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Improvement of basic food crops in Africa through plant breeding, including the use of induced mutations

Report of the Third Research Co-ordination Meeting of FAO/IAEA/ITALY Co-ordinated Research Programme, held in Nairobi, Kenya, 20-24 September, 1993

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JOINT FAO/IAEA DIVISION
OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE

INTERNATIONAL ATOMIC ENERGY AGENCY
FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

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FOREWORD

Food production in Africa depends upon the cultivation of many local crops which have been used by the native farmers for thousands of years. Several of these crops, such as sorghum, African rice (*Oryza glaberrima*), and bambara groundnut, represent local varieties and land races, which are well adapted to the native agro-climatic environment, but have undergone little or no improvement in their yield and quality. Many of these crops are not known outside the limited regions where they are cultivated; hence have been neglected in their genetic improvement for yield and quality. Others crops, such as cassava, banana, plantain and sweet potato were introduced into Africa long time ago, but from limited germplasm, and have a narrow genetic base. There is thus a need to increase the genetic potential of the local varieties and land races yet retaining their genetic diversity and adaptability to the climatic conditions. Induction of mutations in these cultivars offers the possibility to change one or two characters at a time without disrupting the original genetic blue-print. Since, several of these crops, such as cassava, banana, plantain, sweet potato are propagated from vegetative parts, *in vitro* culture techniques in combination with mutagenesis can speed up the improvement of these crops through breeding. Hence, a Co-ordinated Research Programme, on "Improvement of basic food crops in Africa through plant breeding including the use of induced mutations", funded by the Italian Government, was initiated in the Joint Division of the Food and Agriculture Organization and International Atomic Energy Agency, Vienna. The primary objective of this CRP was to breed improved varieties of staple food crops of Africa with main emphasis on the indigenous species and local cultivars. The Third Research Co-ordination Meeting (RCM) under the FAO/IAEA/ITALY Co-ordinated Research Programme was held in Nairobi, Kenya, 20-24 September 1993 in which 24 persons participated and 18 scientific reports were presented. These included reports from 10 Research Contract holders from Africa, 3 Technical Contract holders from Italy and the update on the backstopping of research carried out at the IAEA Laboratories, Seibersdorf. The reports, and conclusions and recommendations made by the participants are presented in this publication.

This publication has been edited by the Scientific Secretary, Dr. B.S. Ahloowalia. The retyping of the manuscripts was undertaken by Ms. Daniela Panzenboeck and Ms. Katayon Entekhabi without whose patience and diligence, this publication would be still sitting in the files.

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INTRODUCTION

Self-sufficiency in food production in many of the African countries is a challenge to the modern technology and the agricultural scientists. The use of improved varieties of crop cultivars is a small but an important factor in the effort to sustain food production. Seed technology is the easiest and the cheapest of all technologies to transfer to the farmer. The transfer of this technology is often self-sustaining through seed exchange between the farmers where seed multiplication and distribution does not either exist or is marginal. The upgrading of local and well adapted cultivars by breeding genotypes with short height, early maturity, drought tolerance or disease-resistance, can increase the yield and quality. Induction and selection of mutations in such local cultivars offers a simple, efficient, rapid and cheap method to alter the genetic make-up, and obtain desired genotypes without disrupting genomes of ecotypes otherwise well-adapted to the edaphic and agro-climatic conditions. Hence, a Research Co-ordinated Programme on the "Improvement of basic food crops in Africa through plant breeding including the use of induced mutations", was established to improve a number of local crop varieties.

At the Third Research Co-ordination Meeting, several progress reports were presented. Of the crops targeted for improvement, in most cases, progress in research was excellent, and counterpart scientists obtained promising results in the improvement of cassava, yam, sweet potato, plantain, sorghum and African rice. The *in vitro* culture of a number of vegetatively propagated crops in combination with radiation induced mutations proved to be a valuable method to produce desired variation and rapid propagation of the selected mutants and parental material in disease-free condition for field evaluation. This method has proven to be particularly important for the improvement of plantain, cassava and sweet potato.

In Mali several stable mutants with short culm, improved grain quality and drought tolerance have been obtained in sorghum. Some of them have been evaluated for morphological, physiological and biochemical characteristics. The drought tolerant line has very deep rooting system. In the M_5 populations of African rice (*Oryza glaberrima*), the lines selected for non-shattering were highly sterile. However, white caryopsis mutants were found. Cultivars with white grain have a higher market value than the normal red grain types.

In Ghana, the improvement of cassava cooking quality through mutation breeding was reported. Of the 37 putative mutants selected for improved cooking quality, most compared with the parental cultivar in yield. A significant improvement was achieved in the protocol to harden *in vitro* cultured cassava plants for transfer into soil, and their establishment in the field. Studies were initiated to develop protocol on somatic embryogenesis in the African cassava clones. The project on the improvement of plantain (*Musa* spp.) and bambara groundnut (*Voandzea subterranea*) using mutation breeding techniques has progressed well. In plantain, 256 M_1V_4 selected plants were grown along with the controls to evaluate short height and resistance of leaves and stems to wind damage. In addition, micropropagated plants have been grown in field trials at 2 locations to test their uniformity and stability. In Bambara groundnut, experiments with limited material but high radiation doses (300 and 350 Gy) produced highly aberrant type plants. Several local cultivars have been collected from the local growers to provide wide genetic diversity in the experimental material.

In Nigeria, 10,000 M_1V_3 plants of yams were tested and showed wide variation. Of these, 110 high yielding clones were selected for preliminary yield evaluation. It is proposed to test the putative mutants in large scale field trials at two locations and to multiply nodal cuttings *in vitro*. In cassava, most of the tissue cultured material was lost through contamination. New material has been initiated from stem cuttings and from *in vitro* cultured material.

In Congo, the project on the control of rust disease in baselle through the creation of rust resistant or tolerant mutants progressed extremely well in the short duration. The vegetatively propagated variety was established in microculture and irradiated. The doses of 150-200 Gy were found to be optimal for the irradiation of tissue cultured material. Similar studies have been planned with the seed propagated varieties.

In Kenya, seeds of local cowpea varieties were irradiated, but this project did not progress beyond growing M_1 material. In pigeonpea screening for resistance to diseases and insect pests was continued. Irradiated material, which had originated from unadapted germplasm, had been grown to M_4 populations, however, without any positive gains.

In Uganda, research on *in vitro* culture was initiated to obtain virus-free plants of cassava, and cultures were established. In addition, research has been initiated on sweet potato which has similar problems of disease infection with viruses. Local sweet potato cultivars have been established in tissue culture, but the problem of bacterial contamination still persists in sweet potato cultures.

In Cote d'Ivoire, attempts were made to develop an *in vitro* culture technique for yam but were not successful; hence there was no progress in the project.

Under the Technical Contracts, all reports indicated an excellent progress. For the genetic improvement of root and tuber crops, a protocol was developed for somatic embryogenesis in sweet potato to establish methods for *in vitro* irradiation. In potato, the response to artificial inoculation with *Phytophthora infestans* in clones insensitive to fungal culture filtrate was studied. Clones were selected for insensitivity to culture filtrates. Protocols have been developed for *in vitro* selection for tolerance to late blight and this proved to be stable in the glasshouse tests.

In chickpeas, studies were carried out to transfer insect resistance genes (*a*-amylase and proteinase inhibitors) by co-culture of meristematic cells with virulent and hyper-virulent *Agrobacterium tumefaciens* strains. The frequency of explants showing the expression of GUS (β-glucuronidase) ranged from 3 to 15%, but no transformed shoots were obtained. In cowpea, regeneration and transfer of genes expressing insect-resistance proteins (*a*-amylase and proteinase inhibitors) was investigated. Regeneration was obtained in the local and African cultivars from explants of immature leaflets and hypocotyl segments from mature embryos. The regeneration frequency ranged between 0.8 to 17.1%. Histochemical studies showed that regeneration initiated form the epidermal cells. Genetic transformation was obtained by electro-injection of the plasmid DNA directly into the shoot meristem, in which transient gene

expression was observed. After co-cultivation of hypocotyl segments with two strains of *A. tumefaciens*, 5 to 82% transformed tissue with stable expression of GUS gene were obtained. However, no transgenic shoots were obtained.

In the FAO/IAEA Laboratories, Seibersdorf, Austria, studies were continued on peroxidase and chitinases to develop a selection system for resistance to *Fusarium* in *Musa* spp. Banana clones, which show varying degree of resistance to *Fusarium oxysporum sp. cubense*, were analysed for peroxidase activity after inoculation. The highest constitutive enzyme activity for peroxidases was found in the resistant clones. All clones of banana and plantain screened for the presence of constitutive chitinases showed the presence of at least two to three bands in the electro-isofocusing analysis.

In vitro culture of African cassava cultivars was undertaken to compare sensitivity to irradiation in acute and fractioned doses of gamma rays of 15, 30, 45, and 60 Gy. Radiation damage increased with the dose, and splitting an acute dose into fractions gave better recovery. Explants from the shoot tips were more sensitive to gamma rays than the first or second node buds at all doses tested. Backup activity to the project was also provided by irradiating samples of different crops such as sorghum, cowpea, rice, groundnut, kidney beans, soybeans, sunflower, maize and cotton from Africa.

The participants suggested that there was need to promote the exchange of mutant germplasm produced by various research groups. Investigations on the biochemical characters for consumer acceptance and yield stability were required before the release of mutants. The continued support for equipment, training and visits was essential to implement the programme.

Mr. S.B. King, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Nairobi, suggested the possible future collaboration between ICRISAT and Co-ordinated Research Programme. He emphasized the need to breed for insect resistance (pod borer and pod fly) in pigeonpea by using transgenic and mutation techniques as was being carried out under the present research programme. Conventional breeding has had little or no success in this area. He suggested the role of exchange of mutant germplasm for evaluation in other parts of Africa and other regions, and to induce male sterility in finger millet (*Eleusine coracana*) and its evaluation, and to produce pigmented markers in finger millet to identify cultivated types from the wild weedy types, and investigate the role of pigeonpea mutants in sustaining soil fertility through biological nitrogen fixation and crop rotation.

List of Publications

O. SAFO-KANTANKA, OWUSU, J. (1992) Cassava varietal screening for cooking quality: relationship between dry matter, starch content, mealiness and certain microscopic observations of the raw and cooked tuber. J. Sci Food Agric. 60:99-104.

G.Y.P. KLU. (1993) Induced dwarf-type mutant of yam, *Dioscorea rotundata* Poir. Trop. Agric. (Trinidad) 70: 289-290.

OPENING ADDRESS

HAROLD L. NORTON
Resident Representative
Food and Agriculture Organization of the United Nations
Nairobi, Kenya

Mr. Chairman, Distinguished guests, Participants, Ladies and Gentlemen,

I welcome you on behalf of the sponsoring Organizations of FAO and IAEA to the Third Research Co-ordination Meeting on the Improvement of Basic Food Crops in Africa under the auspices of FAO/IAEA/ITALY project.

I am also grateful to the Government of Kenya for hosting this meeting and to the organizers, particularly the Director, Kenya Agricultural Research Institute (KARI) and his staff members who have made all the arrangements for this meeting.

As you are aware, the First Human Right is the Right to Food, which is under threat because of increasing human population and shrinking land resources. In many lands, it is compounded with political unrest making basic food a luxury item. Hence, the problems of food production and food security in some parts of Africa are even more critical than the rest of the world.

As it is well known to you, plants such as cassava, banana, plantain, sweet potato, yams, sorghum, African rice, legumes such as cowpea, pigeon pea, and chickpea are among the most important food crops in African agriculture. Many of these plants were domesticated by the African farmers since the dawn of agriculture. Some of these plants are tolerant to stress and well suited to the eco-climatic zones of Africa, and are the main source of food in the native diet of millions of African people. These crops have not received the same attention in improvement and breeding as given to wheat, maize, rice and potato in other parts of the world. However, both the national and international organizations have become increasingly aware that the solution to food shortages in Africa and indeed in rest of the developing world, is not in importing food, but in producing home grown crops. This can be achieved by making available improved cultivars and seeds of local crops, and by growing such cultivars with optimal agronomic inputs. This approach has proven to be successful worldwide, and plant breeding has left an imprint on the Green revolution in South East Asia which was based on improved high yielding, short height, photoperiod insensitive, short duration wheat and rice cultivars. Hence, the Joint FAO/IAEA/ITALY project was initiated to improve the basic food crops of Africa for increased productivity under stress for disease and insect resistance, increased yield and improved quality.

Mutation breeding offers several advantages in plant improvement by upgrading a specific character without disrupting the original genetic make-up of the cultivar, e.g. short height or early maturity in wheat and sorghum, or white grains from red/brown in rice or non-climbing habit in some legumes. In that sense, it provides a fast and rapid method to improve local crop varieties, without going through extensive hybridization and back-crossing used in the conventional breeding.

This project has attempted to develop links and to share know-how in the methods of mutation breeding in the African region and to interact with the International Agencies and Institutions. It has set targets to strengthen the research on plant breeding, genetics and tissue culture techniques in the national institutions participating in the program. It has provided a forum for the flow of information on technology of plant breeding among the participants who share the common goal of producing more productive and better yielding crop varieties. We hope that their efforts will be rewarded through increased food production and thereby better living standards and health of their nations.

It is now well recognized that the increased food production is linked to research and technology which must reach from laboratory to land. This requires dedicated teams of trained scientists and extension personnel. By promoting research and development, this Joint FAO/IAEA/ITALY project has tried to support the national requirements for carrying out research by providing equipment, training personnel and experts.

The project has resulted in the production of useful germplasm some of which could be used either directly as new varieties or incorporated into the new varieties by conventional back-crossing. Promising genotypes have been isolated in sorghum, African rice and legumes. In vegetatively propagated crops, cassava, plantain and sweet potato, several genotypes were produced by irradiation of *in vitro* propagated material. Large scale experiments were performed with advanced generation mutants of sorghum with short height and high yield. Promising mutants were also isolated from irradiated populations of *Oryza glaberrima*, the wild African rice, which normally has short, red grains. Some of the isolated mutants have white and thin long grains, which are important traits in domestication of this wild species. These desirable characters can also be introduced in rice germplasm, adapted to the local eco-climatic conditions.

In Kenya, populations of pigeon pea have been evaluated for drought tolerance, plant height, early ripening, insect tolerance of the harvested pods, and disease resistance.

In Ghana, a suitable protocol has been developed for micropropagation of the local varieties of plantain and irradiation of the *in vitro* material. Likewise, in Nigeria, *in vitro* culture of yam has been carried out for irradiation.

The research carried out by the Italian participants has provided critical information on the starch quality of African food crops. The study of grain quality of sorghum seeds has shown that there is a large variability among the sorghum varieties for lipid and amylose content, cold water binding capacity, swelling power value and visco-amylographic properties. Likewise, cooking quality of cassava tubers of several African cultivars and early generation mutant populations has been studied in Ghana and Italy in cooperative research studies which have shown that starch granule size, which determines starch mealiness, can be modified by mutation induction.

The Italian Counterparts carried out *in vitro* culture of sweet potato clones from Africa, and have determined the optimal radiation dose which has given a high frequency of mutant types in the subsequent propagations. They have also shown that it is possible to insert new genes in chickpea and cowpea by using recombinant DNA techniques.

International cooperation in the project has also been provided by the IAEA Laboratories, Seibersdorf in training, lectures, and development of techniques for *in vitro* induction and isolation of mutants for disease resistance in banana and cassava. Irradiation services were provided by treating 13 different grain crops from ten African countries. The second phase of the project may also include in future to initiate exchange of germplasm for national and regional trials.

So, Ladies and Gentlemen, this morning, you may be able to appreciate the small but important contribution of this project towards increasing the food quality and quantity in Africa. You would be able to hear a lot more of the technical aspects in the scientific sessions during the next few days.

May I conclude by wishing all the participants a very successful meeting.

WELCOME ADDRESS

B.S. AHLOOWALIA
Scientific Secretary
Joint FAO/IAEA Division of Nuclear Techniques in Agriculture
International Atomic Energy Agency
Vienna, Austria

Mr. Chairman, Dr. Norton and Gentlemen,

I wish to welcome you all on behalf of the sponsoring Organizations of Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency to the Third Research Co-ordination Meeting on the Improvement of Basic Food Crops in Africa under the auspices of FAO/IAEA/Italy project.

Research and development and application of technology are essential to sustain increase in food production. However, regional problems have regional solutions. Where as some of the technology can be transferred without reinventing it, most of the borrowed technology in agriculture has to be modified and reshaped to adapt to the needs of each region. This is particularly true in the improvement and breeding of new varieties which must be selected at the local level to fit in with the agronomic practices of the region.

Crops such as cassava, banana, plantain, sweet potato, yams, sorghum, African rice, cowpea, pigeon pea, and chickpea are important for African agriculture. Most of these crops have been grown for centuries by the local farmers and are well adapted to the climatic, edaphic and biotic conditions. These crops need to be improved by breeding cultivars with early maturity and high yield, improved quality, resistance to diseases and insect pests, and tolerance to stress. The impact of Green revolution in South East Asia is well known. This was based on breeding high yielding, short duration wheat and rice cultivars. A similar approach is now required to enhance the productivity of the local African crops. We hope that the Joint FAO/IAEA/Italy project shall help to achieve this goal by changing the genetic make-up of the local crops by producing useful and valuable mutants of the local cultivars and land races, and by incorporating these in the conventional plant breeding through hybridization and selection. In this meeting, progress reports of the participants shall be discussed, and conclusions shall be drawn to formulate recommendations for future action.

May I conclude by thanking the Government of Kenya for hosting this meeting and the organizers, particularly Dr. Rono, Kenya Agricultural Research Institute, Machakos, and his colleagues for making all the local arrangements for this meeting.

I thank you for your attention.

AN OVERVIEW OF FAO'S FOOD CROP DEVELOPMENT PROGRAMME FOR AFRICA - A PLANT BREEDER'S PERSPECTIVE

E.A. KUENEMAN
Field Food Crops Group
Plant Production and Protection Division
FAO, Rome

1. INTRODUCTION

My colleagues at Joint Division FAO/IAEA in Vienna contacted me last month and asked me if I would be able to attend this Research Co-ordination Meeting and suggested that I speak to you in the opening address about the role of plant breeding in sustainable development of Africa. Last week I understood that what was intended was, in fact, a more general description of FAO's activities in Africa. The scope of this topic is really beyond my level of institutional responsibility. I will, however, attempt to give you an overview of some of the thrusts and philosophies of FAO's activities in Africa related to Sustainable Development, and I will summarize some of the projects of the Crops and Grassland Service of the Plant Production and Protection Division with the view to give you flavor of what FAO is doing in projects directly focused to assist its Member Countries in Africa in crop production.

2. FAO AND RESEARCH

This Steering Committee Meeting principally involves scientists and I would like to say a few words about what FAO is and about what it is not, *vis-a-vis*, research and development. While FAO employs some scientists for posts at headquarters, in its laboratory at Seibersdorf in Austria and in its field projects, *FAO is not, in the conventional sense, a research organization. FAO is a development institution in the broadest possible sense of the word*, which since its conception in 1945, has had, *inter alia*, the mandate to assist and advise member countries on agricultural research, and to "promote and recommend national and international action with respect to scientific, technological, social and economic research relating to nutrition, food, and agriculture[1]". In this presentation, I will later highlight some of the crop-oriented, research- related activities of FAO currently ongoing in Africa.

FAO assists its member nations providing information on matters ranging from: remote sensing, to projections on food availability to land-use-planning to extension to crop and animal production methodologies (including variety development and germplasm preservation) to marketing and processing to nutrition needs and policies. FAO is also a forum where member nations can present different opinions on regional and global needs as they relate to food, agriculture and sustainable development.

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[1] *Source: Article I of FAO's Constitution*

As it was not possible to have this text reviewed prior to this presentation, it must be clearly understood that the text does not necessarily reflect FAO's official position.

FAO is not a bank or a granting institution. Its core budget covers its vast Regular Programme of Work that is approved by FAO's Conference every two years. From this FAO has a small financial resource base to catalyze some country or region-specific development activities. The great majority of funds which FAO uses for direct country assistance are from multi-national donors such as UNDP and World Bank and from bilateral "trust fund' projects where funds from a donor country are ear-marked for assistance in another country(ies), under the guidance of FAO. This Resource Coordination Meeting is a product of just such a trust fund project sponsored by the Government of Italy.

FAO operates under great pressure. Prior to, but especially following the United Nations Conference on Environment and Development (UNCED) held in Rio de Janeiro on 1-12 June 1992, there is a growing awareness that we must find a means to feed and provide the other basic needs for 8.5 billion human beings that are expected to populate the planet by the year 2025. This is about 3 billion more people than now inhabit the planet and their needs will have to be met from a natural resource base which is already seriously threatened by unsustainable farming practices and environmental pressures arising from other human activities (Den Bosch Declaration, April 1991). By year 2025 more than 80% of the population will be living in developing countries. Thus, affordable protein and energy rich foods will be in demand in the future and consequently the role of the plant breeder will be pivotal in optimizing production on a finite land resource.

The current situation and trend of agriculture in Africa[2] are:

1. While sub-Saharan Africa has shown a reasonable long-term growth of nearly 3% in agriculture production, the problem in the region is the population growth which has outstripped the growth in production. Many countries in Africa are showing a decrease in per capita food production and the forecast for 1994-95 is not much improved.

2. The total debt was more than $183 billion in 1992 and exceeds the 1992 annual GDP by 6%. Interest arrears on long-term debt alone were $14 billion. Nearly one-fifth of the hard-earned export revenue was used to service debt in 1992.

3. External assistance flows to agriculture have tended to decline in real terms in recent years. World Bank commitments for 1992 were 19 percent lower. Bilateral aid has also declined during the last 5 years. The conclusion, in part, is that countries will need to take greater financial responsibility for agriculture development in the future if this trend continues.

4. Sub-Saharan Africa continues to be a major recipient of food aid. As much as 60% of the food aid to this region is intended to meet emergency needs of refugees and displaced persons and needy population in drought-affected countries.

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[2]*This section was deawn from FAO's Publication, "The State of Food and Agriculture 1993"*

Crop production activities in Africa

The following is not meant to be a complete list of current activities, and the examples are only extracted from FAO's Group at Headquarters focusing on Food Crop Production; related activities in extension, protection, marketing, and in the Joint FAO/IAEA/ITALY are numerous, but not provided herein. The examples provided are organized under various themes (focal elements).

Focal Element 1.Technology adaptation, verification and demonstration

1.1. A Vietnamese rice husk stove was introduced to three Sub-Saharan countries in Africa, namely, Burkina Faso, Mali, and Senegal in 1991. In 1992, the stove was promoted and locally built stoves are being widely adopted and as a result people have access to a cheap source of fuel for cooking and consequently less fuel wood was used. This initiative is a product of a Regular Programme thrust called 'Thriving Rice' technology, where all aspects and all components of the rice plant are exploited for the benefit of the rice farmer and consumer. Currently our 'Thriving Rice' initiatives in Africa are receiving additional financial support from a Japan-funded trust project. Other aspects of the 'Thriving Rice' package include the introduction of new cone-type weeders, new single animal-pulled plows, 8-tooth harrow, Hampasan thresher, legume rotations, introduction of Azolla algae to provide part of the rice plant's nitrogen requirement, etc.

1.2. Following the success of our 'Thriving Rice' initiative a Maize version, (Thriving Maize Technologies) is being contemplated. Studies were initiated in 1992 to explore what components of maize and maize-based production systems in Africa could be enhanced to optimize returns to African maize farmers.

1.3. Support was given to the national programme of Rwanda to conduct on-farm demonstrations of improved wheat production practices; in addition, an information bulletin for extension persons and farmers was prepared and distributed.

1.4. On-farm cowpea/cotton inter-cropping trials were established in Zambia and Malawi to determine if the routine insecticide sprays applied to cotton would provide protection to the companion legume crop, which if not protected, is devastated by pod-sucking pests. The legume crop's contribution to the cotton crop in terms of biologically fixed nitrogen is also being assessed. Trials established in 1991 were lost due to the regional drought; the 1992 trials were established in December; results are pending. Similar trials will be initiated in Nigeria and Ghana in 1993.

1.5. A previous (1990) Regular Programme initiative to promote the coupling of improved small-holder crop production with appropriate home and village-level processing is beginning to bear fruit. UNDP has funded in 1992 a FAO-promoted project on small farmer soybean production and utilization in the Philippines. FAO will continue its technical involvement through a new TSS-2 arrangement. Models for Nigeria, Cameroon, Ivory Coast and Ghana are still under government and UNDP deliberations.

Focal Element 2. Promotion of research and development networks

2.1. A global research and development network on tropical and sub-tropical soybeans has been under consideration and formulation since 1989. Regional planning workshops were held in Africa, Asia, and in Latin America; the regional workshop for Asia took place in 1992, and draft project documents for all three regional sub-networks and the global coordination center were prepared and submitted for donor consideration.

2.2. Thriving Rice Network for West Africa (Guinea, Senegal, Mali, Burkina Faso) was established through the Regular Programme. Funding support now comes from a Japanese Trust Fund. FAO staff provide technical guidance (See section 1.1 of this report for more information). A regional workshop on this activity was held in Mali in November 1992 to exchange information and experiences among experts, national staff and farmers.

2.3. FAO contributed both financially (contract LOA) and through advice to the steering committee for the new 'International Research Network on Drought Tolerance in Legumes' that is under the initial co-sponsorship of ICRISAT and ICARDA.

2.4. FAO held several discussions in 1992 with the staff and management of ICARDA on the promotion of winter-sown chickpea technology package in the WANA region. As a result, a joint FAO/ICARDA formulation mission is being sent in 1993 to 5 countries to develop a document to procure funding to accelerate demonstrations of the new technology package(s) to farmers.

Focal Element 3. Support to Governments on planning (including formulation) and policy analysis

3.1. Support was provided to assist in the formulation of various food crop projects in Africa such as a. For West Africa a formulation mission was sent to develop a trust fund project with the view to transfer new technologies to maximize the benefits maize-based production systems under the theme "Thriving Maize Technologies" (see section 1.2 above). 2. Integrated Production Systems: Food and Oilseed Legume Improvement for Zambia.3. Crop Production Research in Malawi and in Mozambique. 4. Hybrid Rice Development -A Network for Mediterranean Climate Rice, including North Africa. (PRODOC for consideration of France).

3.2. The Governments of Kenya, Zambia and Zimbabwe were assisted in assessing the production potentials and constraints related to root crop production. The consultants' mission formulated proposals for the development of these crops in each country.

Focal Element 4. Promotion of agro-ecosystem based research and development

4.1. Numerous discussions were held with various FAO divisions and with IITA on the organization of a special think-tank workshop designed to develop a coherent approach to sustainable development of the favorable savannas of Africa. The workshop, to be co-sponsored by FAO and IITA is scheduled to be held at IITA in August 1993.

Within the favorable African savannas, major agro-ecozones such as the lowland hydromorphics and the derived savannas will receive special emphasis.

Focal Element 5. Promotion of strategic crop research

5.1. In December 1991, FAO organized an expert consultation to review the biochemistry and genetics of post-harvest deterioration of cassava to see if modern molecular methods (biotechnology) could develop varieties with improved storability. In 1992 follow-up, FAO has provided a contract to produce proceedings of the expert consultation with a thorough review of what is currently known. A consultant contract was provided to develop the first draft of a proposal for a multi-laboratory integrated research programme to clarify the biochemistry, develop the molecular probes and libraries and to transform cassava germplasm with the view to enhance its post-harvest storability. Staff attended the first technical workshop of the International Cassava Biotechnology Network where this initiative was discussed extensively. By mid 1993, the multi-institutional research proposal should be finalized and plans for a joint CIAT/FAO presentation be made to UNDP.

5.2. A unique phosphorous uptake mechanism in pigeonpea was identified by Japanese scientists working at ICRISAT in 1991. By root excretion of pisidic acid this crop is able to extract phosphate from the iron-bound phosphate pool, not normally available to crop plants; the Fe-P is the dominant form of soil P in several major tropical soils. A joint FAO/ICRISAT expert panel is shall explore the possible application of molecular genetics to incorporate this characteristic to other crop plants. This could be as important as BNF in sustainable production systems in the future.

5.3. In a few plants, the embryonic tissue of a seed that develops into the new plant, comes from somatic cells instead of a fertilized egg. These somatic apomictic seeds have the exact genetic constitution of the plant that produced the seed. It is probable that apomictic reproduction will be genetically incorporated in many crop plants in the next two decades. The implications of controlled apomixis in crop production will be far reaching. For example, farmers will be able to keep their own hybrid seed. The FFCG began discussions internally and with other research and development institutions on the need to organize a high-level workshop to examine the probable effects (positive and negative) on agriculture of the widespread application of apomictic reproduction. The feeling is that if we are more proactive than reactive, we might be able to avoid some of the negative consequences that come from any rapid change, e.g., the green revolution.

Focal Element 6. Support to international commissions, congresses and conferences

6.1. The Secretariat for the International Rice Commission is located within FAO/AGPC and in 1992 Regular Programme resources were used for the preparation and publication of the IRC Newsletter vol. 40 (1992). Regular Programme funds were also used for an author's contract to prepare a keynote paper for the IRC newsletter.

6.2. The IRC Steering Committee was convened in 1992 to discuss alternative uses of the marginal ecologies (deep water and mangrove rice). Themes for the 18th Session of the IRC to be held in Italy 1994 were also reviewed.

6.3. Funding support and advice was provided to the organizers of the International Food Legume Conference II, held in April 1992, Cairo. FAO staff have been requested to be on the steering committee for the next conference that will be held in 1997.

Focal Element 7. Support to externally- funded field projects

The FFCG provided the lead technical coordination for about 35 field projects in 1992 with involvement of 32 professional field staff. These projects varied from regional projects such as the UNDP-funded, FAO-implemented Asian Network on Coarse Grains and Food Legumes involving 14 countries, the Mediterranean Climate Rice Research Network involving 12 countries, the Asian and Pacific Root Crops Development project involving 11 countries to projects that give specific input to strengthen a given national programme, such as support to applied agricultural research in Mozambique, where a team of 5 international experts have assisted the Government to establish a crop research agenda, protocols, and have trained local staff on maize, cassava, food legume, and rice farming systems, and supported national projects executed by the government but with technical assistance from FAO to ensure sound implementation. An example can be found in support for accelerated small-holder soybean production and processing in the Philippines. In addition to the 35 projects referred to above, the FFCG is involved in more than 40 other projects in which it is not the designated lead, but nevertheless, reports are read, comments provided and meetings attended.

Focal Element 8. Information/Publications

8.1. A new book on soybean production in the tropics is being published. The book was badly needed in that many significant breakthroughs on the production of the crop in the tropics have taken place since the last FAO publication of 1982. The new book has been prepared by the scientists of the Soybean Research Center, Brazil under a letter of agreement between FAO and EMBRAPA. The English version should be printed in February 1993; Spanish and French versions will be published soon.

8.2. A 'manual on the methods and merits of transplanted maize technology' is under preparation.

8.3. A 'manual on use of plastic mulch to enhance maize production' is under preparation.

8.4. The proceedings of the Expert Consultation on Post Harvest Deterioration in Cassava, held at FAO, HQs, Rome, are under preparation.

8.5. The International Rice Commission Newsletter vol. 40 was produced and distributed in 1992.

African science to solve African problems

It has become abundantly clear that the onus of sustainable development in Africa rests on the shoulders of Africans. The international development community has a role to play as facilitator. For example, it is the objective of this project to assist African plant breeders to acquire and utilize useful technologies in breeding food crops for traits where conventional cross-breeding methods are not the methods of choice. Already some of the participants are demonstrating their mastery of these technologies through creative vision and hard work, which is always required in plant breeding, realizing impressive progress in the development of germplasm that will be most useful to farmers when it ultimately reaches them in the form of improved varieties. In two or three cases, on-farm tests are already underway.

On behalf of the Director General of FAO, I wish to express FAO's appreciation for your participation in this Co-ordinated Research Programme and in this meeting. I wish you success in your deliberations, and look forward to learn from you about your research programmed and to explore with you how new developments can be accelerated and how they can be effectively transferred to farmers.

INDUCED MUTATION BREEDING IN CASSAVA (*Manihot esculenta* Crantz) CULTIVAR 'Bosom Nsia'

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1. INTRODUCTION

Cassava is one of the most important staple food crops in the lowland tropics. In most cassava producing countries, it is mainly utilized for human consumption. It is used for 'fufu', 'banku', 'yakayake', and dried for use. Cassava remains a valuable source of energy and is cheaper in economic terms than many alternative foods. Cassava leaves are a good source of protein and vitamins, and are used as food in Africa [4]. In Ghana, 'Bosom Nsia' is one of the most widely grown cultivars probably because of its good cooking quality and fast maturation in six months. However, this cultivar is highly susceptible to cassava mosaic virus disease (CMV), hence the need to improve its resistance to the disease.

Various *in vitro* techniques have been developed for cassava research [1, 2, 5, 6]. Klu and Lamptey [3] reported irradiation doses of 25 and 30 Gy to be ideal for *in vitro* mutagenesis of cassava. These doses were applied to *in vivo* and *in vitro* mutation for breeding CMV resistance in the cultivar 'Bosom Nsia'.

2. MATERIALS AND METHODS

2.1. *In vitro* techniques

Two batches of 150 shoot tips each were irradiated with 25 and 30 Gy gamma rays.. Meristems were isolated and cultured on a two-stage media. Plantlets (M_1V_1) were hardened and transferred to field conditions for selection. Preliminary studies were made to generate somatic embryos from meristematic tissues and young leaf-lobes.

2.2. *In vivo* techniques

Three batches of 350 stakes of cassava, 15 cm long with about 20 axillary buds were irradiated at 20, 25 and 30 Gy, and planted in a field to observe and select resistant variants. Eleven local cultivars were collected for future studies.

A system based on 0-9 score was used to assess disease incidence. 0 = no symptoms; 2 = 1/4 of plant showed symptoms; 4 = 1/2 of plant showed symptoms; 6 = 3/4 of plant showed symptoms; 8 = whole plant infected; 9 = dead plant.

3. RESULTS AND DISCUSSIONS

Field performance of plants in relation to disease incidence is as shown:

	In vitro		*In vivo*	
Irradiation dose (Gy)	(25)	30	(20)	25 30
Score	No. of plants	% plant population	No. of plants	% plant population
0	-	-	-	-
2	(1) 2	(0.47) 0.68	-	-
4	(6) 9	(2.87) 3.0	-	-
6	(36) 44	(17.22) 15.0	(14) 10 6	(5.3) 3.9 3.14
8	(88) 163	(42.10) 55.82	(162) 110 64	(61.8) 43.833.50
9	(61) 52	(29.18) 17.80	(53) 56 68	(20.2)22.3135.50
10	(17) 22	(4.7) 7.5	(33) 75 53	(12.59) 29.88 27.74

Preliminary studies on cassava somatic embryogenesis using meristem and adaxial portions of young leaf-lobes showed that both explants have the potential of somatic embryogenesis, but meristem explants were found to be more consistent in generating somatic embryos.

4. CONCLUSIONS

In vitro mutagenesis seems to have a better potential for generating resistant or tolerant variants than *in vivo* mutagenesis. Other aspects of *in vitro* techniques need to be exploited in the search for a genotype which would be resistant to CMV. Irradiated somatic embryos are potential explants that could generate the desired genotypes.

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MICROPROPAGATION OF VEGETATIVELY PROPAGATED CROPS

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Abstract

Crops such as banana, plantain, cassava, potato, sweet potato and sugarcane are conventionally propagated from corms, stem cuttings, tubers and roots. Many of these plants do not produce seed, and when they do, the progeny does not resemble the parent. It is possible to multiply these plants through micropropagation. As a result of the *in vitro* culture conditions, the plants multiply rapidly, in a small space, short duration and disease-free situation, and produce clones - exact genetic copies of the original plant. For example, in potato, a 10 mm long stem cutting can produce a complete plant with roots and a shoot with three leaves within one week of culture. This plant when cultured again can give rise to 3 plants in one week. If a plant doubles once a week, then repeating the process 20 times can produce 1.06 million plants in 21 weeks. Such plants, when grown *in vitro* for an extended duration, produce microtubers, ca. 2 to 10 mm diameter. Alternatively, 6 to 8 week-old micropropagated plants, transferred into soil, produce minitubers ca. 5 to 25 mm diameter after 65 to 80 days of growth. Micro- and mini-tubers can then be grown as Super Elite, Elite seed tubers, and used for the production of Certified seed tubers. A modular system of micropropagation has been developed for seed tuber production by using disposable plastic containers in which the container can be used to grow the plants to maturity. Using this system, large scale production can be undertaken by establishing low cost micropropagation units. This system can also be adapted for the micropropagation of other vegetatively propagated plants and *in vitro* irradiation of propagules. It is also possible to produce complete plants from single plant cells through a different but related process - regeneration. In this process, cells are cultured on several complex media and in a step-wise manner. In many crops, plants can be regenerated from cells by producing somatic embryos. These embryos are similar to those formed in seeds, except they originate from vegetative rather then reproductive cells. During this process, the cells grow first into a callus, which on further culture produces complete plants. Often, some of the plants produced from the callus differ from the donor plants from which cells were taken - a phenomenon called somaclonal variation. The technology of cell and tissue culture has wide implications in the production of new and improved cultivars of vegetatively propagated plants, induction of mutants, their rapid multiplication, and distribution as disease-free propagules to the growers and farmers.

IMPROVEMENT OF PLANTAIN, *Musa* spp. USING MUTATION BREEDING TECHNIQUES

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Abstract

Tissue culture regenerated plants of 'Apantu' and 'Asamienu' varieties of plantain were acutely irradiated using gama rays at 0, 30 and 40 Gy, rooted in soil, and transferred to a field for agronomic evaluation and selection. A proposal to select dwarf mutants is reported.

1. INTRODUCTION

Plantain is a major staple crop and a cheap source of energy in the diet of Ghanaians. It is cultivated throughout the forest zones and as a starchy food is superseded only by cassava in terms of acreage covered. Cultivation is usually carried out by small-scale farmers, who inter-crop it with cocoa and coffee or maize and cowpea. Large scale production in pure stands has recently started in response to governments' policy aimed at increased production of "non-traditional crops" for export. The preferred types are varieties derived from the 'French', 'False Horn' and 'Horn' plantains, called 'Apem', 'Apantu' and 'Asamienu', respectively. Two major problems associated with their production are the lack of planting materials for establishing new farms and wind damage during unfavourable weather conditions [1], which usually follows drought.

As established methods of vegetative propagation of plantain are slow and hybridization is impracticable, efforts at solving these problems have relied on the combined use of micropropagation and *in vitro* mutagenesis [2,3]. Modified protocols, reported elsewhere [4], were used to establish reliable procedure for rapid multiplication. Radio-sensitivity tests were also conducted to determine a suitable dose for mutation breeding work. This paper reports on the field performance of tissue culture regenerated plants of 'Apantu' and 'Asamienu' following acute irradiation.

2. MATERIALS AND METHODS

Approximately, 65 plants from each treatment (0, 30, 40 Gy) of the two varieties were planted in a field following acute irradiation and serial sub-culture to M_1V_4 *in vitro*. The plants were weaned in a step-wise manner in artificial rooting medium (perlite) and then in a sandy-loam soil for duration of two weeks and five months, respectively. They were planted in the field on 21/6/93 along with "control material" consisting of suckers of approximately the same size excised directly from mother corms growing in multiplication plots of the University of Ghana Agricultural Research Station, Kade. The trial was laid out in replications using the Randomized Complete Block design. There were four replicates per treatment.

3. RESULTS AND DISCUSSION

Morphological characteristics of plants at time of planting are presented in Table I. There was not sufficient data to identify aberrant types or selecting dwarf mutants. It is, therefore, proposed to take records on plant morphological characteristics till fruiting.

TABLE I. MORPHOLOGICAL CHARACTERISTICS OF FIELD GROWN PLANTS

Variety	Dose (Gy)	Mean number of leaves/plant	Mean plant weight (cm)	Remarks
APANTU	0	16	142.0	Vigorous
	30	14	132.0	Vigorous
	40	14	132.0	Vigorous
	Control	13	134.0	Vigorous
ASAMIENU	0	17	148.0	Vigorous
	30	14	125.0	Yellowish Leaves
	40	15	137.0	Vigorous
	Control	15	130.0	Vigorous

Further data collection will include the following characters: 1. Number of months to flowering; 2. Mean height at flowering; 3. Number of effective photosynthetic leaves (green) at flowering; 4. Mean length of leaf petiole; 5. Number of months to fruit maturity; 6. Number of hands per bunch; 7. Number of fingers per hand; 8. Bunch weight per plant. Two generations of ratoon crops (i.e. M_1V_5 and M_1V_6) shall be produced from these plants in the field and screened for dwarf habit, but without reduction in bunch weight. The desirable plants will be selected and multiplied as a new clone.

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IMPROVEMENT OF BAMBARA GROUNDNUT PRODUCTION USING INDUCED MUTATIONS

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1. INTRODUCTION

Bambara groundnut (*Vigna subterranea* (L.) Verdc. syn. *Voandzeia subterranea* (L.)) is a fairly drought-tolerant, tropical crop which grows well in hot dry regions with poor soils regarded as marginal for other pulses [4,5]. It is cultivated in the coastal and Northern regions of Ghana where the low yield (not exceeding 300kg/ha) and the long cooking time [6] have made it a subsistence crop. It ranks third after groundnut (*Arachis hypogaea* L.) and cowpea (*Vigna unguiculate* L.) as a source of plant protein in the diet of Ghanaians. The crop has indeterminate flowering and fruiting habit which contributes significantly to its poor yield [1,3,6]. Desirable variation for this trait is not available in the popularly cultivated varieties. Mutagenesis has, therefore, been attempted as a means of creating the desired variation from which determinate-flowering types would be obtained to improve yield.

Induction of variation in bambara groundnut using gamma radiation has been tried before [2,6]. However, no mutants with the desired determinate flowering habit and synchronous pod maturity were obtained. Instead, highly aberrant phenotypes (albina, xantha, crinkled leaf, vegetative, dwarf and sterile types) were obtained in field-tested M_3 plants. Reasons that may be attributed to these are the use of a limited number of genotypes and application of too high doses of gamma radiation.

A number of under-exploited landrace varieties of the crop exist, some of which probably harbour this rare but desirable trait. In view of the results obtained by earlier workers [2,6], there is need to embark on an extensive field exploration exercise to assemble the available germplasm for incorporation in the breeding programme.

The project is aimed at: i. conducting a nationwide exploration exercise to collect germplasm of bambara groundnut for agronomic evaluation with respect to flowering and fruiting characteristics and their effects on yield. ii. applying the technique of mutation induction to create variability (if this is not found in the germplasm. to be collected) from which mutants with determinate flowering and fruiting habit may be selected for use in breeding.

2. MATERIALS AND METHODS

The following workplan is proposed for this project:

First year:
Germplasm collection and agronomic evaluation:

A nation-wide germplasm collection programme will be carried out to assemble as many genotypes as possible. Seeds collected will be multiplied and planted in the field to study flowering and fruiting behaviour of the various genotypes and their effects on yield.

Second year:
Radiosensitivity test:

Four varieties exhibiting flowering and fruiting characteristics together with high yield will be selected for radiosensitivity test. For this, fifty seed lots of each will be irradiated at the following doses using gamma radiation: 0 (control), 100,150, 200, 250 and 300 Gy. Plant height at full expansion of leaves and survival at 30 days after planting will be used to determine germination and LD_{50} respectively. Useful doses for mutation induction in the four varieties will be chosen based on results obtained from this test.

Mutation induction:

Five thousand seeds per variety will be irradiated at the selected dose and planted in the field. Following flowering and fertilization, M_1 plants will be observed for signs of early maturity. The 10 most mature seeds will be harvested from each surviving plant and bulked as M_2 seed. The bulked seed will be sown in the field as M_2 generation.

Third year:
Selection of desirable aberrant types:

A phenotypic appraisal of M_2 plants will be made with regard to plant architecture, synchronous flowering and prolonged grain filling. Desirable plants will be harvested individually for progeny testing in M_2. Seeds of normal looking M_2 plants will also be harvested and sown in plant progeny rows as M_3. Data on flowering date and duration, maturity pattern and yield will be collected in M_3. Selection of desirable plants will be continued in this generation, and they will be tested further during the next two generations and compared with parent cultivars.

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USE OF MUTATION BREEDING FOR SORGHUM IMPROVEMENT IN MALI - THE CASE OF A DROUGHT TOLERANT MUTANT

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In Mali, sorghum is the second most important crop after pearl millet. Breeding of new varieties is important to increase sorghum production in Mali. The breeding program, based on crosse between local varieties (guinea type) and introduced caudatum types has been of limited success, hence all the cultivated varieties are based on the local germplasm. The hybrid varieties although have give high yield, but the grain quality is poor and not acceptable to the farmers. It seems that the caudatum germplasm is not good source to improve the local sorghum.

A sorghum breeding program was initiated in 1986, based on induced mutations in the local and caudatum types to enhance genetic variability. Several mutants with improved characters were developed and are now in the advanced stages of evaluation in the field trials. These mutants differ from the parental types in plant height, grain colour, vitriousness and size, panicle size, shape, compactness, glume colour, and yield component characters. Of these, one mutant, MIG-SORB86-30-3, seems exceptionnaly good. This mutant is drought tolerant with deep rooting system, and has short stem (1.6 m), good grain quality, with panicles which resemble the local guinea type. This mutant could be used in crosses to improve the local types.

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IMPROVEMENT OF LOCAL VARIETIES OF RICE (*Oryza glaberrima*) FOR RESISTANCE TO SHATTERING AND GRAIN QUALITY BY INDUCED MUTATIONS

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Abstract

In Mali, a large area of rice is planted with the local rice, *0. glaberrima* which under conditions of low rainfall and flood water is more hardy to stress than the introduced *0. sativa* cultivars. A program to improve the local varieties of *0. glaberrima* by induced mutations was started in 1988. Ten local varieties were irradiated with 20 and 30 krad. In M_4 of cv. 'Gorbal', irradiated with 20 krads, 13 variants were selected. Five of these were evaluated in M_5 for their agronomic performance. The induced mutants in the remaining 9 varieties were highly sterile with 90% or more sterility in the M_2 and M_3. Irradiation with 20-30 krad gave high survival (70-95%), and several mutants with white kernel were obtained from the red seeded types. Nearly two-third of the identified mutants had white caryopsis. There is better consumer acceptance of the white seeded type of rice than the red seeded varieties in Mali and the white seeded mutants may have an added premium in the market.

The field performance of the M_5 mutants was investigated. Preliminary results showed that some of mutants derived from cv. 'Gorbal' were early in maturity and had more panicles per plant, but had a lower 1000-kernel weight, and did not differ from the parent in grain yield. Additional trials are planned to establish potential of the mutants for yield and quality. Three more cultivars of *0. glaberrima* - 'Haira', 'Tombo' and 'Yele' - were irradiated with 20 and 60 krads, and gave 75, 81 and 72% seed viability, respectively. M_1 showed reduced plant height. Selection for non-shattering of grains shall be carried out in M_2. Any plants which are non-shattering but sterile shall be crossed with the parent to recover the mutant types. The taxonomic status of cv. 'Gorbal' is not very clear. Isozyme patterns suggest that this cultivar may belong to *0. sativa* and not to *0. glaberrima*. To establish its taxonomic status, crosses shall be made with *0. sativa* and *0. glaberrima*

I. INTRODUCTION

Mali is one of the rare countries where *Oryza glaberrima* is still cultivated. The area grown under this species is more important than that *Oryza sativa* species introduced in the early 1950's. Under normal conditions, the later species yields better than *Oryza glaberrima*. Under low rainfall and flood water during the last decade, the introduced varieties have become less and less productive because they are not adapted to such conditions. This is why the improvement of local varieties by mutation is necessary. The local varieties improvement program has been carried out since 1988 with the support of International Atomic Energy Agency. The main problem in these varieties is that they are prone to seed shattering. The

first objective of this project is to get mutants resistant to shattering and with high yield potential. This report presents the preliminary results obtained and the future prospects to improve these varieties..

2. MATERIALS AND METHODS

The first mutagenesis programme was carried out with 10 local varieties, all cultivated under natural flooding conditions. They were irradiated with 20 and 30 krad. In cv. 'Gorbal' a number of mutants were obtained from the 20 krad. From successive segregation and selection, 13 M_4 mutants were retained. Of these, 5 were evaluated in M_5 for their agronomic value. Mutants were also obtained in M_2 from each treatment of the 9 remaining varieties, but their sterility was more than 90%. This sterility persisted in M_3 generation. Unfortunately, no crossing program was planned with their parents to restore the fertility.

3. RESULTS AND DISCUSSION

This study showed that irradiation with 20 and 30 krad reduced seed viability of *Oryza glaberrima* only slightly and survival was high 70 to 95%. It was easy to change the red color of the caryopsis of all the varieties of *Oryza glaberrima*. About 2/3 of the identified mutants had white caryopsis. The results of the enzymatic analysis of cv. 'Gorbal' at Montpellier, France in 1992 suggested that this variety may belong to the species *Oryza sativa* even though it has some common morphological characteristics of the varieties of *Oryza glaberrima*. A crossing program between 'Gorbal' and a variety of *Oryza sativa* is planned to investigate this hypothesis.

3.1. *Performance of M_5 mutants*

The objective was to compare yield potential of 5 selected mutants with that of their parent.

Site: Agronomic Research Station of Mopti (medium and shallow zones).
Number of treatments: 6
Experiment design: randomized complete block design with 3 replications.
Individual plot size: 5 x 1.8 m
Fertilization: 100 kg/ha ammonium phosphate at sowing, and 50 kg/ha urea one week before floods.

In general, mean yields were low (Table I and II). This may be attributed to the low rainfall at the beginning of the rainy season which did not permit a good establishment of rice. The analysis of variance did not show any significant difference between treatments. Therefore, no clear conclusion can be drawn between the yield of the mutants and the control. Nevertheless, some of these mutants had the following characteristics :

1. All of them had white caryopsis while the control had the red one which has low marketable value compared with the *O. sativa* varieties with white caryopsis.

2. They were resistant to flooding but they had the same floating ability as the control. Thus, they may be suitable for cultivation under natural floating system like *O. glaberrima* varieties, which are very hardy.

TABLE I. AGRONOMIC PERFORMANCE OF MUTANTS IN MEDIUM ZONE

Variety	Days to maturity	Plant height (cm)	No. of tillers /m	No. of panicles /m	No. of grains /p	1000 gr weight (g)	Yield kg/ha
Control (Gorbal)	163	189	38	29	160	29.6	2310
SMMG88-9	143	161	43	37	148	26.7	2260
SMMG88-13-1	153	156	37	32	140	26.2	2100
SMMG88-15-2	162	169	45	34	178	24.1	2235
SMMG88-20-1	144	187	46	36	162	27.0	2509
SMMG88-20-2-1	153	158	48	38	191	27.3	2680
F							NS
CV (%)							22.8

Sowing date: 9/07/1992
Maximum water levels: 80 cm
F test: not significant at 5% level

TABLE II. AGRONOMIC PERFORMANCE OF MUTANTS IN SHALLOW ZONE

Variety	Days to maturity	Plant height (cm)	No. of tillers /m	No. of panicles /m	No. of grains /p	1000 gr weight (g)	Yield kg/ha
Control (Gorbal)	163	184	38	30	154	29.3	2105
SMMG88-9	143	150	35	26	135	27.1	1634
SMMG88-13-1	153	142	36	30	134	25.9	1833
SMMG88-15-2	162	158	41	36	165	24.6	1712
SMMG88-20-1	144	179	43	37	141	27.0	2350
SMMG88-20-2-1	153	149	47	38	188	27.5	2417
F							NS
CV %							29.8

F test: not significant at 5% level
Sowing date: 11/07/1992
Maximum water levels: 50 cm

3.2. *Irradiation of additional varieties*

The objective of this study is to obtain mutants resistant to shattering and with high yield potential. Three varieties prone to shattering but more productive in natural submersion condition have been used. These varieties are 'Haira', 'Tombo' and 'Yele'. Each variety was irradiated with Cobalt 60 with dose of 20 krad and planted in plots 21m x 5m, with 0.30m

between rows, and 0.20m between hills. Data shall be collected on date of seedling emergence, plant survival after germination, date of 50% flowering, plant height at maturity, plants reaction to diseases and insects. The main panicle from each bush will be harvested at maturity and threshed. Then, the seeds will be sown in M_2 generations. Only the percentage of survival is now available. It was 75% for 'Haira', 81% for 'Tombo' and 72% for 'Yele'. These results confirmed those obtained in 1988. Radiation reduced seed viability of *O. glaberrima* varieties. Also any reduction of plants M_1 height compared to the control has been recorded.

3.3. *Future program*

As mentioned above, all of the identified mutants in the first mutagenesis program except one, were male sterile. The male sterile mutants identified in M_2 will be crossed with their parents to restore their fertility. From February 1994, one part of M_1 will be grown as M_2 under irrigation to advance generation.

CHICKPEA AND COWPEA GRAIN IMPROVEMENT USING MUTATION AND OTHER ADVANCED GENETIC TECHNIQUES

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1. INTRODUCTION

To improve resistance of chickpea and cowpea against diseases and pests, it is necessary to find sources of durable resistance in cultivated and wild gene-pools. Most of these traits have been found in wild species which are generally cross-incompatible with the cultivated ones. Furthermore, breeders need some years to release new genotypes, since the long process based on crosses, back-crosses and selection makes difficult to respond to the evolution of new disease and pest strains.

The use of genetic engineering methodologies in breeding programmes seems to be very promising a. to find new resistance-related genes present in other phyla, to clone and transfer them into plants, and b. to shorten the time to obtain an improved genotype since only a single gene is involved in this process. The main "bottle-neck" to apply this scheme in chickpea and cowpea is the absence of a reliable protocol of regeneration and genetic transformation. In this frame, following some pilot experiments on these grain legumes to induce regeneration and gene transfer, we attempted a. to find a regeneration medium, assay the effect of different hormones on young tissues, and b. to select the best procedure for transfer of genes into the plant genome.

2. MATERIALS AND METHODS

Two cowpea genotypes, cv. 'Cornetto' and TVu83D442, were used in the experiments. Immature leaflets and hypocotyls detached from mature seeds after sterilization were used as explants in all experiments.

Regeneration of cowpea

Different combinations of two callus-inducing media and three regeneration media were tested. Growth regulators added to callus-inducing media were 0.5 mg l^{-1} kinetin and 0.5 mg l^{-1} NAA or 0.5 mg l^{-1}Picloram, whilst 0.1-4.0 mg l^{-1} kinetin, 0.05 mg l^{-1} 2,4-D, 0.05 mg l^{-1} BAP, and 1-3 mg l^{-1} IBA were tested in combination with coconut milk. In some experiments, explants were placed directly on the regeneration medium.

3. RESULTS AND DISCUSSION

Regeneration of new shoots was obtained from hypocotyl explants (3.1%) and from immature leaflets (3.7%) of TVu83D442, after 70 days of culture from 301 explants. No regenerants were obtained in cv. 'Cornetto', not even when explants were left to grow directly

on a regeneration medium. This result confirms our hypothesis that to induce regeneration, it is necessary to shock plant tissue explants with a pulse of hormone or growth regulator. Furthermore, callus growth is necessary to obtain regeneration, but a profuse differentiation of roots seems to inhibit shoot differentiation. Histological analysis was performed. We found that regenerants occurred on the surface of callus with differentiation of new buds, suggesting true differentiation, though at a low frequency

Genetic transformation of cowpea

Direct and indirect methods of gene transfer were studied to get the highest percentage of transformed tissues with a stable expression of the transgene. Since regeneration is still a low frequency event in cowpea and reliable protocols need to be established. We have tried to transfer genes into already formed meristematic cells to obtain chimeric shoots. Thus, electroporation of pDNA into meristematic cells was tested as direct gene transfer method, and *Agrobacterium tumefaciens* co-culture with meristems was investigated as an indirect method.

Electro-injection of pDNA into cowpea meristems

The rationale of this method is to apply a high voltage field to plant cells to produce micropores in cell walls and cell membranes, through which pDNA present in the electro-injection solution can pass into cell cytoplasms. If enough PDNA molecules enter into meristematic cells, there is a chance of integration of at least one pDNA molecule into the cell genome, thus giving rise to a transgenic cell. In the presence of an appropriate marker and/or reporter gene, it is then possible to find and/or select transgenic cell clusters, and finally obtain transgenic shoots.

Pilot experiments were performed to study the resistance of cowpea mature embryos to high voltage field. After applying voltages between 200 to 1,600 V cm^{-1}, we found that 600 V cm^{-1} was the voltage that did not injure too much apical and lateral meristems; hence this voltage was used in the subsequent experiments. Several cowpea apical and lateral meristems, isolated from embryos obtained from sterilized mature seeds were submitted to electro-injection. A plasmid carrying the *gus* reporter gene (ß-glucuronidase), interrupted by an intron, was added to the electro-injection solution. One week old shoots from explants cultured in a hormone-free medium were analyzed for *gus* expression. About 30% of explants showed apices with an intense blue colour. Histochemical analysis of some explants showed blue crystals of indole inside cells, confirming the transgene expression. However, *gus* expression was not confirmed in the three week-old shoots. This behaviour suggests that pDNA was not stably integrated into plant genome, but only transiently expressed.

Co-culture of cowpea meristems with Agrobacterium tumefaciens

Two strain of *A. tumefaciens* were tested: AT8, a C58C1 derived strain harbouring the plasmid p35S-GUS/INT as binary vector (marker gene - *npt*II, resistance to kanamycin; and reporter gene *gus*, and AT22, a LBA4404 strain carrying binary vector the plasmid pML106, harbouring the *npt*II and *gus* genes as marker and reporter genes, respectively, and an a-amylase inhibitor gene isolated from common bean. This gene has been tested by others as

effective against Lepidopteran larvae. Two explants were tested - apices and hypocotyls of mature embryos obtained from sterilized seeds of 'Cornetto' and TVu83D422.

An unexpected effect of co-culture of the bacteria with 'Cornetto' was the regeneration of immature leaflets and hypocotyl explants which was not obtained in the first set of experiments as previously reported. Regeneration frequency of 'Cornetto' and TVu83D422 explants ranged between 0.8 to 17.1%, and 2.4 to 4.1%, respectively. We hypothesize that the antibiotic cefotaxime used for stopping the *Agrobacterium* growth has a side effect on the regeneration capability of the co-cultured cowpea tissues. This effect has already been observed in other monocotyledon and dicotyledon plants by other authors.

After one month of *in vitro* culture on the selective medium containing 100 mg l^{1} kanamycin, explants were submitted to *gus* histochemical assay. On average, explants co-cultured with AT22 showed a higher frequency of stably transformed tissues than AT8 (58.1% vs 18.9%). No statistical differences were found in the frequency of immature leaflets or hypocotyl explants expressing *gus* gene; however, no differentiated shoots were found to be solid mutants.

RESPONSE TO ARTIFICIAL INOCULATION WITH *Phytophthora infestans* OF POTATO CLONES INSENSITIVE TO CULTURE FILTRATE OF THE FUNGUS

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1. INTRODUCTION

Induction of general resistance to *Phytophthora infestans* (Mont.) de Bary by *in vitro* culture has been reported for cvs. 'Bintje' [1] and 'Kennebec' [2]. In both cases, sporangia suspension of the fungus were used as selective agent. As reported for many host-pathogen interactions [3, 4], culture filtrate or purified toxins may also contribute to the selection of resistant genotypes.

Challenge of mutagenized buds of cv. 'Spunta' with culture filtrate of *P. infestans* allowed selection of insensitive clones [5]. To verify the possible correlation between culture filtrate insensitivity and disease resistance, different inoculation techniques were applied to these clones. Considering that general resistance to late blight is a complex trait, different tests were performed to evaluate the behaviour during various stages of pathogenesis, such as infection, invasion, mycelial growth and sporulation.

2. MATERIALS AND METHODS

Twenty clones insensitive to culture filtrate of *P. infestans*, along with the susceptible mother variety 'Spunta' and the moderately resistant 'Atzimba' were inoculated with an Italian isolate of the fungus. Inoculation of detached apical leaves and stems (6 cm) taken from 40 days old plants were performed in three replications according to the method of Tegera [6]. For the test on detached leaflets, the inoculum concentrations of $8x10^3$ and $2x10^4$ spores ml^{-1} were used and the percentage and the area of lesions as well the mycelium development were measured. The colonization of the mycelium and its growth on the stem were also evaluated. The extent of mycelium growth on the leaf and the stem were respectively assessed from the percentage of mycelium colonization on a 0-4 scale. Sporangia suspensions at concentrations of $8x10^3$ and $2x10^4$ spores ml^{-1} were sprayed on 30 cm tall plants, ten from each genotype grown in a greenhouse. The incidence of *Phytophthora* attack was recorded for individual plants 7 and 14 days after inoculation, using a 0-5 scale (0 = no disease observed; 5 = all leaves and stems dead).

3. RESULTS AND DISCUSSION

Significant differences were found between the genotypes for all the tests, but there was no significant difference between the two inoculum concentrations used. Interactions between genotypes and inoculum concentrations were not statistically significant. The analysis

of variance for each parameter showed that the results were consistent throughout the experiment.

On inoculation of detached leaf and stem, cv. 'Atzimba' was not as severely attacked by late blight as the susceptible control cv. 'Spunta' (Table I). The differences between these two genotypes were statistically significant for all the tests performed. The clones previously selected for insensitivity to culture filtrate showed values intermediate between the susceptible and the moderately resistant controls. The selected clones also showed significantly less symptoms after artificial inoculation of the whole plants.

The response of representative clones to inoculation of detached parts and whole plant are reported in Table I. In particular, reaction for resistance was significantly different for the genotypes 3 and 5 in all tests compared to cv. 'Spunta'. In clones 11, 21 and 25, some infection frequency was combined with good resistance to invasion (lesion area and stem colonization) and a slow mycelial growth. These two last factors may be considered valuable components of general resistance to late blight, and may prevent effectively the colonization of pathogen in the host tissue [7].

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TABLE 1. REACTION TO DIFFERENT INOCULATION PROCEDURES WITH *P. infestans* IN CLONES INSENSITIVE TO CULTURE FILTRATE OF THE FUNGUS

Genotype	Detached leaf			Detached stem		whole plant
	lesions (%)	lesion area (CM^2)	colonization (%)	colonization (%)	mycelial growth (0-4)	(0-5)
5	0.00 E	0.00 C	0.00 C	0.00 E	0.00 E	3.00 AE
3	3.45 DE	0.01 C	0.00 C	16.03 E	1.67 AE	2.60 CE
11	10.23 AE	0.15 BC	2.15 BC	0.00 E	0.00 E	2.30 DE
21	23.10 AE	0.03 C	4.31 BC	26.89 AD	0.00 E	2.55 CE
25	14.56 AE	0.20 BC	0.00 C	0.00 E	0.00 E	2.30 DE
Mean clones	17.80	0.17	3.12	13.20	1.05	2.88
Spunta	32.32 A	0.98 A	35.58 A	46.55 A	2.67 AD	3.77 A
Atzimba	4.01 CE	0.00 C	2.15 BC	3.49 DE	0.00 E	-
F Genotypes	1.7*	1.81*	5.4***	3.5***	4.1***	5.2***
F Conc.(a)		0.3 n.s.	0.2 n.s.	2.7 n.s.		108.4
F GxC	1.5 n.s.	0.7 n.s.	1.3 n.s.			0.5 n.s.

*significant P=0.05 ***significant P=0.001 n.s.=non significant (a) inoculum c

PRODUCTION OF *Basella* PLANTS RESISTANT TO RUST BY IRRADIATION OF SEEDS AND VEGETATIVE TISSUE

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1. INTRODUCTION

Basella is classified in the family Chenopodiaceae or Basellaceae. Also known as African spinach, this plant is consumed in Central Africa and several other African countries. There are two types of varieties grown in Congo:

i. a local variety characterized by red leaves and stalks in which the principal way of propagation is from cuttings;

ii. a group of varieties which have green or purple leaves and stalks. These varieties are called *Basella alba* and *Basella rubra*. These varieties have sexual reproduction.

Among the two groups of varieties, the local variety is propagated vegetatively but is resistant to rust, while varieties with green leaves or with purple leaves (*B. alba* and *B. rubra*) that are propagated from seed are susceptible to rust. Since hybrid cannot be made by conventional crossing, the following procedures have been adopted to produce plants with disease tolerance: 1. production of resistant variants by irradiation of *Basella alba* seeds with Cesium 137; 2. production of resistant variants by irradiation of vegetative tissues obtained by culture of meristematic cells of *B. alba*; and 3. obtaining resistant plants through somaclonal variation.

2. MATERIALS AND METHODS

Basella alba seeds were irradiated with various doses of gamma rays from a Cs^{137} source and seed germination was recorded.

3. RESULTS AND DISCUSSION

3.1. *Effects of irradiation dose on seed germination*

Seed germination ranged between 61 and 62% (Table I). Seeds irradiated with 50 to 150 Gy had germination between 60 and 57%. Seeds irradiated with 200 to 300 Gy had germination between 40 and 46% and those irradiated with 400 to 500 Gy between 29 and 23%.

TABLE I. EFFECT OF RADIATION DOSE ON SEED GERMINATION, SEEDLING SURVIVAL AND HEIGHT

Radiation dose Gy	No. of seeds irradiated	Seed germinated		Seedling survival after 24 days		Mean seedling height	Inhibition of growth
		No.	%	No.	%	cm	%
T1	246	152	62	152	100	46	0
T2	246	150	61	150	100	45	0
50	246	148	60	148	100	45	0
100	246	134	54	134	100	30	34
150	246	132	54	132	100	24	48
200	246	113	46	54	48	11	77
250	246	115	41	60	52	8	83
300	246	93	42	36	39	2	95
400	246	71	29	39	53	5	89
500	246	56	23	19	33	3	94

3.2. *Effects of radiation dose on plant mortality*

There was no mortality among plants obtained from seeds irradiated with 50 to 150 Gy. Seedling mortality ranged from 33 to 53% among plants obtained from seeds irradiated with 200 to 500 Gy.

3.3. *Effect of radiation dose on plant growth*

Inhibition of plant growth was very high in plants obtained from seeds irradiated with 100 and 500 Gy.

3.4. *Irradiation of tissue cultures*

A suitable culture medium was developed to obtain *in vitro* plants from meristematic cells of *B. alba* (rust sensitive variety). The *in vitro* plants will be irradiated to test their radio-sensitivity.

The preliminary results showed that doses between 150 to 200 Gy reduced 50% germination of seed, seedling survival and growth. These two doses will be used in the next cycle.

PROGRESS REPORT ON MUSA AND CASSAVA

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1. INTRODUCTION

The Plant Breeding Unit develops nuclear methods and related technology, such as *in vitro* culture techniques, molecular biology and carries out physiological studies on the genetic improvement of crop plants. The Unit transfers these technologies by providing training in plant breeding, mutagenic treatment service and technical advice. The following activities were undertaken in relation to the CRP "Improvement of basic food crops in Africa through plant breeding including the use of induced mutations".

Musa spp.

In *Musa* species, two main enzymatic systems were investigated. Peroxidase (E 1.11.1.7) is a multiple purpose enzyme that, besides other functions, catalyses the condensation of phenolic compounds derived from phenylpropanoid pathway as insoluble polymers. Several authors have demonstrated that peroxidase plays an important and specific role in the hyper-sensitive containment of the pathogen. The current model for the banana-*Fusarium* interaction considers the species-specific plant response to infection through the build-up of exopolysaccharide mechanical barriers and the infusion with phenolic compounds, and condensation to lignin-like compounds.

Another proposed mechanism involved in the plant response to a pathogen is the activation of chitinase, an enzyme responsible for the degradation of chitin - a major component of the fungal cell wall. The role played by this enzyme in the plant response is not clear. However, two main effects have been proposed 1. the chitosan fragments produced by enzymatic cleavage could act as specific elicitors or 2. the enzyme could be directly active as an antifungal weapon by disrupting the fungal cell walls.

2. MATERIALS AND METHODS

Plant material

Seven banana clones (Table I) showing different resistance to *Fusarium oxsporum* f.sp. *cubense* (FOC) Race 1 and 4 were used for the peroxidases experiments. For chitinase several clones were screened; however, data on the induction of enzymatic activity after infection with *Fusarium* were obtained using three reference clones, namely SH3362, 'Pisang Mas' and 'Grande Naine'.

TABLE I. BANANA CLONES TESTED FOR RESISTANCE

Clone	Race 1	Race 4
SH3362	resistant	resistant
PISANG JARY BUAYA	resistant	?
SH3142	resistant	resistant/susceptible
HIGHGATE	susceptible	susceptible
GRANDE NAINE	resistant	susceptible
DWARF PARFITT	resistant	resistant
PISANG MAS	resistant	susceptible

Fungal culture and plant inoculation

FOC was cultivated on PDA (potato, dextrose and agar) medium and incubated under light at 28°C. After three weeks, conidia of Race 1 and Race 4 were collected and adjusted to a concentration 5 x 10^5 conidia/ml. Plantlets were obtained and rooted as reported (Novak 1992). Roots were trimmed and dipped in the conidial suspension for 10 min. under air flow to promote evapotranspiration and mechanical uptake of fungal propagule. Only distilled water was used as control. Inoculated and non-inoculated plants were incubated in 0.3% guaiacol, 2 mM hydrogen peroxide in 0.01 M sodium phosphate buffer, pH 6.8. After two minutes, absorbance was read at 470 nm and expressed per unit protein content.

Peroxidase activity

Corn tissue was ground in a mortar and 0.1M sodium phosphate buffer pH 6.8 (1:2 w/v) was added. The resulting homogenate was centrifuged at 14,000 rpm in a refrigerated Eppendorf centrifuge, and the supernatant assayed for protein content according to Bradford (1962). Enzymatic activity was measured in 1ml final reaction volume. Five µl of corn extract were incubated in 0.3% guaiacol, 2 mM hydrogen peroxide in 0.01 M sodium phosphate buffer, pH 6.9. After two minutes, absorbance was read at 470 nm and expressed per unit protein content.

Peroxidase IEF

Samples with equal protein content were loaded on a gel containing Pharmalyte 3.5 - 9.5 (Pharmacia) as carrier ampholyte. The gel was pre-focused for 30 min. Running conditions were 8 Watt fixed power, 33 mA current and up to 25000 V. The gel was stained after running in a sodium phosphate buffer, pH 6.0, containing 0.6 mg of 4 chloro-1-naphthol per ml and 2 mM hydrogen peroxide.

Chitinase IEF

Gels were cast and run as described above. After running, a second gel (sandwich technique) containing chitinase was overlaid on the running gel, and incubated at 37°C for two hours in dark. The overlay gel was removed, and transferred to staining buffer containing 0.01% of Calcofluor Brightener for 5 min and viewed with a UV source.

3. RESULTS AND DISCUSSION

The seven clones could be divided according to their known resistance/susceptibility field response as follows:

Peroxidase activity

Different clones were analyzed for constitutive peroxidase activity before fungal inoculation. The highest constitutive activity was found in the resistant clones as compared with the susceptible ones. After inoculation with a conidial suspension of FOC Race 1, all clones except 'Highgate' showed increased peroxidase activity but with different time course. Four clones showed in the first two weeks a fast increase in the enzymatic activity. The remaining two clones exhibited a trend toward increased enzymatic activity. When clones were inoculated with FOC Race 4, clones SH3362, 'Dwarf Parfitt' and 'Grande Naine' showed a sudden increase in peroxidase activity. Of these three, 'Grande Naine' subsequently showed a decrease in activity, reaching a negative ratio after three weeks. The other clones showed a more complex pattern in the kinetics of induction. When the banana clones were grouped according to their known field response the following situation was observed. (Fig. 1)

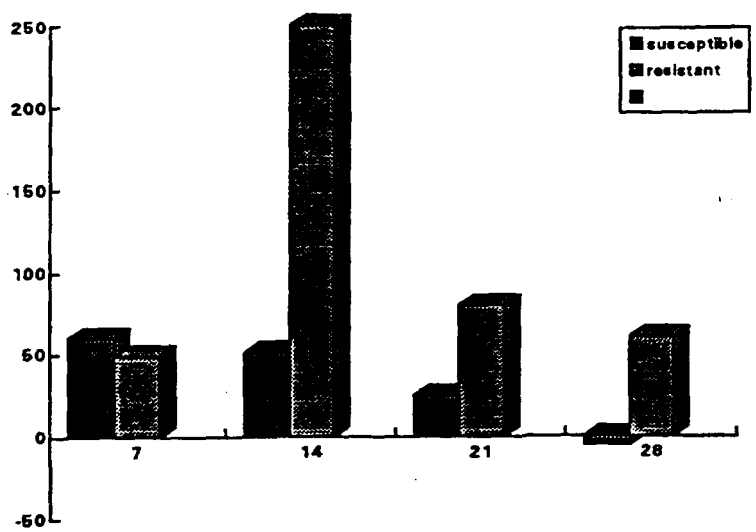

Fig. 1. Peroxidase activity after inoculation with *Fusarium oxysoprum* f. sp. *cubense* Race 4 resistant vs. susceptible

Peroxidase Isoelectrofocusing

Using 4 chloro-1-naphthol, it was possible to separate and count at least 12 bands of isozyme, common to all clones. Among them, seven anionic bands were detected. No differences were found in isozyme patterns in the treated and non-treated plants. Intensity, i.e. enzymatic expression, increase in both the control and treated plants with time. However, the intensity of some bands increased earlier in inoculated plants than in the control.

In several publications [3,4,5], it has been demonstrated that peroxidase plays an important, early and specific role in the hyper-sensitive containment of the pathogen. The results suggest that the resistant genotypes show a general trend in increased enzymatic activity to FOC challenge, although the induction kinetics differed between the genotypes. The fact that peroxidase activity increased rapidly in the compatible interaction suggests that in banana this enzyme might be involved in the defence response. These observations are in agreement with the current model of banana resistance to FOC. The speed and the magnitude of the defence mechanism activation appears to be critical for the expression of resistance. Moreover, the measurement of constitutive peroxidase activity had a positive correlation between high enzymatic activity in non-infected plants and resistance to FOC. To be effective, the defense mechanism should be constitutively present or rapidly elicited to reach a certain threshold in response to the pathogen infection. All but one clone ('Highgate') are able to reach this threshold, although some of them react more promptly than others when infected with FOC Race 1. When these genotypes were inoculated with FOC Race 4, two of them responded ('Dwarf Parfitt', SH3362) faster than others. One ('Grande Naine', susceptible) showed an early increase in activity, but after two weeks, the enzymatic activity was lower than that in the control; this is a possible indication of some damage in the cellular mechanism for resistance. This trend was similar to the one expressed by the other two clones ('Highgate' and 'Pisang Mas', susceptible). A third class was defined by the remaining clones SH3142 and 'Pisang Jary Buaya', both considered resistant, at least to some FOC Race 4 biotypes. In these clones, after an initial enzymatic activity depression, a slow increase in activity was observed over the time.

Chitinase Isoelectrofocusing

All the banana and plantain clones screened for the presence of constitutive chitinases showed at least two or three bands, except 'Burro CEMSA', 'Burro Criollow' (ABB) and 'Saba' (BBB). Chitinase was induced in a preliminary experiment with FOC Race 4 and Race 1 only in SH3362, while the chitinase activity remained unchanged in the other two banana clones, 'Highgate' and 'Pisang Mas', used in this experiment. This resistance is based on multiple biochemical factors that interact with each other. One of the possible roles depicted for chitinase, beside the direct effect on the fungal cell wall, is to elicite other defence mechanisms. Although, these are only preliminary results, it could be speculated that chitinase in banana may have an active role in this respect. The chitinase could act upon the fungal chitin producing elicitors that in turn would activate a cascade of biochemical events leading to resistance in banana.

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***Manihot* spp.**

1. INTRODUCTION

Cassava is a multiple purpose crop, and its role as a staple food and as a livestock feed is well established. Traditional cross breeding is somehow constrained by the high levels of heterozygosity and sporadic flowering in certain clones. Moreover, hybrid breeding using inbred lines is not possible due to severe inbreeding depression. However, its vegetative propagation allows desirable traits to be fixed, which originate from the lengthy breeding process.

Mutation induction aims to optimize genetic variation with minimal plant injury. At the Plant Breeding Unit, the work or cassava mutation induction aims to optimize the mutagenic treatment and to study parameters of injury in tissue cultures which are indicative of a mutagenic response and permit estimates of mutation frequency. Other objectives are to compare the effects of acute vs fractioned gamma dose application and to investigate whether mutagenic efficiency, i.e. the ratio between mutation and primary damage, could be increased by split dose application. Preliminary studies were done on the primary injury caused by gamma radiation and the influence of the source of explant.

2. MATERIALS AND METHODS

Explant source

Nodal cuttings of cassava were cultured in liquid medium (see below). After three weeks in culture, the plantlets were removed and single nodal cutting were taken for radiation treatment. The nodal cuttings were divided into three lots according to their position on the stem (shoot tip, first node and second node), and treated with four doses of gamma radiation (15, 30, 45 and 60 Gy). Radiosensitivity was based on the number of shoots, number of nodes, fresh weight and survival rate.

Acute vs. split gamma doses

Four clones of cassava, i.e. M.Mal-2, M.Mal-3, M.Thai-1 and M.Col-1390 were mass propagated on modified MS medium supplemented with 100 mg/l inositol, 0.1 µM BAP, 0.01µM NAA, 0.1 µM GA, 30 g/l sucrose, pH 5.8 and B5 vitamins. The top two nodes were aseptically excised, and a representative population cultured on hormone-free medium was treated with acute or fractioned ^{60}Co gamma radiation at a rate of 4.99 Gy/min. Dose fractionation implies splitting of the total dose in three fractions where the first dose was the lowest, effectively an enhanced level of radio-resistance (dose fractionation 20 Gy = 5+5+10, 30 Gy = 10+10+10, 40 Gy = 10+15+15). A recovery period of 4 hours was allowed between split doses. The irradiated material was immediately transferred to the liquid culture medium. Radio-sensitivity was based on the number of proliferating explants, fresh weight and shoot height.

3. RESULTS AND DISCUSSION

Effects of acute gamma radiation

Radiation damage increased with dose and followed a sigmoidal pattern of primary injury for all the radio-sensitivity parameters and cassava clones tested. However, a significant influence of the explant source on the primary damage was observed; shoot tips were less sensitive to gamma radiation than the first and second nodes at all doses tested.

Application of fractionated gamma ray doses

Splitting an acute dose into three fractions separated by at least four hour intervals produced consistently superior recovery compared with two acute dose fractions and shorter recovery intervals between doses. The magnitude of recovery was influenced by the level of the first or primary dose which activates repair phenomena. Recovery effects were most pronounced at dose levels which induce severe radiation damage or lethality after acute irradiation. The radio-biological effects of split dose radiation and their potential positive impact on an improved mutation induction methodology are only the first step within the scope of efficient *in vitro* mutation induction for breeding objectives. Large populations of homohistont plants with desirable traits must be grown for a complete study of the correlation between primary injury and the induced genetic variation. Further systematic studies on mutation induction in different tissue culture systems are required to correctly define and estimate mutation frequency based on primary injury.

TRAINING

Scientists and plant breeders from African Member States were awarded IAEA fellowships for in-service training in the Plant Breeding Unit, Seibersdorf Laboratories. The training periods were from six to twelve months. The training programmes were individually designed to ensure direct application of the technologies learned. A total of 32 man months were awarded to scientists from Africa over a period of 2 years. This constitutes 50% of the fellows trained in the Plant Breeding Unit. Of this, more than 60% of the fellowship were to African scientists participating in the CRP.

During the training, the following techniques were taught to the participants: 1. Mutation induction; 2. Micropropogation of banana, plantains, cassava, kenaf, Enset, yams; 3. Somatic embryogenesis in banana and plantains; 4. Polyploidy induction and screening through flowcytometry

Interregional Training Course

Several scientists from Africa participated in the 12th and 13th FAO/IAEA Training Courses, organized at the IAEA's Agriculture Laboratory. The Plant Breeding Unit participated in the implementation of a FAO/IAEA/ITALY Workshop on "Breeding for Stress Resistance" held at IITA, Ibadan, Nigeria.

SERVICES

On request from Member States, cost-free treatment for mutagenesis was provided to plant breeding institutes involved in CRPs from Cote d'Ivoire, Nigeria, Mali, Cameroon, Ghana, Kenya and Uganda. A total of 55 samples which included *Sorghum bicolor, Vigna unguiculata, Oryza glaberrima, Orzya sativa, Sorghum vulgaris, Arachis hypogea, Phaseolus vulgaris, Glycine max, Heliantus annuus, Zea mays, Gossypium hirsutum,* were treated with gamma ray at the Seibersdorf Laboratory.

IMPROVEMENT OF CASSAVA FOR RESISTANCE TO INSECT PESTS AND DISEASES

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Abstract

The African cassava mosaic virus and cassava mealybug are devastating the cassava crop in Uganda. Because of the severe and widespread occurrence of the virus and mealybug, *in vitro* cultured cassava plantlets instead of stem cuttings will be irradiated. In addition, the project has incorporated sweet potato. Installation of tissue culture laboratory at Namulonge was completed in early 1993. Work is in progress to establish efficient *in vitro* culture micropropagation techniques for the two crops. Small numbers of cassava plantlets of varieties 'TMS 30337' and 'TMS 4(2)1425' and sweet potato entry 39 are *in vitro* culture. Mass irradiation of plantlets is planned in future.

1. INTRODUCTION

Root and tuber crops continue to play a major and increasing role as basic food crops in Uganda. However, the crops have major production constraints. During the last three or four years, the status of pests has changed dramatically, especially in cassava. Although, green spider mite, *Mononychellus tanajoa* Bondar and cassava bacterial blight (CBB) are important (2,3,4), more attention is being given to African cassava mosaic virus (ACMV) and cassava mealybug *Phenacoccus manihoti* which are almost devastating the crop. The objective of the conventional cassava breeding is to select for resistance to ACMV by screening local and introduced breeding populations. In addition, the release of two natural enemies of the mealybug, *Epidinocarsis lopezi and Hyperaspis notata* was started in April 1992. Because of the high severity and widespread occurrence of ACMV and the presence of the mealybug, it was decided to use *in vitro* cultured plantlets as starting material for irradiation. Installation of the tissue culture laboratory was completed early 1993. Currently work is in progress to establish *in vitro* culture micropropagation techniques of cassava and sweet potato. Following the change in work plan the current objectives are: 1. To establish efficient micropropagation protocols of cassava and sweet potato, and 2. To induce genetic variation in cassava and sweet potato by X-ray irradiation of *in vitro* cultured plantlets for selection to pest and disease resistance in cassava and desirable root traits in sweet potato.

2. MATERIALS AND METHODS

Surface sterilization of two cassava varieties 'TMS 30337' and 'TMS 4(2)1425' and sweet potato entry 39 was done using a range of concentrations (10-80% V/V) of locally available sterilizing agents (Parazone, Domestos and Jif). Explants of deleafed, 1-3 nodded cuttings, grown in the screen house, were dipped in 70% alcohol for 2-5 seconds, rinsed in sterile distilled water and then soaked in the sterilizing agents with a few drops of Tween 20

for 10 to 30 minutes. The explants were rinsed three times in sterile distilled water. The explants were cultured on Murashige and Skoog medium (1) containing 3% sucrose and supplemented with 0.05 mg/l benzylaminopurine (BAP) and 0.01 mg/l naphthalene acetic acid (NAA) for cassava and 0.05 mg/l BAP for sweet potato. The pH was adjusted to 5.8 and the medium was solidified with 0.8% Difco-Bacto Agar. The cultures were maintained at about 27 °C under 16 h day length.

3. RESULTS AND DISCUSSION

Only a small numbers of plantlets of 'TMS 30337' and 'TMS 4(2)1425' and sweet potato entry 39 were produced *in vitro*. The main problem has been contamination of the cultures, frequently reaching 100%, especially in sweet potato.

Future plans

1. The material for irradiation will be *in vitro* culture plantlets, not mature stem cuttings as planned initially.

2. Establish efficient techniques for virus elimination by thermotherapy (35-38°C for 30-40 days) and culture apical meristem for micropropagation.

3. Carry out radio-sensitivity test on cassava 'TMS 30337' and 'TMS 4(2)1425' and sweet potato entry 39 by X-ray irradiation using 0, 1, 2, 3, 4, 5 krad on 4 plantlets per treatment to give 20 single node cuttings/treatment, and

4. Mass irradiate *in vitro* cultured plantlets after establishing optimum dose and subculture M_1V_1 through M_1V_5 plantlets.

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IMPROVEMENT OF PIGEONPEA FOR DROUGHT, DISEASE AND INSECT TOLERANCE/RESISTANCE THROUGH INDUCED MUTATIONS

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Abstract

Pigeonpea *(Cajanus cajan* L. Millsp) is the second most important grain legume after cowpea *(Vigna unguiculata* L. Walp) in the semi-arid areas of Kenya. At the farm level, the grain yield of pigeonpea is lower than that of other grain legumes and cereals. The low grain yields are mainly attributed to the late-maturity of the local land-races which are prone to drought, insect attack and disease damage. Recently, a mutation breeding programme was initiated to augment the conventional breeding approaches to alleviate some of these constraints. Three varieties, namely, Kat 60/8, Kat E31/4 and Kat 777, representing early, medium and late maturing groups were irradiated with three doses of gamma rays, namely, 80-100 Gy, 110-125 Gy, and 140-150 Gy. Single plant progenies from the M_2 and M_3 generations of were screened and selected for tolerance to drought, tolerance/resistance to *Fusarium* wilt and insect tolerance in the field. Selections were advanced to M_4 generation. In this paper, preliminary results of these studies reported.

1. INTRODUCTION

Pigeonpea *(Cajanus cajan* L. Millsp) is the second most important grain legume after cowpea *(Vigna unguiculata* L. Walp) in the marginal dry areas of Kenya. The crop is grown on 116,000 ha annually, mainly in Eastern, Central and Coastal provinces [5]. It is normally cultivated in mixtures either with cereals such as maize *(Zea mays),* sorghum *(Sorghum bicolor)* millets *(Pennisetum sp)* or with other grain legumes such as cowpea, common bean (*Phaseolus vulgaris*), and mung bean (*Vigna radiata*). Pigeonpea grown by farmers in these areas is a mixture of local land races that are large-seeded, tall, upright and of long duration, taking up to 10 months to mature [2]. These plant types yield on average about 500 kg/ ha [4] which is low compared to the yields of the other grain legumes.

There are several reasons as to why the potential grain yield of the pigeonpea types has not been realized. One of the constraints is the long-maturing trait of the traditional pigeonpea which makes it vulnerable not only to the terminal droughts common between the two bimodal seasons, but also to the intermittent dry spells common during the growing season. Moreover, the crop utilizes most of the limited soil moisture and nutrients during vegetative growth rather than grain development. Another reason of the low yields is the heavy damage caused by insects such as the pod-fly, pod-borer and pod-sucking bugs. However, early-maturing pigeonpea is more disadvantaged than the late-maturing pigeonpea, in that it is exposed more to these and other insects, whose life cycle and population dynamics coincides with the development phase of the crop. Thirdly, the crop is continuously

cultivated in the same fields for many years with crop rotation being seldom practiced. Under such conditions, it is heavily attacked by *Fusarium* wilt, though this may not be evident in some fields due to natural selection for wilt tolerant types. The pigeonpea growing areas are characterized by a bimodal rainfall pattern with seasonal peaks in April and November [1]. This delineates the crop growing season into what are referred to as long (March/June) and short rains (October/December). A long intervening dry period is present during the growing season. Pigeonpea is traditionally sown in the October/November rain and harvested before the next years' short-rain. In the past, the breeding strategy at the National Dryland Farming Research Centre (NDFRC), Katumani, has been to optimize productivity by matching the phenology with the existing rainfall regimes. In this respect, three maturity groups namely, early (150 days), medium (150 - 200 days) and late (over 200 days) have been introduced in the different agro-ecological zones[2]. However, the reproductive phase of the early and late maturing groups are greatly affected by terminal drought. The early maturing group come to anthesis in February whereas the late-maturing group flowers in July/August, giving the two peaks, when drought stress is at its highest. These two maturity groups are more prone to damage by insects whereas the medium and late maturing groups are more affected by *Fusarium* wilt.

Conventional plant breeding method is being used to improve pigeonpea for the wide range of environmental and biotic stress in semi-arid regions of Kenya. However, it also been realized that induced mutations may be an effective tool in generating new variation, which can be utilized to overcome some of these constraints in the local types. Since 1991, we initiated a mutation programme [1] using gamma rays, with the objectives to select mutants with desirable characters of earliness, drought tolerance, high yield, *Fusarium* wilt and insect tolerance, either for direct use or for cross-breeding. This paper reports on the preliminary studies undertaken during the last two years, and on the on-going research programme.

2. MATERIALS AND METHODS

Three elite pigeonpea lines, namely, Kat 60/8, Kat 777 and Kat E31/4 were irradiated with, namely, 80-100, 110-125, and 140-150 Gy gamma rays. The M_1 seed was sown at Katumani (1° 35'S, 37° 14'E, 1570 m above sea level) and Kibwezi (2° 28'S, 38° 15'E, 765 m above sea level) during March-April rains, 1990. About 200 single plant progenies were selected at each site on the basis of grain yield. The M_2 single plant progenies of Kat 60/8 were sown at Kiboko (2° 17'S, 37° 50'E, 997 m above sea level) and those of Kat 777 and Kat E31/4 were sown at Katumani during October-December rains. The total amount of rainfall received in both short and long rains at Kiboko was 280 mm in 64 rain days compared with the average of 550 mm in 100 days. The growing season was, therefore, fairly short and the plants were exposed to both intermittent and terminal droughts. At Katumani, sowing of the M_2 progenies was delayed by about 2 months from the normal time because the season was abnormally wet and no irrigation was applied thereafter. However, the crop received 238 mm of rainfall during the short rains and also 183 mm from the long rains. Visual scoring using a scale of 0 - 9 was adopted for three characters; leaf fall as a sign of drought stress, (0 - no leaf fall, 9 - all leaves fallen), wilting as a sign of *Fusarium* attack (0 - no wilting, 9 - very heavy wilting), podding as a sign of insect attack (0 - no insect damage, 9 - very heavy damage). At Kiboko, 30 single plants of Kat 60/8 that maintained green leaves at harvest were selected for further evaluation. The M_3 generation was sown during long-rains 1991 in a randomized block design trial, replicated twice and ratooned in the short-rains 1991. This trial

received a 360 mm rainfall of which 120 mm was received by the ratoon crop. The trial was not.protected against insects. From the M_3 generation, 15 single plant selections based on grain yield and tolerance to wilt were harvested, and sown in December 1991 for further evaluation. At Katumani, 15 single plant progenies - selections from the M_2 generation of Kat 777, based on earliness, yield and wilt tolerance - were sown in an artificially made wilt sick plot in the short-rains 1991. Scoring was done for wilt tolerance, insect damage and grain yield in long-rains of 1992.

3. RESULTS AND DISCUSSION

The visual scoring for leaf-fall as a sign of both drought stress and susceptibility to leaf spots, maturity and grain yields of best selected M_2 progenies of Kat E 31/4 and Kat 777 are presented in Tables I and II. Of the 200 progenies in the M_2 generation of Kat E31/4, only 10 progenies gave higher yields compared with the check. The highest yielding progenies were T1P8 and T3P4. Progeny T1P8 produced more pods than the rest but all progenies were more or less equally affected by wilt. However, progenies T1P3 and T2P27 produced the heaviest leaf-fall. Three progenies, namely, T1P6, T3P4 and T3P26 flowered earlier than the local check.

TABLE I. VISUAL RATING FOR LEAF-FALL, MATURITY, PODDING AND YIELD OF E31/4 PROGENIES IN THE M_2 GENERATION

Progeny	Wilting signs	Leaf fall	Maturity	Podding	Yield g/plot
T1 P3	5	7	M	4	1404
T1 P8	5	3	L	2	2078
T1 P6	6	6	E	3	1068
T1 P26	5	5	M	5	1133
T2 P6	5	6	M	4	1235
T2 P27	6	7	L	5	952
T2 P42	4	3	L	3	1480
T3 P6	5	4	L	4	1054
T3 P4	5	4	E	4	2078
T3 P26	6	4	E	4	1133
Check	6	6	L	5	920

T - treatment dose: T1 = 80-100; T2 = 110-125; T3 = 140-150 Gy.
P - progeny: M - medium (compared to check); E - early; L - late.
Rating Scale: 1.leaf fall - 0-9 (0 - no leaf fall; 9 - all leaf fall); 2. podding - 0-9 (0 - heavy podding; 9 - no pods); 3. wilting - 0-9 (0 - no wilting; 9 very heavy wilting)

In the M_2 generation of Kat 777, 13 of the 200 progenies yielded higher than the local check (Table II). Progeny T2P14 was the highest yielder. This progeny and the another produced more pods than the rest. Four progenies, namely T2P6, T2P9, T2P12 and T2P20 matured earlier than the local check variety. T2P14, T2P18, and T3P6 showed less leaf-fall. In general, M_2 progenies of Kat 777 showed less leaf-fall than those of Kat E31/4 probably because Kat E31/4 is more susceptible to *Cercospora* leaf spot, besides the effects of droughts.

TABLE II. VISUAL RATING FOR MOISTURE STRESS, LEAF FALL, MATURITY AND PODDING AND GRAIN YIELD OF SELECTED M_2 PROGENIES OF KAT 777

Progeny	Wilting sign	Leaf fall due to stress	Maturity	Podding	Yield g/plot
T1 P2	5	4	M	3	1381
T1 P3	5	5	M	5	1105
T1 P10	5	6	M	5	1000
T1 P6	6	5	E	6	923
T2 P9	5	4	E	5	1045
T2 P12	5	3	E	4	1197
T2 P14	4	4	M	2	1812
T2 P18	4	3	M	2	1605
T2 P20	5	4	E	3	1589
T3 P5	6	3	M	6	1089
T3 P6	4	4	M	5	1115
T3 P7	5	4	M	3	1447
T3 P19	5	5	M	4	1457
Check	6	5	M	5	911

T - treatment (T1 = 80-100; Gy; T2 = 110-125 Gy; T3 = 140-150 Gy). P - progeny- M - medium maturity (compared to check); E - early maturity; L - late maturity.
Rating Scale: 1. leaf fall - 0-9 (0-no leaf fall; 9 - all leaf fall); 2. podding - 0-9 (0 - heavy podding; 9 - no podding); 3.wilting - 0-9 (0 - no wilting; 9 - very heavy wilting)

Twelve of the best single plant selections in the M_3 of Kat 60/8 at Kiboko produced grain yields that did not differ significantly from those of the check variety Kat 60/8 (Table III). Except for T1P16, none of the other progenies was flowered earlier than Kat 60/8. In the M_4 generation of Kat 60/8, two progenies, namely, T351 and T2P112, were the highest yielding (Table IV). These progenies also flowered and matured earlier than the local check variety, Kat 60/8. However, T1P76 was the earliest maturing and the shortest.

The results of the best performing eight progenies in the M_3 generation of Kat 777 and of seven progenies of Kat E31/4 are presented in Tables V and VI, respectively. All the irradiated progenies gave higher grain yield than Kat 777, four of which were significantly better. Progeny T3P13 was the highest yielding. However, none flowered or matured earlier than the local check variety. Again, all the treated progenies were taller than Kat 777. In the M_3 generation of Kat E31/4, three progenies, namely, T1P21, T2P17 and T2P28 out-yielded the check variety. Nevertheless, their flowering and maturity did not differ significantly from that of Kat E31/4.

TABLE III. PERFORMANCE OF SINGLE PLANT SELECTIONS OF M_3 GENERATION OF KAT 60/8 AT KIBOKO DURING SHORT-RAINS 1990

Entry	Days to flower	Days to maturity	Plant stand	Yield (kg/ha)
1. T1P6	87	146	40	1405
2. T1P16	82	144	43	1583
3. T1P38	90	146	38	1435
4. T1P73	89	147	45	1515
5. T1P78	89	146	39	1413
6. T2P75	88	146	44	1405
7. T2P106	90	145	38	1470
8. T2P111	86	145	45	1503
9. T2P112	89	147	38	1425
10. T3P25	86	145	36	1416
11. T3P51	88	146	42	1494
12. T3P59	87	146	35	1423
13. KAT60/8	84	147	40	1424
S.E.	2.4	0.9	5.0	164.0
CV%	3.3	0.9	15.0	29.0

Most of the Kat 777 progenies in the M_2 generation, sown in the *Fusarium* wilt sick plot showed serious symptoms of wilting 42 days after sowing (seedling stage), and also at a later stage, 84 days after sowing (reproductive stage). Of the 10,040 irradiated plants, only 245 survived. Table VII shows that most of the surviving plants were from the treatment dose 80-120 Gy.

TABLE IV. PERFORMANCE OF SINGLE PLANT SELECTION OF M_4 GENERATIONS OF KAT 60/8 AT KIBOKO DURING SHORT RAINS 1991

Progeny	Days to 50% flower	Days to 75% maturity	Plant height (cm)	100 seed weight (g)	Grain yield (kg/ha)
1. T3P51	86	119	92	10.3	2260
2. T2P112	86	121	96	10.5	2240
3. T2P75	87	119	100	10.2	2070
4. T1P82	87	121	87	10.1	1870
5. T1P76	84	120	83	10.3	1750
6. T1P18	90	125	102	9.8	1690
7. T2P11	90	125	102	11.5	1670
8. T1P71	90	125	103	11.0	1640
9. T1P38	89	125	104	11.1	1510
10. T1P73	91	127	100	11.2	1460
11. T1P95	91	126	93	11.4	1230
12. T1P16	91	126	93	10.4	1190
13. T3P15	87	125	93	11.3	1090
14. T1P38	89	126	89	11.0	1042
15.KAT 60/8	83	121	94	10.3	2115
S.E.	1.6	1.8	6.9	0.4	250
CV%	3.2	1.7	12.5	5.7	26.8

TABLE V. PERFORMANCE OF SINGLE PLANT SELECTIONS OF M_3 GENERATION OF KAT 777 AT KATUMANI 1991 - 1992

Progeny	Days to 50% flower	Days to 75% maturity	Plant height (cm)	100 seed weight (g)	Grain yield (kg/ha)
1. T1P3	120	177	119	16.7	1600
2. T2P9	120	176	143	14.8	1320
3. T2P7	121	176	139	16.9	1776
4. T2P12	117	168	115	16.8	1768
5. T3P8	119	168	118	16.6	1906
6. T3P13	117	176	119	16.4	2150
7. T1P10	125	172	106	15.2	1486
8. T3P19	131	182	166	16.1	2033
9. Check KANT 777	101	161	97	16.2	1294
SE	2.3	3.9	16.3	2.2	461
CV%	2.4	2.7	15.6	16.5	31.8

Treatment: T1 - 80-100 Gy; T2 - 110-125 Gy; T3 - 140-150 Gy.

TABLE VI. PERFORMANCE OF SINGLE PLANT ELECTIONS OF M_3 GENERATION OF KAT E31/4 AT KATUMANI DURING SHORT-RAINS 1991 - 1992

Progeny	Days to 50% flower	Days to 75% maturity	Plant height (cm)	100 seed weight (g)	Grain yield (kg/ha)
1. T1P21	149	189	143	16.4	1900
2. T2P17	159	184	138	17.6	1728
3. T2P28	147	195	126	17.6	1533
4. T2P7	144	187	120	17.2	1412
5. T2P6	144	189	120	15.7	1306
6. T1P31	143	188	137	18.1	1247
7. T3P4	153	207	147	16.3	1177
8. KANT E31/4 Check	140	199	138	17.2	1080
S.E.	6.4	6.9	13.7	1.4	242.6
CV%	5.3	4.4	12.6	10.1	62.7

Treatment: T1 - 80-100 Gy; T2 - 110-125 Gy; T3 - 140-150 Gy.

TABLE VII. NUMBER OF M_4 PROGENIES OF KANT 777 SHOWING WILT SYMPTOMS AT FORTNIGHTLY INTERVAL UP TO MATURITY AT KATUMANI WILT SICK PLOT DURING SHORT-RAINS 1991 - 1992

	T0	T1	T2	Check
Seedling count 14 DAP	57	1973	1959	68
No. of plants wilting				
28 DAP	24	743	360	28
42 DAP	8	206	199	15
56 DAP	4	167	116	8
70 DAP	3	149	127	14
84 DAP	10	171	81	3
98 DAP	8	305	665	-
112 DAP	-	62	236	-
Total wilted plants	57	1803	1784	68
No. without wilt signs	-	170	75	-

DAP - Days after planting.
Treatment: T0 - No treatment; T1 - 80 - 100 Gy; T2 - 110 - 125 Gy.

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MUTAGENESIS IN YAM, *Discorea rotundata*: CLONAL EVALUATION OF M_1V_3 YAM PLANTS

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Abstract

Ten thousand plants of M_1V_3 population of the white guinea yam, *Dioscorea rotundata* Poir, were evaluated. There were no consistent trends in variations in plant height, number of branches, branching heights and number of leaves of the treated tubers. However, plant height were lower in the irradiated than in the control. The coefficients of variations (C.V.) were higher in the irradiated than in the control populations, indicating wider variations in the former population. Based on yield performance, 110 tubers were selected for preliminary yield trials. Considering that diplontic selection may occur in the irradiated micro-tubers, the experiment will also be carried out using nodal cultures *in vitro*.

1. INTRODUCTION

The genetic improvement of white guinea yam, *Dioscorea rotundata* Poir, through conventional breeding methods is difficult. This is because only about 5% of the cultivars flower and even these are shy in flowering with poor fruit and seed set. Also, the incidence of heterozygosity and high ploidy levels make yam seedlings segregate so widely that the probability of recovering useful recombinants in very low. Mutation breeding is a useful option for the genetic improvement of this crop. During the 1989 and 1990 cropping seasons, the radio-sensitivity was established for yam micro-tubers which ranged between 20 to 40 Gy. This report presents the results of evaluation of the M_1V_3 population, derived from micro-tubers irradiated with 10, 20, 30 and 40 Gy gamma rays.

2. MATERIALS AND METHODS

About ten thousand M_1V_3 plant population which had undergone clonal multiplication during 1990 (M_1V_1) and 1991 (M_1V_2) seasons was evaluated in a progeny-to-row experiment during 1992. The M_1V_2 tubers, used to establish the M_1V_3 plants, were cut into mini-sets (25-30 g) and planted in rows 1 m x 0.25 m. Cultural practices included staking and weeding. Data were collected on plant height, number of branches, branching height and number of leaves per row. The harvested tubers were grouped into classes of over 250, 200-249, 150-199, 100-149, 50-99 and less than 50 g.

3. RESULTS AND DISCUSSION

There were no consistent trends in variations for plant height of treated tubers (Table I). However, the plant height of the control was more than those of the treated. The same trend was evident when data collected on number of branches, branching height and number

of leaves per hill were compared. The coefficients of variation (C.V.) was higher in the treated than in the control population indicating a wider variations in these populations. This is agreement with the observed trends in mixed populations resulting from hybridization including mutation induction. When tuber-harvest data were analyzed, the C.V. was found to be higher in both the treated and the control (Table II). There was no consistent trend in their yields when the mean tuber yields per stand of controls and treated plants were compared. On the other hand, 110 tubers, each weighing more than 250 g were selected from populations treated with 10, 20, 30 and 40 Gy, for preliminary yield trials. Future trials will include screening for major pests and diseases of yams and also some biochemical qualities such as starch content and quality. Since diplontic selection may not be ruled out in the irradiated micro-tubers, this experiment will also be carried out using nodal cultures.

TABLE I. VEGETATIVE CHARACTERS OF M_1V_3 POPULATION OF YAM

Gamma ray dose (Gy)	Plant height (cm)			Number of branches			Branching Height (CM)			Number of Leaves			Sample No. (n)
	Mean	S.E.	C.V. (%)	Mean	S.E.	C.V. (%)	Mean	S.E.	C.V. (%)	Mean	S.E.	CV (%)	
0	74.1	4.2	31.3	2.3	0.2	48.6	5.1	0.3	34.1	54.8	6.4	63.7	30
10	63.9	4.8	46.7	2.9	0.2	50.2	5.3	0.6	66.0	42.5	4.8	69.3	38
20	71.1	6.1	78.3	2.7	0.2	46.9	5.2	0.3	53.0	37.0	2.6	65.3	84
30	67.5	5.9	65.6	2.7	0.2	58.2	6.3	0.3	95.0	32.3	3.2	73.5	56
40	64.5	7.5	59.5	2.2	0.2	55.0	5.3	0.5	50.3	27.1	3.8	71.1	26

TABLE II. TUBER YIELD OF M_1V_3 POPULATION

Gamma ray dose (Gy)	Tuber yield per row (g)			Tuber Size distribution (g)			100-149	50-99	Less than 50	Total
	Mean	S.E.	CV (%)	Over 250	200-249	150-199				
0	113.3	15.1	100.0	5	85	-50	270	399	200	1 009
10	113.8	10.1	83.8	25	164	234	600	410	500	1 933
20	144.5	8.9	81.5	40	200	328	720	960	800	3 054
30	94.6	8.6	99.0	30	70	120	400	730	1 195	2 545
40	79.8	9.9	122.0	15	-	100	250	210	420	995

IMPROVEMENT OF CASSAVA QUALITY THROUGH MUTATION BREEDING

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1. INTRODUCTION

Ghana has not been able to take advantage of the high-yielding cassava varieties developed by the International Institute of Tropical Agriculture (IITA) because these varieties generally do not have the desired cooking quality. The major emphasis of this project therefore is to use mutations to produce varieties with the desired starch characteristics while maintaining the disease-resistance and high-yielding characteristics of the IITA varieties.

2. MATERIALS AND METHODS

The following two approaches reported during the last RCM have been followed during the past two years: 1. selection within irradiated populations for disease and pest resistance, high yield and good cooking quality. 2. research to understand the physico-chemical properties of cassava starch.

In 1991, we reported the screening of the M_1V_2 generation of varieties 'ISU-W', '1425-LB', '1425-W' and '518-DB'. A number of promising mutants were identified especially in ISU-W. Much effort has been subsequently concentrated on the M_1V_3 and M_1V_4 of this variety.

3. RESULTS AND DISCUSSION

During 1991, in 'ISU-W' M_1V_3, 197 plants were individually examined for tuber yield and cooking quality. Only those plants with yield above 2 kg, and which showed mealiness, smoothness and elasticity of pounded paste score of 3 or above were selected. Thus, 32 plants were selected and five cuttings were planted of each selected plant. The yield and cooking quality and the characteristics of the plants selected are shown in Tables I and II.

During 1992, the M_1V_4 generation was examined as in the previous year; in addition, dry matter content was also determined. The yield, dry matter and cooking quality characteristics of the lines selected are shown in Table III. The selected lines were planted in 0.5 acres. The 1993 harvest, which is not completed yet shows that all the plants have very good cooking quality and high tuber yield. The characteristics of those harvested are shown in Table IV. The high yielding ability combined with good cooking ability of this new variety has brought tremendous benefit to the workers of the Arable Crops Section who have known the poor cooking quality of the initial introduction, and are now witnessing the improved cooking quality. The Director, Regional Crop Services was invited to witness the high yield and improved cooking quality.

TABLE I. FREQUENCY DISTRIBUTION OF YIELD AND COOKING QUALITY SCORES OF M_1V_3 ISU-W POPULATION

Tuber Yiled/Plant (Kg)	Frequency	Cooking Quality Score*	Frequency Mealiness	Frequency Smoothness	Frequency Elasticity
0.5	26	0 - 1	6	6	3
0.6 - 1.0	47	1 - 1.9	5	1	2
1.1 - 1.5	40				
1.6 - 2.0	36	2 - 2.9	28	22	13
2.1 - 2.5	15	3 - 3.9	89	71	72
2.6 - 3.0	10	4	70	101	112
3.1 - 3.5	8				
3.6 - 4.0	4				
4	1				

*Score
0 - 4 None mealy texture to very mealy texture
0 - 4 Lumpy pounded paste to increasing degree of smooth and elastic paste

Cuttings from this variety have been given to the Crop Services Department and have been included in this year's regional multi-location trial in farmer's fields. We are following these trials to monitor the results. A multi-location trial would be carried out next year. If the variety maintains its performance in these trials, it could be released for cultivation by 1995. Other irradiated varieties which are being examined include 60142, 30474, 30001 and 1425-LB.

Studies on the cassava starch

The studies on cassava starch and cooking quality reported at the 1991 RCM have been published [1]. Under a six-month IAEA fellowship at the National Institute of Nutrition, Rome studies on the physico-chemical properties of cassava starch in relation to cooking quality were carried out. The proximate composition, starch content, amylose content, changes in viscosity on heating using the Brabender amylograph, the swelling-power and solubility and the water-binding capacities of the IITA and local varieties in relation to their cooking qualities were studied. A lot of data was generated which showed that the characteristics of the starch rather than the total amount has a great effect on the cooking quality.

A related study on the effect of age at harvest on the cooking quality, dry-matter and starch characteristics of four cassava varieties was completed last June. The starch characteristics studied were swelling-power, solubility and water-binding capacity. The results showed how these starch characteristics affect the cooking quality which falls with the onset of the rainy season.

TABLE II. YIELD AND COOKING QUALITY CHARACTERISTICS OF PLANTS SELECTED IN M_1V_3 1991

Plant No.	Top growth (Kg)	Tuber yield (Kg)	Harvest Index	No. of Tubers	Cooking Quality		Elasticity
					Mealiness	Smoothness	
1.	2.0	3.0	1.50	4	4	4	4
2.	2.4	3.0	1.25	5	3	4	4
3.	1.2	2.0	1.67	4	3	3.5	3.5
4.	1.5	2.2	1.47	6	3	4	4
5.	1.4	2.2	1.57	5	4	3	3
6.	2.7	2.7	1.00	4	4	4	4
7.	1.9	2.7	1.42	7	4	3	3
8.	1.9	2.5	1.32	4	3.5	4	4
9.	2.1	2.2	1.05	6	4	4	4
10.	2.6	2.7	1.04	7	3.5	4	4
11.	2.7	3.8	1.41	12	3	3.5	4
12.	1.1	2.0	1.82	6	3	4	4
13.	5.8	6.5	1.12	4	4	4	4
14.	3.2	3.2	1.00	8	3.5	4	4
15.	1.5	2.3	1.53	3	4	3	3
16.	1.5	2.3	1.53	3	4	3	3
17.	1.8	3.2	1.78	9	4	4	4
18.	1.7	2.1	1.24	6	4	3	3
19.	2.3	3.4	1.48	8	4	4	4
20.	1.6	2.5	1.56	8	3	3	4
21.	2.3	3.1	1.35	5	3.5	4	4
22.	1.5	2.9	1.93	5	4	4	4
23.	3.0	3.7	1.23	5	4	4	3.5
24.	1.3	2.1	1.62	6	3.5	4	4
25.	2.9	3.8	1.31	7	3.5	4	4
26.	1.9	2.9	1.52	5	3	4	4
27.	1.2	2.0	1.67	5	3.5	3	4
28.	2.1	3.0	1.43	3	3	4	4
29.	1.9	2.9	1.53	5	4	4	4
30.	2.6	3.7	1.42	5	4	4	4
31.	1.9	3.5	1.35	9	3	4	4
32.	1.8	3.5	1.94	9	3.5	3	4

Studies on *Solanum torvum*

Solanum torvum is a wild species from which berries are used in stews and soups, and are reported to have lactogenic properties. Therefore, lactating mothers and anaemic patients are encouraged by doctors and nurses to include it in their diets. It also has many other medicinal uses. However, its sharp, hard spines or thorns on the stems and leaves make its cultivation difficult. It also has a large number of tiny indigestible seeds, and is a perennial. It is our objective to obtain a thornless mutant with reduced number of seeds and annual growth habit. Radio-sensitivity study showed 60 Gy as the appropriate dose. In M_2, a chlorophyll-deficient plant was observed but no useful mutations were obtained. M_3 seeds were re-irradiated and gave an M_1 thornless chimera. The M_2 will be studied to obtain solid mutant. This work was presented as a poster at the International Genetics Congress, Birmingham, U.K.

TABLE III. YIELD AND COOKING QUALITY CHARACTERISTICS OF PLANTS SELECTED IN M_1V_4 - 1992

Plant No.	Tuber Yield (Kg)	% Dry Matter	Cooking Quality		
			Mealiness	Elasticity	Smoothnes
1.	3.24	40.64	2.3	2.5	2.3
2.	1.55	39.90	2.3	2.8	2.8
3.	7.38	41.07	2.5	2.8	2.7
4.	1.89	42.49	2.3	2.7	2.3
5.	1.23	36.96	2.2	3.0	2.7
6.	1.54	43.20	2.3	2.8	2.7
7.	2.00	39.12	2.5	3.0	2.8
8.	0.64	39.17	2.4	2.8	2.8
9.	2.46	37.29	1.8	2.5	3.0
10.	0.28	34.20	1.3	2.0	1.8
11.	1.10	39.41	2.1	2.9	2.8
12.	0.55	41.18	1.7	2.3	2.2
13.	1.89	40.23	1.3	2.7	2.1
14.	1.06	39.06	1.6	2.1	1.9
15.	0.73	39.52	2.4	2.8	2.3
16.	0.70	42.64	1.3	2.2	1.8
17.	0.56	43.05	1.6	2.8	2.0
18.	0.78	39.77	2.2	2.8	2.4
19.	0.99	44.95	2.5	2.3	2.5
20.	0.83	42.03	2.5	2.7	2.6
21.	1.17	40.87	1.8	2.8	2.7
22.	1.08	36.20	1.7	2.4	2.4
23.	0.68	44.03	2.2	3.0	3.0
24.	0.77	41.73	2.2	2.8	2.7

TABLE IV. YIELD CHARACTERISTICS OF M_1V_5 PLANTS HARVESTED - 1993

	SET A	KG	SET B	KG	SET C	KG
Row No.	Av. No. of Tubers/Plant	Av. Tuber Yld./Plant	Av. No. of Tubers/Plant	Av. Tuber Yld./Plant	Av. No. of Tubers/Plant	Av. Tuber Yld./Plant
1.	4.4	1.87	8.6	4.46	7.0	5.61
2.	8.9	7.08	11.5	6.13	7.7	5.05
3.	7.8	3.83	12.8	6.28	7.5	5.51
4.	8.4	7.72	7.1	4.13	7.1	8.03
5.	8.8	5.87	11.0	6.81	6.2	3.90
6.	7.8	4.89	9.1	5.59	6.9	4.49
7.	7.4	7.25	9.1	6.91	7.9	6.74
8.			7.6	4.80	7.1	4.65
9.			6.8	4.62	8.0	9.52
10.			7.0	4.26	5.9	2.64
11.			7.4	5.20	7.9	5.85
12.			10.8	7.93	8.8	5.98
13.			6.8	4.75	7.5	3.81
14.			7.0	4.78		
15.			8.0	6.89		
16.			6.6	4.88		
17.			6.8	6.41		

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EMBRYOGENESIS IN SWEET POTATO, *Ipomoea batatas* (L.) LAM

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1. INTRODUCTION

Sweet potato *(Ipomoea batatas* (L.) Lam) ranks sixth among the cultivated crops of the world. In fact, it represents a major staple food in many tropical countries. Recently this crop has been proposed as a source of starch for industrial utilization. Somatic embryogenesis could prove useful as an alternative to traditional propagation by cuttings, which is labour intensive and can transmit diseases. Somatic embryos are reported to originate from single cells, so that, if regenerated from mutagenized tissues, should give rise to solid mutants.

2. MATERIALS AND METHODS

In a previous work [1], five clones of sweet potato were tested for their somatic embryogenesis capability. The Q 23728 clone showed the best performance with 483 regenerated plants. To verify their genetic uniformity, morphological traits of 81 regenerated plants and 6 test plants of Q 23728 clone were studied. Of these 81 plants, 45 were previously micropropagated, so that a total of 126 plants plus 8 test plants were tested. Plants were transferred in pot from test tubes, and then transplanted in field in May, 1992 at a density of one plant per square meter and standard agronomic practices were followed during growing season. For evaluation, we used descriptors for sweet potato [2].

3. RESULTS AND DISCUSSION

In case of 10 out of 14 morphological characters, related to green parts of the plant, there was neither difference between regenerated and test plants nor among the regenerates. Variation was found for abaxial leaf vein pigmentation in 19 plants (15%), for mature leaf colour in 9 plants (7%), for immature leaf colour in 48 plants (38%) and for petiole pigmentation in 14 plants (11%). Observations on flowers were not possible because no flowering occurred. Many plants, including among the tests, had roots with irregular shape, possibly resulting from the rather compact structure of soil. The mean weight of storage roots of regenerated plants was 95% higher than in test plant.

Root skin colour showed slight change in 31 regenerated plants (29%) and marked change in one plant; six regenerants (5%) showed more coloured flesh, while 23 (21%) showed one or more coloured rings in flash. Six of the 45 micropropagated plants showed a secondary flash colour, being different in two plants which originated from the same regenerant. This difference may be due to chimerism during ontogenesis. If this is the case, somatic embryo did not always originate from a single cell. Many authors have reported the occurrence of somaclonal variation after somatic embryogenesis in sweet potato. To our knowledge, this is the first report of occurrence of variation among sweet potato plants

derived from somatic embryo. The observed variation should be confirmed by molecular and biochemical analyses; however, its occurrence has an important consequence in the multiplication and breeding of sweet potato.

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UTILIZATION OF MUTAGENESIS FOR IMPROVEMENT OF YAM

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1. INTRODUCTION

Yam (*Dioscorea* spp.) is an important vegetable crop in tropical countries of Africa, the Caribbean, South America, and South East Asia. The tubers which contain 2-3 times more protein than sweet potato, cassava and plantain, constitute a major staple food for about 200 million people in West Africa. This area contributes 96% to the world production of yam [9].

Genetic improvement is needed to solve numerous problems that reduce yam production. Many yam varieties do not flower or have irregular flowering or have only female or male flowers, are heterozygous and polyploid. Consequently, for many years from the hybridization programs which were initiated in Nigeria [10,11] and Gouadeloupe [6] only promising families were selected, and no improved varieties were released. Therefore, mutation breeding can be used in good varieties to obtain some desirable characters in short duration of 1-3 years, without altering the genetic background. Mutation work in yam so far is very limited. Abraham [1,2] obtained high yielding mutants by treating yam tubers with gamma-rays. Koo and Cuevas-Ruiz [12] treated aerial tubers with 20 Gy gamma-rays at 10 Gy/mn, and observed solid shoot mutants. With 150 Gy X-rays, Pal and Sharma [16] Murthy and Ramarao [14] increased diosgenin content in tubers. Recent studies at IAEA Laboratories and Nigeria showed that yam microtubers, buds and *in vitro* plantlets respond to gamma radiations and to ethyl methanesulfonate [7,8,13].

The goals of this mutation breeding program is to obtain from selected yam varieties, plants that are tolerant to viruses or to mealy bugs, are erect or semi-erect in habit (bushy architecture), are able to grow without staking and have a short growing season.

2. MATERIALS AND METHODS

In this study (Fig.1), the selected varieties will be propagated by *in vitro* single nodes culture. Mutation treatment will be done on *in vitro* plantlets. The treated plantlets will be multiplied 2-3 times from *in vitro* single node culture before planting in field. Selection will start after 2 years in the field. One goal of *in vitro* clonal propagation of yam from meristems and single node stem cuttings is to multiply rapidly the needed variety. This technique is now well established in many yam species [3,4,15,16,17].

Young stems, apical sections of stem or branches were collected from plants grown in a field or nursery. The vines were cut in the midpoint of the internode and the leaves were moved by cutting the petioles from the node. The segments were pre-treated with detergent solution for 5 mm, and washed with tap water. For surface sterilization, the segments were

first dipped in 70% ethanol for 30 sec and rinsed 3 times with sterile distilled water. Small end of the internode and petiole were cut before immersion in 5% Ca (OCL)2 solution with a few drop of detergent for 25 min. After rinsing 3 times with sterile distilled water, each end of the petiole and nodal segments was trimmed again to obtain about 1-2 cm explant, and kept in sterile distilled water. The explants were inoculated one per culture tube or 2-3 per flask.

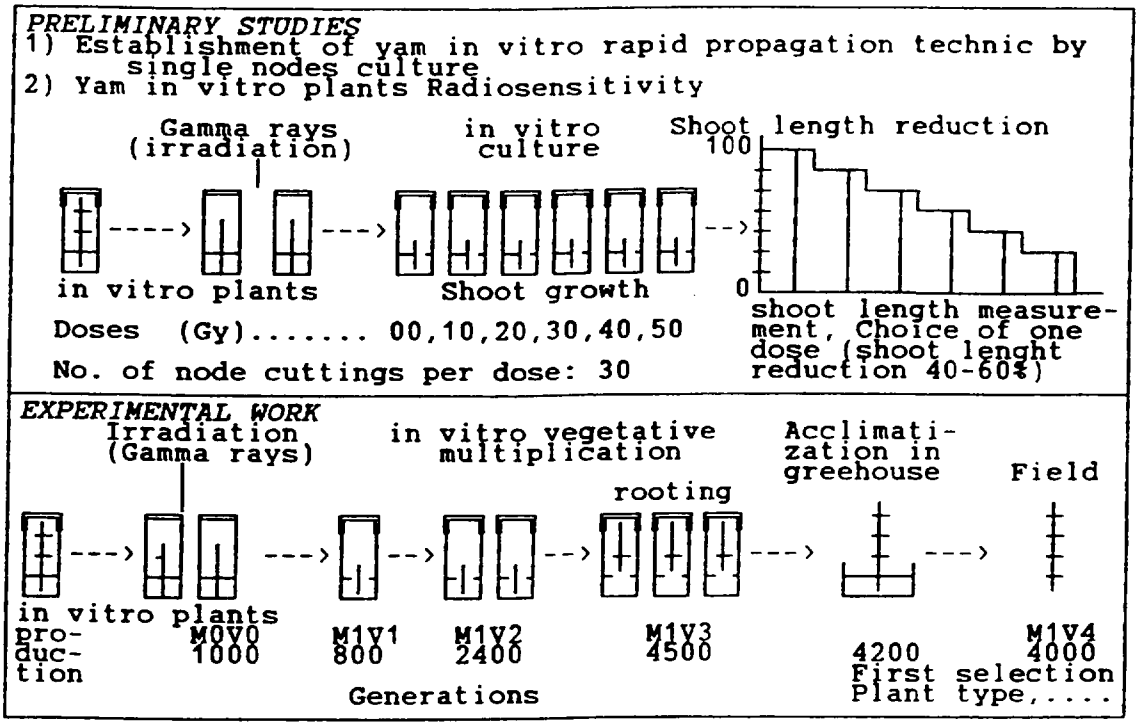

Fig. 1. Yam mutation breeding by clonal *in vitro* propagation from single nodes culture

The basal medium (Table 1) used was Murashige and Skoog's salts The medium M_1 with increased levels of thiamin 5 mg/l, pyridoxin 1 mg/l, nicotinic acid 1 mg/l, was not supplemented with growth regulators. The medium M_0 with a lower level of thiamin 0.4 mg/l was supplemented with IAA 0.17 mg/l and NAA 0.19 mg/l. The medium M_2 differed from M_3 in growth regalators all media were supplemented with 30 g/l sucrose, solidified with 8 g/l agar, and adjusted to pH 5.7. The media were dispensed in test tubes (20 cm^3) or flasks (40 cm^3), and sterilized by autoclaving at 121°C at 1.05 kg/cm^2 for 15 min. The un-used media were stored in a refrigerator. After inoculation, the cultures were incubated at room temprature (27 °C under daylight or laminar flow light).

3. RESULTS AND DISCUSSION

The first culture experiments were done on three media, M_0, M_2 and M_3 (Table I). After several weeks, no response was noted on the three media. A few days after inoculation, many cultures we infected mainly by fungi. The infection rate was about 80%. Several colours of mycelium were noted showing that there we several types of fungi. A few cultures were also infected by viruses. Some explants dried up, others stayed green.

TABLE I. COMPOSITION OF THE MEDIA USED FOR THE *IN VITRO* CULTURE

Ingrédients	M0 (mg/l)	M1	M2	M3
Major salts				
KNO3	1900.000	+	+	+
NH4NO3	1650.000	+	+	+
CaCL2.2H2O	440.000	+	+	+
MgSO4.7H2O	370.000	+	+	+
KH2PO4	170.000	+	+	+
FeSO4.7H2O	27.800	+	+	+
Na2EDTA.2H2O	37.300	+	+	+
Minor salts				
MnSO4.H2O	22.300	+	+	+
H3BO3	6.200	+	+	+
ZnSO4.4H2O	8.600	+	+	+
KI	0.850	+	+	+
Na2Mo4.2H2O	0.250	+	+	+
CuSO4.5H2O	0.025	+	+	+
CoCL2.6H2O	0.025	+	+	+
Vitamins, Amono acids				
Glycin	2.000	2.000	2.000	2.000
Thiamin.Hcl	0.400	5.000	1.000	1.000
Pyridoxin.Hcl	0.500	1.000	0.500	0.500
Nicotinic Acid	0.500	1.000	0.500	0.500
Myo-Inositol	100.000	100.000	100.000	100.000
Ascorbic Acid	20.000	20.000	20.000	20.000
Growth regulators				
IAA	0.170	0	3.500	3.500
NAA	0.190	0	3.720	0.000
Zeatin	0	0	0.500	0.500
Kinetin	0	0	0.500	0.500

The culture contamination by fungi may have resulted from surface sterilisation of the explants or from the culture media.The drying or the non-sprouting of the explants in uncontaminated cultures may have resulted from the damage by the chemicals used for surface sterilization. At the beginning, the explants were disinfected with 100% ethanol and 9% CA(OCL) which could be toxic to the tissues. To avoid the toxicity during surface sterilization, 70% ethanol and 5% Ca(COL)2 were used in the subsequent experiments. The media with growth regulators and low level of thiamin (0.4-1mg/l) were not suitable.

Based on the previous observations, the surface sterilisation of the explants was improved by cutting the end of the internode and petiole after dipping in alcohol and Ca(COL)2. The basal medium (M_1) of Murashige and Skoog with increased level of thiamin (5mg/l), pyridoxin (1 mg/l) and nicotinic acid (1 mg/l) and without growth regulators were used subsequently.

Based on the new sterilization protocol and the use of medium M_1, and about 3 months culture, the following results were obtained (Table II). The improved surface sterilisation was more effective in controlling contamination. Only 7% of cultures were infected by *Penicillium* spp. The contamination was more in the explants obtained from old stem section. About 39.2% of the nodes sprouted, but the rate of non-sprouted explants was still too high, 60.7%. Generally, the explants from very young stem sections or apical nodes did not sprout.

TABLE II. EFFECT OF IMPROVED STERILIZATION AND CULTURE ON M_1 MEDIUM ON EXPLANTS OF *D. rotundata* CV. 'KPONA' THREE MONTHS AFTER CULTURE.

Observation	Percent
Fungus infection	7.0
Sprouted explants	39.2
Sprouted explants with leaves	35.0
Non-sprouted explants	60.7

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CONCLUSIONS AND RECOMMENDATIONS

GENERAL

In the projects based on mutation induction, it is better to select for a single trait than for several traits at the same time. Mutant germplasm produced by various research groups should be exchanged with the participants, and field tested in their National programs. Rapid distribution of improved germplasm in African countries should be promoted, and scientists should create awareness of the newly developed germplasm to other scientists through personal contacts and Mutation Breeding Newsletter. Efforts should be made to transfer traits from the mutants to other varieties, especially to the local varieties. Particular attention should be given to the biochemical characters for consumer acceptance, and to yield stability of the genetic material to be released as varieties. Other species with similar problems which can be improved through mutation techniques include Bambara groundnut in Mali for selection for late maturity, synchronous flowering, cooking quality and white kernels; micropropagation of nitrogen fixing leguminous trees in Ghana, and improvement of *Dolichos* with reduced phenolic compounds in Kenya. Adequate population size should be used in the experiments as recommended in the IAEA manuals. As far as possible, explants for tissue culture should be taken from virus-free materials. There are now established tissue culture laboratories in several participating countries. In addition to research on *in vitro* irradiation, these laboratories should also be used to clean up plant material for propagation and distribution to the farmers.

In general, equipment was not a limiting constraint in the implementation of the programme, and all the equipment provided in the project was very valuable. Support for equipment, training and visits should be continued during the second phase of the programme. Whenever possible, scientists must depend (purchase and use) on locally available equipment and consumables. Training should be provided for the maintenance of equipment, and funds should be made available for repair and servicing of the equipment. Training should be provided in anther culture, embryo rescue, selection for drought tolerance, genetic characterization of mutants, and other advanced genetic manipulation techniques. It is requested that a training course should be organized in one of the French speaking countries.

Links between well established laboratories participating in *in vitro* and other techniques in relation to mutation breeding should be encouraged. Links between breeding and tissue culture laboratories working in this programme especially on the same crop should be strengthened. This should also include exchange of germplasm. Scientists should seek and continue co-operation with International Agricultural Research Centers such as ICRSAT, IITA, IRRI for germplasm resources and technology for crop improvement. Where ever simple conventional methods and techniques are available, these must be used in preference before undertaking sophisticated technologies.

IAEA Laboratories should continue to provide back-stopping activities for the development of protocols in relation to requests made e.g. the development of somatic embryogenesis and virus eradication techniques. Scientific information should be published; when possible summaries should be sent to Mutation Breeding Newsletter. Agency shall distribute such published material to all other participants.

LEGUMES AND GRAIN CROPS

CHICKPEAS

Studies have been carried out on transformation of chickpea to transfer insect resistance genes (α-amylase and proteinase inhibitors) at the Department of Agronomy and Plant Genetics, University of Naples, Italy. Several experiments were carried out to transfer genes by co-culture of meristematic cells with virulent and hyper-virulent *Agrobacterium tumefaciens* strains with two chickpea cvs. 'Sultano' and 'Principe' from Italy. The frequency of explants showing the expression of GUS (β-glucuronidase) ranged from 3 to 15%, but no transformed shoots were obtained.

It is recommended that this program should continue by using different explant sources as well as other co-culture conditions to obtain transgenic shoots with stable gene expression.

COWPEA

Two groups have been carrying out research on this crop. The aim of the group at the Department of Agronomy and Plant Genetics, University of Naples, Italy is to regenerate cowpea *in vitro* and to transfer genes expressing insect-resistance proteins (α-amylase and proteinase inhibitors). Regeneration has been obtained in a local and an African cultivars from explants of immature leaflets and hypocotyl segments of mature embryos. The regeneration frequency ranged between 0.8 to 17.1%. Histochemical studies have shown that regeneration initiates form the epidermal cells. Genetic transformation was obtained by electro-injection of the plasmid DNA directly into the shoot meristem, in which only transient gene expression was observed, or after co-cultivation of hypocotyl segments with two strains of *A. tumefaciens*, which gave 5 to 82% transformed tissue with stable expression of GUS gene. However, no transgenic shoots were obtained.

It is recommended that the research on the regeneration of the transgenic tissues should be continued by changing the medium composition and explant source, and develop a reliable protocol for the regeneration of solid transformed shoots from transgenic calli.

The objective of the group at the National Dryland Farming Station, KARI, Machakos is to improve the local cultivars for resistance to diseases, insects and drought. Seeds of the local varieties were irradiated, but so far this project has not progressed beyond growing M_1 material.

It is recommended the experiments should be repeated by irradiating new seed material in which selection should be carried out only for drought tolerant genotypes while maintaining the grain quality.

PIGEONPEA

The research on pigeonpea is carried out at the National Dryland Research Station, Machakos. The objective of this project is to screen for resistance to diseases and insect pests. Irradiated material was grown to M_4 populations without any positive gains.

It is recommended that elite local material adapted to Kenyan climate should be irradiated and selected for resistance to *fusarium* and drought as well as for improved cooking quality.

BAMBARA GROUNDNUT

The research on this crop has been carried out in Ghana with the objective to induce determinate flowering habit. Previous experiments with limited material but high doses (300 and 350 Gy) produced highly aberrant type plants in M_1, and the M_2 and M_3 populations were too small to be meaningful. Several local cultivars have been collected from the local growers to provide wide genetic diversity in the experimental material.

It is recommended that collected germplasm should be studied for phenotypic differences in seed and plant characters, flowering habit, maturity and yield. It is recommended that at least 5000 seeds from the selected material should be irradiated with 200 to 250 Gy, and then plants should be selected for early maturity in M_2 and other desired phenotypes such as determinate flowering.

RICE

The main objective of the research, carried out at the Institut d'Economie Rural, Mopti, Mali, has been to obtain non-shattering of grain in the local cultivars of African rice, *Oryza glaberrima*. In the M_5 populations, a number of lines selected for non-shattering were highly sterile. In addition, white caryopsis mutants were found. Cultivars with white grain have a higher market value than the normal red grain types. Three selected mutant lines will be multiplied during 1994 for field trials at five locations in 1995. Meanwhile, a second cycle of mutagenesis has been started to induce non-shattering mutants.

It is recommended that the sterile mutants should be crossed with fertile types to avoid loss of such mutants. The possibility of incorporating anther culture technique to obtain homozygous mutant lines from selected M_2 plants should be considered to speed up breeding.

SORGHUM

Several stable mutants with short culm, compact panicle, changed glume colour, improved grain quality and drought tolerance were obtained at the Polytechnique Rural de Kotibougou, Mali. Some of these lines were evaluated for morphological, physiological and biochemical characteristics in France. The drought tolerant line has very deep rooting system. So far this material has not been evaluated in the National trials. Difficulties have been experienced to release and spread these genotypes through the national system. The next phase of this programme should be the field evaluation of these mutants through the National system. The financial support necessary to accomplish this phase should be provided by the project to ensure that all the entries are incorporated in the field trails. These genotypes should also be used as a source of new genes to transfer the desired characters in the local cultivars through conventional crossing. Further evaluation of the grain quality shall be carried out.

It is recommended that the mutant lines should be tested at multi-location trials in Mali through the National System. Special attention should be paid to the grain quality. It is also planned to initiate a second cycle of mutagenesis to obtain late-flowering genotypes for areas with a long rainfall season.

ROOT AND TUBER CROPS

CASSAVA

In Ghana, the objective to improve the cooking quality of cassava has been achieved. Of the 37 putative mutants selected for improved cooking quality most compare in yield with the parental cultivar.

It is recommended that multi-location trials should be carried out with the selected mutants in the different ecological zones of Ghana. It is also essential to develop a simple and reliable technique to determine cooking quality based on dry matter. Additional cultivars should be radiated for improving cooking quality. Non-traditional uses of cassava starch and flour should be investigated with the mutants with improved cooking quality.

At Legon, Ghana, protocol has been established to harden the *in vitro* cultured plants for transfer to field. Previous tissue cultured material which was M_1V_2 population was lost. New material has since been established *in vitro*. Additional germplasm has been collected for screening resistance to viruses and high yield. Studies were initiated to develop protocol on somatic embryogenesis in the African cassava clones, but have not been successful beyond callus induction.

It is recommended that nodal cuttings of the new material should be irradiated and material should be planted as M_1V_2 plants in the field for selection to cassava mosaic virus.

In Nigeria, most of the tissue cultured material was also lost through contamination. New material has been initiated from stem cuttings and *in vitro* culture. In Uganda, most cassava material is highly infected with viruses. Microcultures of African cassava varieties have been established to obtain disease free material.

It is recommended that this material should undergo thermotherapy and indexing to obtain virus free material before any experiments are undertaken with mutation induction. This would require establishment of disease indexing facility at the Station. Further irradiation is not possible because the radiation source is broken down. It is also recommended that the Agency Laboratories at Seibersdorf should establish a protocol on somatic embryogenesis in cassava material from Africa. The polyploid material of cassava generated in Seibersdorf is available for field trials in Africa.

YAM

In Nigeria, 10,000 M_1V_3 plants were tested and showed wide variation. Of these, 110 clones were selected for preliminary yield evaluation. So far, no erect-type variants have been found which can stand without staking.

It is recommended that the putative mutants should be tested in large scale field trials at two locations. Irradiation of *in vitro* nodal cuttings should be continued and attempts should be made to establish protocols for somatic embryogenesis in the local yam cultivars.

In Cote d'Ivoire, there has been a major problem in developing a suitable method of *in vitro* propagation of yam, and hence no material was irradiated. It is recommended that the conventional material (microtubers) rather than tissue cultured material should be irradiated to induce mutations. The protocol of yam micro-propagation needs to be developed.

SWEET POTATO

At ENEA, Policaro, Italy, a protocol was developed for somatic embryogenesis in 5 different cultivars. The plants derived from somatic embryos showed genetic variation.

It is recommended that this protocol should be refined further to include a wide range of genotypes, and that experiments should be carried out on irradiation of embryogenic callus cultures to establish protocols for *in vitro* irradiation.

In Uganda, local sweet potato cultivars have been established in tissue culture, but the problem of bacterial contamination still persists in the cultures.

It is recommended that the technique of micropropagation should be further refined to eliminate bacterial contamination, and to carry out thermo-therapy for obtaining virus free material to establish *in vitro* populations for irradiation and multiplication.

POTATO

In Italy, clones have been selected for insensitivity to culture filtrates of the pathogen *Phytophthora infestans*. Protocols have been developed for *in vitro* selection for tolerance to late blight; the selected clones have proven to be stable in the glass house tests.

It is recommended that the stability for insensitivity of the selected variants grown in the glass house should be tested under field conditions. The genetic basis of the insensitivity should be determined through dihaploidy and selfing.

PLANTAIN

In Ghana 256 M_1V_4 plants were grown along with the controls for selection for short height and resistance of leaves and stems to wind damage. This material has not flowered yet. In addition, field trials have been carried out with micropropagated plants at 2 locations. So far, no variation has been detected among these clones; however, the plants have not yet fruited. The selected material shall be further multiplied and distributed to the farmers.

It is recommended that the selected mutant clones should be multiplied conventionally and tested for uniformity, stability, and distinctness in field trials.

At the FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, seven banana clones were analyzed for peroxidase activity after inoculation with *Fusarium*. Studies have been initiated on the production of chitinases to clarify plant-pathogen reaction.

It is recommended that work on enzyme related response should be continued and if possible, a rapid method of screening be developed for detecting resistance to *fusarium* based on β-gluconase and chitinases.

BASELLE

In Congo, dose of 150-200 Gy was found to be as the optimum irradiation of the local cultivar which is normally propagated from vegetative cuttings.

It is recommended that by using the established doses, large scale material should be irradiated and examined for mutations. Micropropagation and *in vitro* irradiation should be undertaken to induce mutations for improving disease resistance. It is suggested that systematic studies should be carried out to establish the taxonomic status of the seed and vegetatively propagated cultivars. Physiological studies should be initiated to induce flowering in the non-flowering types. Collection of additional germplasm from neighboring countries and others sources through IPGRI is recommended. The rust causing pathogen should be studied in collaboration with a plant pathologist.

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