PLANT BREEDING AND GENETICS NEWSLETTER

Joint FAO/IAEA Division
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TO THE READER

We are approaching the end of yet another year and it is logical to take stock of the activities undertaken by the sub Programme during 2000.

Our primary focus has continued to help Member States in improving crops by using radiation-induced mutations. Many allied technologies, particularly tissue culture and molecular biology have been added to enhance the efficiency of mutant induction, isolation, multiplication and their release as new varieties. We have continued to bring the latest developments and innovations in these rapidly advancing technologies for integration into the conventional breeding of mutation-derived varieties. This is reflected in the on-going Coordinated Research Projects (CRPs) on new and traditional industrial crops, creation of new useful banana genotypes, molecular characterization of mutated genes controlling important traits in seed crops, radioactively labeled DNA probes in crop improvement and the improvement of local food crops in Low Income Food Deficit Countries, and in the two newly initiated (CRPs), one on the analysis of root characters in annual food plants related to plant performance and the other on the improvement of tropical fruits.

The necessity to have an inter-disciplinary approach to solve problems is also reflected in the implementation of Technical Co-operation Projects (TCPs). For example, in North Africa, date palm has a unique role in food security and the eco-system. However, 'Bayoud' disease has killed over 15 million trees and is posing a serious threat to date palm production in Tunisia. Under a regional TCP to control the disease, irradiation of embryogenic cultures was investigated and low dose gamma radiation was found to increase the formation of somatic embryos. Date palm trees can also be multiplied *in vitro* through either shoot formation or somatic embryos, which allows irradiation of large populations.

Another example is fruit crops, many of which have not benefited from the use of radiation-induced mutations. A new CRP on the improvement of tropical and subtropical fruit trees through induced mutations has been initiated. The overall objective of this CRP is to generate and characterize radiation induced and natural genetic diversity in tropical and subtropical fruit trees for improving nutrition balance, food security, and enhancing economic status of growers. The project aims to produce mutants with traits such as resistance to biotic and abiotic stress, and fruit with reduced seed number or seedless.

Transfer of new technologies through training and courses continued. For example, a regional training course was organized on "New Frontiers of developing and handling Mutants" that included insertion of plant transposable genes and retro-transposons to induce and identify mutants and gene tagging at the Institute of Nuclear Agricultural Sciences, Zhejiang, China.

Development of low cost technology for *in vitro* culture is important for developing Member States. In the experiments carried out at the FAO/IAEA Agriculture and Biotechnology Laboratories, natural daylight was used successfully to replace artificial light for *in vitro* culture of banana, thus eliminating the need and cost of electricity. A prototype system to capture natural light in the culture room has been developed.

The continued use of radiation-induced mutations by Member States was reflected in the FAO/IAEA database on mutant varieties. During 2000, the number of mutant varieties released reached 2252 in 163 species spread over 62 countries.

As the latest issue of Newsletter goes to the press, we wish all the readers a happy and successful New Year!

Miroslaw Maluszynski

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B. FORTHCOMING EVENTS

Research Co-ordination Meetings

Final Research Coordination Meeting on "Cellular biology and biotechnolgoy including mutation techniques for creation of new useful banana genotypes", Leuven, Belgium, 24-28 September 2001

Project Co-ordination Meetings

AFRA Regional Project Co-ordination Meeting of RAF/5/042 "Development of improved crop varieties", Arusha, Tanzania, 19-23 February 2001

C. PAST EVENTS

Regional Training Course on "New frontiers of developing and handling mutants", Hangzhou, China, 5-16 June 2000

The Training Course was hosted by the Institute of Nuclear Agricultural Sciences (INAS), Zhejiang University. Fourteen participants from 8 countries plus 3 local participants took part. The training took place in the recently opened Biophysics Building of INAS. The lecture room as well as laboratories are of high standard and very suitable for the organization of regional training courses in the field of application of nuclear techniques in agriculture.

The course was opened by Dr. Mingjiang Ni, Vice President, Zhejiang University and Professor Bujin Xu, the Director of the Institute of Nuclear Agricultural Sciences and the responsible staff member of the sub-Programme. The course was very well organized and implemented following a timetable previously agreed upon with the course director, Dr. Quingyao Shu. The only modification was due to the rainy weather which prevented breeding exercises on experimental fields. They were replaced by an ad hoc workshop, chaired by Dr. J.N. Rutger (invited lecturer). Dr. Rutger, Director of the Dale Bumpers National Rice Research Center, Stuttgart, Arkansas, is the leading rice breeder in the USA, effectively applying mutation techniques for breeding high yielding rice varieties and germplasm enhancement. The field was visited the next day and exercises were performed as planned. Breeding and demonstration plots were very interesting, thanks to Professor Yingwu Xia, the leading rice breeder who developed mutant variety Zhefu 802, the widest grown rice mutant variety in China in the early '90s. All lectures presented by local scientists during the first week of training course were of high scientific value and well prepared. The course was positively evaluated by participants. The evaluation, as well as handouts distributed by lecturers during the Training Course, are available in the Plant Breeding and Genetics Section.

The host institute has a very active rice breeding programme using gamma ray induced mutants. The programme is recently focusing on hybrid rice. However, due to the very good yield of conventional varieties and their higher grain quality in comparison to hybrids, the economic advantage of hybrid rice cultivation seems to be less visible than generally assumed. There are also some symptoms of overproduction of rice in China, especially of early season *indica* varieties. About 15% reduction of rice cultivation area is expected in Zheijang Province and in China, in total, from 33 million hectares to about 30 million this year.

The problem of reduced exchange of crop germplasm among plant breeders from various countries was discussed with Prof. Min Shaokai (previous Deputy Director of the China National Rice Research Institute - CNRRI), the coordinator of rice breeding in China and Professor B. Xu (INAS). They supported the idea to organize a databased-mutant-germplasm collection at Seibersdorf. According to the proposal, collected mutants will be freely available for breeding purposes. However, the use of mutants for patenting or for gene isolation and cloning will need the agreement of the seed donor. DNA fingerprinting of promising mutants should help to follow up distributed material. The mutant collection database should be available through the FAO/IAEA home page.

2nd RCM on "Genetic improvement of underutilized and neglected crops in LIFDCs through irradiation and related techniques" 26-30 June 2000, and FAO/IAEA/UCR Workshop on "In vitro culture techniques for the improvement of vegetatively propagated tropical food crops", 1-5 July 2000, San Jose, Costa Rica,

The 2nd Research Co-ordination Meeting under the FAO/IAEA Co-ordinated Research Project and the subsequent training workshop were hosted by Dr. Francisco Saborio-Pozuelo, University of Costa Rica (URC). Thirteen scientists from 11 countries took part in the RCM, where scientific papers were presented summarizing results achieved under each research contract/agreement. An overview was given at the session pertaining to underutilized species by a local IPGRI representative. Results of the CRP, the present situation and prospects for utilising the respective neglected/underutilized crops in participating countries were discussed. On the basis of the papers presented, induced mutations were used to generate genetic diversity in neglected/underutilized crops in Bolivia, Costa Rica, Ecuador, France, Ghana, India, Indonesia, Mexico, Slovak Republic, South Africa and Thailand. In seed propagated species such as okra, naranjillo, grain and vegetable amarants, Bambara groundnut, grass pea and quinoa, radiation of seeds is mainly practiced. In the vegetatively propagated species cocoyam, yam and bitter potato, mutation techniques were combined with *in vitro* techniques in order to dissociate chimeras more efficiently

Germplasm collection and characterization

New accessions were collected and partly characterized at the morphological and/or molecular level (cocoyam). RAPD and isoenzyme techniques for cocoyam characterization need to be further optimized.

Mutation techniques

Appropriate radiation treatments were selected after radiosensitivity tests in all underutilized/neglected crops for locally important genotypes. Radiation doses from 4-10 Gy are recommended for shoot tip cultures of cocoyam (Xanthosoma), 15 Gy for yam and 22-28 Gy for bitter potato. For seed treatment the recommended radiation doses are generally higher: okra (600 Gy), naranjilla (50 Gy), grain amaranth (175 Gy), leaf amaranth (160 –200 Gy), quinoa (200-250 Gy), bambara groundnut and grass pea (150-250 Gy).

In vitro techniques

An efficient protocol for the propagation of cocoyam using the small-clusters technique was adapted for use in *in vitro* mutagenesis in Costa Rica. Starting from radiation treatment of 100 *in vitro* plantlets, an M_1V_4 population of 2700 plants for screening can be produced in less than one year. Other projects will adapt these techniques, which were also demonstrated during the associated training workshop. In yam and bitter potato micropropagation methods were successfully combined with mutation techniques.

In amaranth, adventitious buds were generated from epicotyl explants of seedlings. Calluses were established as source tissue for cell suspension cultures. For bambara groundnut, regeneration of plantlets from excised embryonic axes/cotyledonary nodal explants was obtained. The techniques for shortening the breeding cycle in pea using *in vitro*/*in vivo* systems were successfully adapted to bambara groundnut. For grass pea and pea, progress was made in plant regeneration from hypocotyls explants and protoplasts. After fusion of protoplasts from both species, callus production could be achieved from somatic hybrid cells. Molecular markers were established by fluorescence *in situ* hybridization to help recognize grass pea from pea genomes.

Screening techniques

Field screening is done for morphological traits (erect plant type, pod quality in okra). For drought screening of amaranth a number of physiological parameters are measured in greenhouse trials.

Screening of cocoyam for resistance to diseases (root rot, leaf blight) is done in the glass house by soil inoculation or has still to be established.

Induced biodiversity for improvement of neglected/underutilized crops

Early mutant generations of all species were produced. In cocoyam the mutant population of one of the projects is sufficiently large to start with mutant screening. For cocoyam, yam and bitter potato large populations should be available for mutant identification by the end of 2000.
In seed propagated crops, segregating populations of okra, amaranth and quinoa are available. Others have to be further advanced (bambara groundnut, grass pea) and increased.

Conclusions and Recommendations

The FAO/IAEA sub-programme on genetic improvement of underutilized and neglected species plays a strategic role in complementing work being carried out on a global level in the promotion of these species. Mutation studies on a wide range of such crops have been initiated.

1. IPGRI should provide - similar to banana - material transfer agreements for the crops in this project to be used for exchange for seed/plant material among participants.
2. It is recommended that close links be strengthened and/or established with relevant partners particularly within international agricultural research institutes (IARCS) such as IITA (for yam, bambara groundnut) and CIP (for bitter potato). Links should also be ensured with existing international networks and research groups working on the species selected by participants of the FAO/IAEA project.
3. IPGRI's services should be used for relevant information on where the germplasm of the selected crops is being conserved along with information on the person to contact to request material. It is recommended that IPGRI assist in the publication of short articles in its regional newsletters on the various research activities that will be carried out within the framework of the FAO/IAEA project. It is recommended that IPGRI provide relevant descriptor lists to participants and encourage the preparation/revision of new/old descriptor lists if needed. It is recommended that FAO/IPGRI assist/advise participants on the safe movement of germplasm, particularly for those vegetatively propagated crops (e.g. *Dioscorea*, *colocasia*, *xanthosoma*).
4. Considering the present day evolution of patenting of genetic materials the group agrees that measures should be taken and/or awareness of the situation obtained by institutions interested in the conservation and use of plant genetic resources. This will ensure that such materials as might be collected or obtained within this CRP will remain accessible for further research and use by farmers without any need for royalty payment or patent contesting. Molecular markers should therefore be developed for the reliable identification of the materials. Groups without the opportunity to undertake the molecular characterization of the genetic novelties produced, should apply for assistance in this domain to international organisations. The latter should be encouraged by governments to collaborate in this respect. FAO and IAEA could also contribute through the Seibersdorf Laboratory to assist in DNA fingerprinting of minor species.
5. The presentation of research results at regional and international conferences as well as their publication in peer-reviewed journals should be strongly encouraged as additional means of promoting the wider publicity of the crops studied in this CRP. The free transfer of information among participants will ensure the efficient transfer of technology.
6. Since it is becoming more and more difficult to exchange germplasm and breeding material between breeders across borders, IPGRI and FAO should create awareness in Member States that this might hamper or delay the development of improved varieties needed to sustain and improve crop productivity.
7. The group was successful in obtaining additional funds for research from national, regional and international sources. Other sources were also identified. For some crops

studied in this CRP various private companies have shown interest in their promotion and use.

8. Technology transfer between participants should be facilitated by the Agency's manpower development programme in providing expert services, scientific visits and training activities. Requests for such support should be submitted through the national authorities.

After the RCM a training workshop was held for five RCM participants and 12 other scientists from the region. The majority of other participants had no previous experience in the use of radiation-induced mutation techniques for crop improvement but showed strong interest in the technology.

First RCM on "Improvement of tropical and subtropical fruit trees through induced mutations and biotechnology", Vienna, Austria, 25-29 September 2000.

Fourteen participants from Australia, China, India, Indonesia, Iran, Israel, Malaysia, Pakistan, South Africa, Thailand, United Kingdom and the USA attended this RCM.

Improvement of tropical and subtropical fruit trees has been difficult using conventional breeding techniques but the challenges can be met by application of biotechnology. The crops are characterised by long juvenile period and large tree size, and the available genetic information is very limited. In many crops such as avocado, mango, litchi and others, controlled crosses are difficult to perform due to the massive fruit drop. Developing countries have limited funds for research. Thus, tropical and subtropical trees are lagging behind other plants in terms of availability of genetic information and technologies for the improvement of biological material.

Specific problems in tropical and subtropical fruit trees

The role of these crops for stimulating economic development and political stability involves the removal of a number of obstacles. These include biotic and abiotic stresses, postharvest losses, the supply of improved disease and virus-free plants coupled with efficient propagation techniques. In developed countries most of these problems have been addressed through the use of local cultivars and agro-technologies. Most farmers in the developing world do not have access to these capital and technologically intensive solutions.

Biotic stresses are probably the most important factors limiting the expansion of tropical and subtropical tree crops in the developing world. Diseases caused by *Fusarium, Phytophthora* and *Colletotrichum* combined with insect and nematode pests account for significant losses of produce, and/or trees. The value of the remaining crop is usually further reduced by the influence of the pests and diseases on the quality of the fruit. The presence of some of these diseases or pests on or in fruit can often prevent the export of produce, and hence the loss of much-needed foreign exchange. The use of chemicals to ameliorate these problems significantly reduces profitability and can possess significant health and environmental risks. Many developing countries do not have or enforce legislation that controls the use and disposal of these chemicals. Furthermore, application is often delegated to vulnerable members of societies including children and women. Indiscriminate use of insecticides and pesticides usually results in the breakdown of biological control, resulting in ever-increasing chemical dependence. Chemical residue standards in fruit imported into developed countries are stringent and if alternatives to agrochemicals are not found this may result in the loss of valuable foreign exchange.

The influence of abiotic stresses is exacerbated in developing countries because the infrastructure and equipment needed to moderate these influences are usually not available. In order for developing countries to break into the international market it would be advantageous to produce fruit that has enhanced characteristics such as higher yield, longer storage life, improved taste, fruit colour and seedlessness. Poor transport infrastructure and long distances to ports or airports, significantly exacerbate post-harvest problems. To increase the impact of these crops on local consumption, increased nutritional value is highly important. The expansion of these industries may depend on the development of more appropriate propagation techniques that will ensure the rapid release of disease and virus-free selections of high quality.

This CRP addresses the requirement to close the gap between the limited mutant resource and the full range of phenotypes that is essential to exploit fully tropical and subtropical tree crops, including citrus (South Africa, Iran, Thailand, Malaysia), mango (Australia, India, USA, Israel), Jujube (China), guava (India, Pakistan, South Africa), Cashew (India), avocado (Indonesia), papaya (Malaysia) and other minor fruit crops such as litchi, annona, carambola, pitanga and jaboticaba (South Africa

Global Expected Outcomes

Outcomes in terms of dissemination, training and technology transfer/exchange are expected from all work programmes.

- Dissemination: publications, reports and websites describing progress and technologies useful to breeders, researchers and the public
- Training: transfer of technologies and information to younger researchers and between countries

Conclusions And Recommendations

Many tropical and sub-tropical fruit crops have not benefited from conventional breeding, owing to their long juvenile periods, polyploidy, polyembryony and low frequencies of fruit set. Tropical and sub-tropical fruit cultivars are for the most part vegetatively propagated selections that have been made among open pollinated seedlings. Due to the constraints of conventional breeding with perennial plants, there is a need for alternative genetic approaches for improvement. Appropriate biotechnology techniques can result in the efficient production of new cultivars and that can be assessed for useful horticultural traits under field conditions. Alternatively, if a powerful selection agent is available, it is possible to screen large cell populations for resistance to the agent. The RCM Group is utilising various strategies in order to recover mutants with such traits as resistance to abiotic and biotic stresses, fruit quality, tree architecture etc., for enhanced food security and sustainability.

Exchange of genetic materials. It is evident that exchange of material and information among partners will contribute to the success of the project. At the same time it is recognised that there are serious constraints to international exchange of germplasm and DNA. It is recommended that FAO/IAEA explore ways in which barriers can be overcome, for example through the development of bilateral material transfer agreements, and by agreements that clearly distinguish between research and commercial application.

Collaborative linkages. The application of induced mutations and biotechnology to fruit crop improvement depends on a multidisciplinary approach. This will be best served by close collaboration and cooperation among scientists working within/between these species, and also with those having technological expertise in other crops or model systems. The relevant research areas cover a wide range from traditional plant breeding, crop physiology and genetics, through plant tissue culture to molecular biology. We therefore recommend that new linkages be established, existing ties strengthened, and training (through research) programs set up within the framework of the CRP.

DNA markers. It is highly recommended to choose the DNA marker according to the research goal. In general, the multi-locus markers are better suited for assessment of genetic variation, while the single locus markers are better suited for linkage analysis. Among the multi-locus markers, amplified fragment length polymorphism (AFLPs) are widely used owing to their simplicity and low cost, as well as their reliability. Among the single locus markers, the SSRs are highly polymorphic, quite abundant and very reliable. The main disadvantage is their high cost. In this rapidly changing genomics research area, new high throughput technologies are becoming available which will be efficient and low cost for tropical and sub-tropical fruit crop improvement. Single Nucleotide Polymorphisms (SNPs) are new single locus markers that are very abundant and highly suitable for automation and high throughput. Since their application requires sophisticated equipment, this research avenue offers an opportunity for collaboration between laboratories in developing and developed countries.

Mutagenesis and selection of mutants. *In vitro* and *ex vitro* selection of desired variants through mutagenesis is recommended where there is an appropriate screening agent. It is also recommended as an alternative means for increasing genetic variation, without the intervention of any other genetic material.

***Sustainability and food security*.** It is recognised that preservation and utilisation of biodiversity is of utmost importance. Development of improved varieties by *in vitro* or *ex vitro* mutagenesis is recommended for increasing genetic diversity, both for qualitatively and quantitatively inherited characters (such as disease resistance, stress tolerance, post-harvest disorders, fruit quality and yield). These approaches are environmentally sustainable and can make an important contribution to food security.

Dissemination. The research coordination meeting is a very effective way for the exchange of information and ideas regarding planning for execution of projects. The scientific aspects of the project can be published in the form of technical reports, bulletins, journal articles, proceedings of scientific meetings, popular articles in newspapers or agricultural magazines.

Publication of any aspect of the projects on the Internet, in the FAO and IAEA websites, or participants' websites linked to FAO and IAEA will give fast access to the world community. Establishment of a mailing list devoted to induced mutations for plant breeding would be a very effective way of communication between RCM participants and other interested parties interactively and instantly.

Second RCM on "Molecular characterisation of mutated genes controlling important traits for seed crop improvement", Vienna, Austria, 2-6 October 2000.

Nineteen participants attended this RCM, from Brazil, Bulgaria, Canada, China, P.R., India, Korea, Rep. of, Philippines, Poland, Portugal, Turkey, United Kingdom, USA as well as a representative from the International Centre for Genetic Engineering and Biotechnology.

The participants stressed that mutations as a genomics tool are inadequately quantified. The current status is that serendipitous studies of specific mutants have shown chemical mutagens to produce small deletions, down to base-pair substitutions, while physical mutagens (fast neutrons, gamma and X-rays) also cause larger deletions and, in addition, chromosomal rearrangements such as inversion and translocations. Nothing is known of the frequency at which these alterations occur and no systematic studies have been undertaken in relation to plant material. Knowledge is also needed in order to develop the most effective strategies for using 'knock-outs' mutations either to isolate genes directly or as an aid to map-based cloning by using minimum overlaps to reduce the critical regions to sequencable chunks. Knock-outs are also playing an increasing role in gene function analysis. It is important that we know how many other unrelated (and possibly deleterious) mutations can be expected in the same mutated material selected for a particular trait. The were defined as urgent needs for research which should be undertaken by participants or other plant genomics oriented laboratories:

- Develop methods to address the changes brought about by mutagenesis in the light of our present understanding of the plant genome
- Define 'point mutation'
- Characterise and quantify effects of various mutagens (chemical and radiation) especially related to the following questions:
  - base-pair changes or deletions, what size deletions?
  - how many rearrangements with any particular dose?
  - mutations random within the genome?
  - do they occur more in genes than in inter-genic regions? (if so in what regions of genes?)
  - are there differences between species? Are there differences among large and small genomes?
- Compare induced (chemical and irradiation) with insertional mutagenesis (transposon elements or T-DNA) in different species for different applications

Among other topics participants concentrated on comparison of physical mapping methodologies and their relevance to particular research objectives. Physical mapping is a tool for the analysis of complex genomes and is a means for relating the position of a sequence to its physical position in the genome, and for relating physical distances to genetic distances. Physical mapping relies on different types of methodology. Cytological approaches, such as Fluorescent *In Situ* Hybridization (FISH) or Fibre FISH are used to physically localize DNA sequences onto chromosomal preparations. Molecular techniques used for physical mapping generally revolve around the use of large-insert genomic libraries, such as BAC or YAC libraries. These are used to build 'contigs' of overlapping clones across a region of interest or alternatively, the entire genome of interest. One of the major purposes of physical mapping is for map-based isolation of genes or for building contigs of specific genomic regions for evolutionary studies. Global physical mapping in plants has only been achieved in the model species *Arabidopsis*, and partially in rice. Efforts to map the genomes of other crops are underway, either in a targeted or global fashion. Physical mapping is also a means for comparing the structure of large/complex genomes to smaller model plant genomes and searching for homologous or orthologous genes.

A large part of the discussion was concentrated on problems related to the exchange of plant material and its patenting. The majority of the participants have agreed that the free exchange of biological knowledge has been threatened by the actions of patent offices. The graining of patents for the discovery of genes, genetic sequences and the compositional variation of living organisms has almost halted the free exchange of biological materials. This free exchange was the foundation of the great leap forward in food production made in the 20th Century. The actions of patent offices were made with a superficial knowledge and understanding of biological systems. This has resulted in rules of patenting that were developed for the physical world being adopted for the biological. In addition, governments throughout the world have allowed the basic concepts of patents i.e. novelty, inventiveness and disclosure to be changed in many biological patents to discovery, initiation and obstruction. The traditional purpose of patents was the protection of an invention in exchange for its complete description by the inventor. The description would allow other inventors to make improvements to the invention. The understanding of biological function and inheritance is undergoing rapid change. For example, the use of mutations and molecular mapping for comparative genetics in the Gramineae has shown the tremendous conservation of genes across species. Rice, maize and wheat frequently contain the same genes, located at the same relative chromosomal location in each species. The discovery of the same gene in each species has allowed patenting from each species even though the function of the gene remains the same. No inventiveness was shown, only discovery and imitation. It is suggested that plant breeders and geneticists should promote the development of patenting legislation specific for biological materials, rules that would be based on biological principles, novelty and inventiveness.

The Working Material from the RCM is under preparation and will soon be ready for distribution.

Regional (AFRA) Training Workshop on "Appropriate selection techniques for the development of drought tolerant germplasm", IITA Kano Station, Kano, Nigeria, 9-13 October 2000

Nine participants from Egypt, Ghana, Kenya, Madagascar, South Africa and Sudan, three local participants and six local observers were trained in mutation and drought screening techniques. Participants presented papers on their research work, discussed the results and agreed on appropriate drought screening techniques for regional crop improvement programmes.

The participants commended the Agency, the Local Organizing Committee and IITA Kano Station for organizing and hosting the Workshop. Arrangements, logistics and administrative support were excellent and well organized. The generous support of the Federal Ministry of Petroleum Resources and the Kano State Government contributed to the success of the Workshop and general well-being of the participants. The participants especially appreciated the courtesy call on his Royal Highness, Alh. Ado Bayero the Emir of Kano who presented a historical perspective of the cultural heritage of Nigeria. The participants felt that the tremendous success of this Workshop was partly due to the excellent collaboration between IITA, FAO and IAEA and they expressed the desire that such collaboration with CGIAR centres be continued and extended in future.

Through discussion with Nigerian participants and through a separate meeting with the Commissioner of the Ministry of Agriculture, it was found that there is an urgent need to train junior scientists in the application of induced mutations for the improvement of groundnut, cowpea and sorghum in northern Nigeria.

FAO/IAEA Workshop on "*In vitro* protocols and mutant selection using Bayoud toxin", 20-26 November 2000, Morocco.

The objective of this Workshop was to provide training in tissue culture and mutagenesis, molecular biology, mutagenesis and Bayoud disease toxin. It was attended by nine participants - from Algeria (3), Tunisia (3), and Morocco (3). An internationally well-known tissue culturist and a molecular biologist were invited from Canada and the USA to lecture at the Workshop. Both theory and practical demonstrations were covered. Working groups were formed to discuss project related problems.

Since plant tissue culture is an important component of the date palm project, two lectures were delivered on the application of plant tissue culture technologies and *in vitro* mutagenesis. Various tissue culture technologies were presented and their possible applications in plant improvement were discussed, including induced mutations and somatic cell hybridization and somaclonal variation.

The participants showed a great interest in lectures on micropropagation, cryopreservation and somatic embryogenesis. Somatic embryogenesis technology is well advanced in conifer regeneration, bioreactor culture for somatic embryo production, somatic embryo encapsulation or somatic seed, and cryopreservation for long-term storage. Weyerhauser Company, USA already has over 200,000 somatic embryo-derived plants for field trials at different locations derived from cryo-stored somatic embryogenic cultures. So far, no variation has been observed among regenerated plants and somatic seeds behave just like zygotic seeds. Further tests are in progress. Dr. Gupta, Weyerhauser Company, USA, a lecturer at this Workshop, is the world's leading expert on conifer somatic embryogenesis. This company has obtained 12 patents in this technology and is willing to help in date palm somatic embryogenesis. A recent report on date palm somatic embryogenesis has indicated

5% variation in regenerated plants, which is at the acceptable level. This report is very encouraging. The Tunisian group has developed an excellent system of somatic embryogenesis in date palm. The Tunisians have agreed to train Moroccan and Algerian scientists in somatic embryogenesis

In molecular biology, the main focus was on molecular marker applications, genome mapping, genetic mapping of disease resistance traits, and application of molecular genetics in genetic diversity assessment, conservation, restoration and sustainable management of plant genetic resources.

A series of lectures was given on Bayoud disease: its origin, spread and control; Bayoud pathogen and its toxin *Fusarium oxysporum* f. sp. *albedinis*; *in vitro* and *in vivo* selection against Bayoud toxin; characteristics of Bayoud toxin, and its production, extraction, purification and determination of chemical structure of the toxin. The Moroccan group has already developed technology for Bayoud toxin isolation and production, and also for screening mutants against this toxin. Participants discussed the transfer of Bayoud toxin to Tunisia and Algeria. Morocco agreed to supply Bayoud toxin to Tunisia and Algeria, and help in screening of irradiated date palm material against Bayoud toxin.

FAO/IAEA Workshop on "*In vitro* mutagenesis and Molecular Marker Analysis of Ornamental Plants" Bangkok, Thailand, 17-23 December 2000.

This Workshop is a part of the Technical Cooperation Project THA/5/045. Lecturers have been selected to cover tissue culture techniques and application, *in vitro* mutagenesis and selection as well as molecular markers and their applications. This Workshop will be reported on in more detail in the next issue of the Newsletter.

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 of overcoming 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 (*Colosasia esculenta, Xanthosom*a spp.), yams (*Dioscorea* spp.), grain and vegetable amaranths (*Amaranthus* spp.), Bambara groundnut (*Vigna subterranea*), grasspea (*Lathyrus sativa*), okra (*Abelmoshus esculentus*), bitter potatoes (*Solanum jucepzukii*, *Solanum ajanhuiri*) and naranjilla (*Solanum quitoense*). 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. The second Research Co-ordination Meeting was held in Costa Rica from 26-30 June 2000.

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

This CRP was initiated in 1994 with the general aim of integrating 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 be readily regenerated via somatic embryogenesis for large-scale plant production, which is ideal for *in vitro* mutagenesis and the selection of mutants with desirable agronomic traits. Since 1996, Belgium has been an important contributor to this CRP. Twenty institutions worldwide are involved.

Mutational Analysis of Root Characters in Annual Food Plants Related to Plant Performance

This CRP was initiated this year with the overall objective of assisting Member States to apply mutation techniques and related biotechnology to generate and utilise mutants for the identification of root properties and genes for improvement of crop plants. At the present time there are 21 participating institutes in this project. The first RCM was held in Vienna from 14-18 February 2000.

Molecular Characterization of Mutated Genes Controlling Important Traits for Seed Crop Improvement

This CRP was initiated at the beginning of 1999 with the aim of assisting Member States to apply molecular genetics of mutated genes for improving production in both major cereals and related under-utilised crops. More specifically to collectively develop, characterise and data-base mutant collections of key crops for application in breeding programmes and to molecularly characterize new or existing mutated genes affecting key agronomic traits in major crops using comparative approaches in under-utilized crops with a view to their eventual isolation. The Second RCM was held in Vienna from 2-6 October 2000.

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

This CRP was initiated this year and the first RCM was held in October 2000. The overall objective is to generate and characterize radiation induced and natural genetic diversity in tropical and subtropical fruit trees for improving nutrition balance, food security, and enhancing economic status of growers in Member States.

E. TECHNICAL CO-OPERATION PROJECTS

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

F. ACTIVITIES AT THE PLANT BREEDING UNIT, SEIBERSDORF

Cost reduction in the micropropagation of banana by using tubular skylights as source for natural lighting

Daylight instead of artificial light was exploited for the *in vitro* culture of banana. Tubular skylights re-diverted natural light into an interior enclosed room and could sufficiently illuminate an area of 3 to 5 m^2. The cost of a tubular skylight is ca. US $ 600. The maintenance free system allowed only a minimum of heat transfer and no cooling was necessary. The culture room required no electricity supply and under our conditions savings on costs for electricity of US$ 6 per m^2 per week were achieved as compared to a standard growth room equipped with artificial lighting and controlled photoperiod and temperature regimes. Under natural light conditions, micropropagated plantlets were well developed at mean photosynthetic photon flux densities (PPFD) of 5 to 13 $\mu mol\ m^{-2}\ s^{-1}$ and photoperiods of 9 to 14 hours. Micropropagation rates were either the same or significantly higher than under artificial lighting. Single shoots on rooting medium showed some symptoms of etiolation yet acclimatisation rates averaged 95 %. A step-like culture rack, rather than a vertical one, permitted uniform plant growth on all levels.

Salinity tolerant performance and genetic diversity of four rice varieties

The genetic diversity of three salinity tolerant rice varieties Pokkali, Nonabokra and Bicol was investigated using random amplified polymorphic DNAs (RAPDs). High yielding susceptible variety IR29 was used as check for comparison. The salinity performance of these varieties was tested by using rapid screening techniques at the seedling stage. One hundred primers were tested of which 42 revealed differences between Pokkali & Nonabokra, 43 between Pokkali & Bicol and 50 between Nonabokra and Bicol. Polymorphism differences between IR29 - Pokkali, IR29 - Nonabokra and IR29 - Bicol were 47%, 53% and 31%, respectively. Four primers amplified specific fragments that appeared in all the three salt tolerant varieties but not in the salt susceptible variety IR 29. Primer UBC 9 (5'-CCTGCGCTTA-3') produced a prominent diagnostic fragment of approximately 1600 bp; primer UBC 244 (5'CAGCCAACCG-3') generated a fragment of about 800 bp in the salt-tolerant varieties; primer UBC 251 (5'-CTTGACGGGG-3') amplified one polymorphic band of 1100 bp and primer UBC 267 (5'-CCATCTTGTG-3') yielded a relatively weak polymorphic band of 1100 bp.

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 and 8% in the top. 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.

Studies on Tos17 retrotransposon in rice plants derived from irradiated seeds and in gametoclonal variants

Some transposable elements in plants are known to be activated by stress conditions. In rice it was shown that the transcription of the retrotransposon Tos17 is induced during cell culture (Hirochika et al., 1996, PNAS 93, 7783-7788). As part of our project on mutation induction for rice improvement we are studying if γ-irradiation has an effect on the activation of Tos17 and could thus provide a tool for gene tagging. DNA of 60 plants derived from irradiated seeds of the japonica variety "Taipei 309" was subjected to Southern analysis using Tos17 as a probe. Ten percent of the plants analysed showed integration of new copies of Tos17 into their genome. The number of additional copies varied between 1 and 5. This relatively low number will facilitate a gene tagging strategy because the identification of an affected gene will be easier than with multiple integrations.

Further experiments were performed on gametoclonal variants of the indica variety "Pokkali". These doubled haploid (DH) plants resulted from anther culture and are characterised by semi-dwarfness and photoperiod insensitivity. Using Tos17 as a probe, a polymorphism in the restriction fragment pattern was detected. The gametoclonal variants did not show a 3.5 kb band which was present in the seed derived control as well as in the phenotypically normal DH-plants. The correlation of semi-dwarfness and photoperiod insensitivity with the missing band was also detected in the next generation. The lack of the band was not due to changes in the methylation pattern at that specific site. Our interest is now focused on the DNA which is probably missing in addition to this transposon site. We are currently isolating the 3.5 kb genomic DNA fragment from wild type plants which bear the Tos17 sequence to characterise the integration site of the retrotranposon.

Optimization of in vitro mutagenesis for the genetic improvement of Musa spp.

After colchicine treatment, chimerism dissociation was improved by using the multi-apexing propagation method. This method allowed us to reduce cytochimerism from 30 % (using the traditional shoot-tip culture system) to 7% percent (using the multi-apexing method). This propagation system is being used in mutation induction experiments and should permit us to select stable mutants. To completely overcome the problem of chimerism after mutagenic treatment embryogenic cell suspensions are the material of choice for *in vitro* mutagenesis.

Nevertheless we have shown that old embryogenic cell suspensions become polyploid or aneuploid after several subcultures. Before or after mutagenic treatment, genetic instability in DNA content can be detected by using flow cytometry. Genetic instability in DNA content may interfere with the mutant and make this mutant unusable. The use of flow cytometry has enabled us to eliminate genetically unstable clones to be used in mutation induction experiments.

Eight irradiated Grande Naine plants were selected for their tolerance to Juglone (the main toxin involved in the fungus *Mycosphaerella fijiensis*). The pre-selected plants still need to be inoculated with the fungus itself but if these plants confirm their resistance to the disease black Sigatoka this would have a tremendous impact on environmental, health and food security issues.

Services

Radiation Service using ^{60}Co

15 individual requests from 11 different countries

treatments: 80 (1 *in vitro* 79 seed samples)
plant species: 16 (1 *in vitro* 15 seed samples)

Ploidy determination using flow cytometry

In *Prunus* sp. leaf samples for Vienna University of Agriculture (BOKU), Austria
In banana shoot-tips and cell suspensions for Katholiek University Leuven, Belgium
In banana shoot tips for the Central University, Caracas, Venezuela
In banana leaves for the University of Florida, USA
In *Cornus* sp. Leaf samples for Schonbrunn Botanical Garden, Austria

Training

The following scientists visited and/or received training:

Mr. Md Elagamawy (Egypt)	Cost free expert	Dec.99-Jan. 2000.
Ms. Cresencia Paul Lugendo (Tanzania)	Fellowship program	Jan.-Oct., 2000
Ms Seema Khondokar (Bangladesh)	Cost free intern	Jan.-July 2000
Mr. M.A.K.Azad (Bangladesh)	Fellowship program	March-Oct. 2000
Ms. Yulidar (Indonesia)	Fellowship program	May-October 2000
Mr. Md Ali Azam (Bangladesh)	Scientific Visitor	June2000
Ms. E. Janov (Australia)	Cost free intern	Aug.-Sept 2000
Ms. D. Hjuber (Hungary)	Cost free intern	July 2000

G. PUBLICATIONS

Afza, R., Shen, M., Zapata-Arias, F. J., Xie, J., Fundi, H. K., Lee, K. Bobadilla, E. and Kodym, A. (2000). Effect of spikelet position on rice anther culture efficiency. Plant Science 153: 155-159.

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Jain S.M, PK Gupta and RJ Newton (eds.), (2000). Somatic embryogenesis in woody plants, Volume 5-6. Kluwer Academic Publishers, The Netherlands.

Maluszynski, M., K. Nichterlein, L.van Zanten and S.M. Jain. (2000) FAO/IAEA Mutant Varieties Database - December 1999. Mut.Breed.Rev. 12

Maluszynski, M. and B. S. Ahloowalia, 2000. Mutation techniques in plant breeding. In: Radiation Research Vol. 2: Proceedings. Moriarty,M., C.Mothersill, C.Seymour, M.Edington, J.F.Ward and R.J.M.Fry (Eds.) Int.Assoc.Rad.Res., Lawrence. pp.251-254

Nielen, S., M. Guzman and F.J.Zapata-Arias. (2000). Studies on Tos17 retrotransposon in rice plants derived from irradiated seeds and in gametoclonal variants. 6th International Congress of Plant Molecular Biology, June 18-24 Quebec, Canada, abstract S23-17

Roux N. Optimizing mutagenesis of *Musa* spp. for inducing desired genetic variation. (2000). Haberland Symposium, Abstracts, *In vitro* plant volume 35 No.2

Xie, J., Zapata-Arias, F. J., Shen, M. and Afza, R. (2000). Salinity tolerant performance and genetic diversity of four rice varieties. Euphytica 116:105-110.

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):

|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|

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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 KIND COLLABORATION !