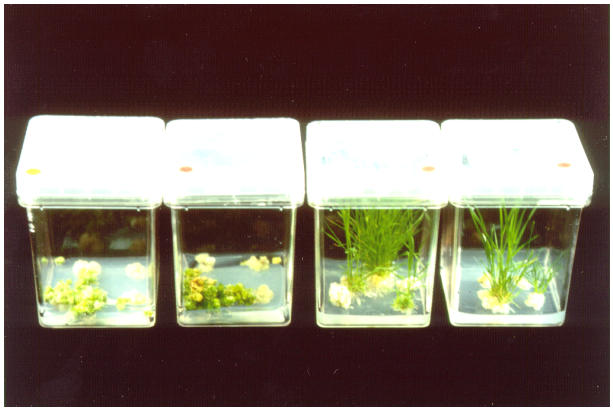

Fig. 1 Androgenesis in the variety Dz-Cr-37. Seeds from donor plants irradiated with 400 Gy.

We studied the effect of radiation on callus induction and green plant regeneration using different *in vitro* culture techniques. Seeds, anthers, spikelets, and young leaves were used as explants. Different induction and regeneration media were evaluated to establish the best culture conditions.

Some of the results are summarized below:

- Highest calli induction and green plant regeneration efficiency was observed for spikelets and anthers from donor plants irradiated with 400 Gy.

- Calli induction was higher for anthers and spikelets plated in semi-solid N6 (calli induction medium) medium with maltose as carbohydrate source instead of sucrose.

No significant difference was found among the three MS regeneration media that were tested, but the highest production of single shoots was observed in the regeneration medium containing 200 mgl^{-1} Myo-inositol, 1 mgl^{-1} BAP, 0.5 mgl^{-1} NAA and 0.45%, agarose Green plant regeneration efficiencies for androgenesis response reached values between 16-35%.

- Production of multiple shoots per calli was observed in almost all the treatments. On average 28 plants per calli were produced, with a maximum of 183 single shoots.

DH1 green plants were transferred to the green house and harvested during March-April (1999) and will be send to Ethiopia for field studies. Some plants in the green house showed plant height reduction.

Gametoclonal variation in the variety Pokkali

Anther culture response of two indica photosensitive varieties Nona Bokra and Pokkali was evaluated. Even though salt tolerant, due to their plant height (140-169 cm) and low yield, they are not suitable for growing in areas with saline soils. From 125 DH green plants regenerated from Pokkali, three gametoclonal variants were identified in the first generation (labelled as. 101, 112 and 113) showing plant height reduction and photoperiod insensitivity (Fig. 2). Self pollinated panicles were single harvested and grown in pots for evaluation of the DH2 generation. All DH lines were planted randomly. Only the three gametoclones with quantitative alterations in comparison with the control population were studied further.

Uniformity of major characters among gametoclones showed on average 45% in plant height reduction. The number of productive tillers per plant, number of grains per panicle, and fertility did not show important variations, and other morphological characters such as grain shape, size and cariopside color remained the same.

RAPD analysis was performed in 18 DH2 gametoclonal lines, in 3 normal doubled-haploid plants from Pokkali and in a seed derived plant from Nona Bokra and Pokkali.

Fig. 2: Gametoclonal variants of Pokkali

Polymorphisms were found among the gametoclonal lines, including both loss of parental bands, and the appearance of novel non-parental bands. There were found clear differences with 5 of 21 primers tested.

A new method to monitor cytochimera dissociation through three different propagation techniques in *Musa* spp.

Genetic improvement methods based on mutation techniques are particularly important for *Musa* species where there is

limited sexual reproduction that could generate genetic variation. However, as in other biotechnology approaches such as genetic transformation, *in-vitro* mutation induction techniques applied to improvement of *Musa* spp. has a major limitation: the treatment of multicellular meristems with mutagenic agents (physical or chemical) results in a high degree of chimeras. The chances of a mutated cell growing out into a sector or layer and manifesting itself depend on its position within the apex as well as its growth rate as compared with the surrounding (non-mutated) cells. It is often suggested to propagate into the fourth vegetative cycle, M_1V_4, in order to dissociate chimeras but fundamental studies have not been carried out to verify this postulation. It is difficult to monitor mutated cells, but genomic mutations can easily be induced using colchicine and detected using flow cytometry.

Our study consisted of inducing ploidy chimerism (mixoploidy) and assessing chimeric dissociation by the fourth vegetative generation after propagating through three different propagation systems (shoot-tip culture, multi-apexing culture and corm slide culture).

The results showed that the greatest percentage of cytochimera dissociation of mixoploid shoots was achieved with the multi-apexing technique followed by corm slide culture technique and shoot-tip propagation technique respectively. Presumably a combination of high proliferation rate and production of adventitious buds could explain why the multi-apexing technique favours chimerism dissociation.

Whether it was applied during the first propagation cycles or after 5 propagation cycles, the multi-apexing technique increased the proportion of cytochimera dissociation. To a less extend after 5 subcultures since probably the selected mixoploid shoots were already stabilised into periclinal chimeras and thus more difficult to dissociate.

We could reduce significantly the percentage of cytochimera after 3 subcultures but not completely. Thus, another propagation system based on single cell origin is still needed to speed up the selection procedure. This study has shown that by mixoploidy induction and flow cytometry detection, it would be possible to verify the unicellular (or multicellular) origin of any propagation system

H. PUBLICATIONS

Working Material: "Evaluation of Cereal Crop Mutants". Report on the Second Working Group Meeting on "Rice Multilocation Trials", Uruguay, 23-27 March, 1998.

Working Material: "Mutational Enhancement of Genetic Diversity in Rice" Report of the Project Formulation Meeting held in Bangkok, Thailand, 8-12 February 1999.

Working Material: Proceedings of the Co-ordination Meeting of Project AFRA III-18 "Development of Improved Crop Varieties", held in Bamako, Mali, 15-19 February 1999.

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

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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.):

N. Name of person contributing this information: _____

THANK YOU FOR YOUR KIND COLLABORATION !

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