IAEA-TECDOC-951

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Proceedings of a final Research Co-ordination Meeting of a FAO/IAEA Co-ordinated Research Programme, held in Naples, Italy, 30 October – 3 November 1995

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FOREWORD

Increase in human population and shrinking land and water resources are threatening the right to food in many regions of the world. In some parts of Africa, this is compounded with political unrest which makes basic food a luxury item. Hence, the problems of food production and food security in Africa are critical.

The use of improved varieties of crop cultivars is an important component to sustain food production. Seed technology is the easiest and the cheapest of all technologies to transfer to the farmers. The transfer of this technology is self-sustaining through seed exchange between the farmers even when seed multiplication and distribution systems do not exist or are only marginally developed. The upgrading of local, well adapted cultivars by breeding genotypes with short height, early maturity, drought tolerance and disease resistance can significantly increase crop yield and quality. Induction and selection of mutants of such local cultivars offers a simple, efficient, rapid and cheap method to alter the genetic make-up, and obtain desired genotypes from otherwise well adapted ecotypes, land races and cultivars.

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 exist only as 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. Other crops, such as cassava, banana, plantain and sweet potato were introduced into Africa long ago, but from limited germplasm, and hence have a narrow genetic base. Therefore, there is a need to increase the genetic potential of the local varieties and land races yet retaining their genetic diversity and adaptability to the eco-climatic conditions. Induction of mutations in such local cultivars offers the possibility of changing one or two characters without disrupting the original genetic make-up. Since several of these crops, such as cassava, banana, plantain and 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.

The Co-ordinated Research Programme (CRP) 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 1989 in the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. The primary objective of this CRP was to breed improved varieties of staple food crops of Africa with the main emphasis on the indigenous species and their local cultivars. The fourth and final Research Co-ordination meeting under the CRP was held in Naples, Italy from 30 October - 3 November 1995. This publication includes the reports, conclusions and recommendations made by the participants. We hope that it will be of value to researchers, students and policy makers alike in their endeavour to promote plant breeding and increase food production in Africa.

This publication was prepared by the Scientific Secretary, B.S. Ahloowalia of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

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CONTENTS

INTRODUCTION

Food production in Africa depends upon the cultivation of many local crops which have been used by native farmers for thousands of years. Plants such as cassava, banana, plantain, sweet potato, yam, sorghum, African rice (*Oryza glaberrima*), and legumes such as cowpea, pigeonpea and chickpea are among the most important food crops in African agriculture. Most of these crop plants have been domesticated by African farmers since the dawn of agriculture, and are tolerant to stress and well suited to the eco-climatic zones of Africa. Hence they are the main source of native diet for millions of Africans. Several of these crops, such as sorghum, African rice and Bambara groundnut (*Voandzea subterranea*) 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 and hence have been neglected in their genetic improvement for yield and quality. These crops have not received the same attention to improvement and breeding as given to wheat, maize, rice and potato in other parts of the world. Other crops, such as cassava, banana, plantain and sweet potato were introduced into Africa many years 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 climatic conditions.

It is now well recognized by national and international organizations that the solution to food shortages in Africa and elsewhere in the developing world does not lie in importing food, but in increasing the production of 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 proved successful and plant breeding has left an imprint as the "green revolution" in south-east Asia which was based on growing of high yield, short height, photoperiod insensitive and short duration wheat and rice cultivars.

Mutation breeding combines 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 rice or non-climbing habit in some legumes. In that sense, it provides a rapid method to improve local crop varieties, without going through extensive hybridization and back crossing used in conventional breeding. Since several of these crops, such as cassava, banana, plantain and 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.

In 1989, a Co-ordinated Research Programme (CRP) 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 FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. The primary objective of this CRP was to encourage the breeding of improved varieties of staple food crops of Africa with the main emphasis on the indigenous species and their local cultivars. In addition, this programme aimed to develop links and to promote sharing of know-how in the plant breeding methods among African scientists and to enhance contacts between the individual scientists with international institutions. To achieve these targets, strengthening of research on plant breeding, genetics and tissue culture techniques in the participating institutions was undertaken. The programme provided a forum for the flow of information on the technology of plant breeding among the participants who shared the common goal of producing more productive and better yielding crop varieties, leading to increased food production and thereby to better living standards and health in

African countries. Increased food production is also linked to research and technology which must reach from the laboratory to the land. This requires dedicated teams of trained scientists and extension personnel. To promote research and development, the programme provided equipment, experts and trained personnel through scientific visits. The results obtained by the participants in the CRP are presented in this publication. It is hoped that these advances to improve basic food crops of Africa will contribute to increased food security in the region.

MUTAGENESIS FOR ACMV RESISTANCE IN A GHANIAN CASSAVA CULTIVAR 'BOSOM NSIA'

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Abstract

Breeding for resistance to the African Cassava Mosaic Virus (ACMV) disease in the Ghanian cassava cultivar 'Bosom nsia' has been on-going for the past four years at the Biotechnology and Nuclear Agricultural Research Institute. Protocols for *in vitro* culture from shoot meristems and acclimation of plantlets were established. Radiosensitivity tests on the regenerated plantlets indicated LD_{50} of 40 Gy, and doses of 25, 30 and 35 Gy were suitable for mutagenesis. These doses were applied to *in vivo* and *in vitro* grown plants, and selection was carried out in three propagations. Four variants, selected under field conditions with high viral incidence, were analysed for virus particles with three virus indexing techniques. Polymerase chain reaction (PCR) product analysis of DNA extracts from *Nicotiana benthamiana* test plants, inoculated with sap from the leaves of variants, confirmed the presence of virus particles in all variants. Inoculation and ELISA tests suggested ACMV tolerance in selected variants .

1. INTRODUCTION

Cassava is one of the most important staple food crops in the lowland tropics. In Ghana, cassava is mainly utilized for human consumption in the form of 'fufu', 'banku', 'yakayake' and dried for use as 'kokonte'. It is also processed as 'gari' and tapioca. Despite its protein deficiency, cassava remains a valuable source of energy; cheaper in price than many alternative foods. Cassava leaves are a good source of protein and vitamins, and are also used as food in Africa [12]. The cultivar 'Bosom nsia' is one of the most widely grown cultivars in Ghana, probably because of its good cooking quality and short maturation period of six months. However, this cultivar is highly susceptible to the African Cassava Mosaic Virus (ACMV) disease; hence, there is a need to improve its resistance to this disease.

The African Cassava Mosaic Virus disease is the most important disease of cassava. It is transmitted by a whitefly vector, *Bemisia tabaci* Gennadius which is prevalent in many parts of Africa [19]. Its etiology has been widely documented in West Africa [4, 5, 6] and also in East Africa [22, 14]. ACMV disease is caused by any one of three distinct Whitefly-transmitted Gemini viruses [7]. Yield reduction from ACMV infection ranges from 24% for highly resistant to 75% for highly susceptible varieties, but can exceed 90% according to some reports [4, 8, 21, 22]. Seif [16] reported that the yield reduction in several Kenyan varieties was 50.7%, and yield loss was highly correlated with disease severity.

The control of ACMV disease can be achieved through sanitation and the use of resistant or tolerant varieties as suggested by Storey [19]. In cassava breeding programme in Africa, resistance to ACMV disease has been a high priority [6, 9, 13]. Six components of resistance (r) are recognized: field r, vector r, inoculation r, virus r, symptom intensity r, and virus diffusion r [5].

Various *in vitro* techniques have been developed in cassava [1, 10, 17, 18]. Klu [11] reported irradiation doses of 25 and 30 Gy to be optimal for *in vitro* mutagenesis of cassava. These doses including 35 Gy were used for *in vitro* and *in vivo* mutagenesis. Meristem culture, in association with thermotherapy for virus elimination [10, 11] was adopted for the

generation of *in vitro* plants, and a protocol for acclimatization as reported by [2] was used for weaning plants and transfer to field. Selected variants were then evaluated for resistance to ACMV, using three virus diagnostic methods.

2. MATERIALS AND METHODS

2.1. *In vitro* and *in vivo* mutagenesis

The cultivar 'Bosom nsia' was procured from germplasm conservation station at Bunso (Ghana). Three hundred stakes measuring 15 cm each with 20 axillary buds were grown in sandy-loam soil at 35-38 C° (provided by high voltage bulbs) in a green house. After two weeks, apical shoot tips were excised with a sterile scapel. Three batches, each of one hundred shoot tips, were irradiated with 25, 30 and 35 Gy from a Cobalt 60 gamma irradiation source. The shoot tips were sterilized for 5 minutes in 70% ethanol and washed twice in distilled water. Shoot tips were then dissected under a light microscope to isolate meristems with 2 or 3 leaf primordia. The meristems were cultured in two stages on Murashige and Skooge basal medium supplemented with various hormones and sugar (per liter) as indicated below:

a. Shoot Initiation Medium.

Murashige and Skooge medium 1/3 strength

Sucrose.....30g
BAP..... 0.5ml
NAA.....0.1ml
GA_3.....0.1ml
Agar.....8g

b. Rooting Medium

Murashige and Skooge medium 1/3 strength

Sucrose.....20g
NAA.....0.1ml
Agar.....8g

The M_1V_1 generated plantlets were subcultured to M_1V_2. This gave on an average 500 planlets per irradiation dose. Plants were acclimatized as described previously [2]. Individual plants were potted in 50:50 sand:soil mix supplemented with 2 ml Raizal 400 starter solution, applied at weekly intervals to the base of each plant. Potted plants were covered with polythene to maintain humid environment, and kept in a well illuminated growth room at 25-27°C. The polythene covers were gradually removed, and temperature increased steadily to achieve complete weaning of plantlets. Hardened plants were transferred to a field with high ACMV incidence.

For *in vivo* mutagenesis, three batches of 500 stakes of cassava were irradiated with the same doses as for *in vitro* shoot tips. These stakes were obtained from disease free planting materials.

2. 2. Selection

Both *in vitro* and *in vivo* irradiated materials were selected for ACMV resistance over three generations. Selection for ACMV resistance was done prior to transfer of *in vitro* materials to field, and continued through three generations with *in vivo* materials; twelve months of growth being one generation. A scoring system as shown below was used to select for resistance to ACMV:

0.....No symptom
2.....1/4 of plant showing symptom
4.....1/2 of plant showing symptom
6.....3/4 of plant showing symptom
8.....Whole plant showing symptom
10.....Dead/Stunted/Morib and plant

2.3. Evaluation for resistance

Four variants were selected using the above disease scoring system. Two stakes measuring 15 cm with 20 axillary buds of each variant and the parent material (labelled VV for *in vivo* and VT for *in vitro* and BN for the parent cultivar "Bosom nsia") were planted in pots filled with a mixture of sand and compost in equal proportions. They were kept in a glasshouse at 30°C and watered daily. After one month of growth, fresh young leaves showing various degrees of disease incidence were taken, and used in the following tests to evaluate the presence of ACMV. Cassava materials were imported into Scotland, UK, under license of the Scottish Office of Agriculture and Fisheries Department.

2.4. Inoculation of test plants

Tobacco, *Nicotiana benthamiana* test plants were inoculated by rubbing leaves previously dusted with 600-mesh carborundum powder with infective sap extracted by grinding 0.25 g of young infected cassava leaves in 0.05 M Tris-HCl, pH 8.0, containing 0.005M EDTA. Excess inoculum was washed off with a stream of tap water. Plants were covered overnight with sheets of paper to prevent scorching of wounded leaves. There were four replicates per treatment. The treated test plants were kept in a glasshouse at 30°C for four weeks, and observed for symptom development.

2.5. Enzyme linked immunosorbent assay (ELISA)

Two forms of ELISA were used. Triple Antibody Sandwich (TAS) using monoclonal antibody, and the Double Antibody Sandwich (DAS) using ACMV polyclonal antibody. The protocol for ELISA was provided by the Virology Dept., Scottish Crop Res. Institute, Dundee.

The following buffer and reagents were used :

Coating buffer (Carbonate buffer), pH 9.6
1.59g Na_2CO_3
2.93g $NaHCO_3$ (dissolved in 1 liter of distilled water)

PBS (Phosphate buffer saline), pH 7.4
8.0g NaCl
0.2g KH_2PO_4
2.9g Na_2HPO_4 (dissolved in 1 liter of distilled water)

PBS - Tween : PBS & 0.05% Tween 20

Extraction buffer (For Gemini viruses)
0.05M Tris - HCl, pH 8.0
0.005M EDTA
2% Polyvinylpyrrolidone (PVP)
0.05% Tween-20

PBS -Tween - PVP : PBS - Tween & 2% PVP

Conjugate buffer : PBS - Tween - PVP and 0.2% Ovalbumin.

Substrate buffer : 10% Diethanolamine, pH 9.8 (with HCl)

2.5.1. TAS-ELISA (With monoclonal antibody, MAbs)

ACMV rabbit polyclonal antibody globulin (1 µg/ml) was diluted in coating buffer to 1/10,000. This was used to coat the wells of polystyrene microtitre plates (NUNC Immunoplate) 100 µl/well. The plates were incubated at room temperature for 2.5 h and were then washed (three short washes followed by three washes each of approximately three minutes duration) with PBS - Tween.

Antigen samples were prepared by extracting leaf tissue of the four variants and a healthy Ugandan cassava cultivar in the extraction buffer (10 ml/g). Sap was passed through muslin, and added to the microlitre plates (100 µl/well) with the extraction buffer as control. Plates were incubated overnight at 4°C.

Wells were washed as before and 5% semi-skimmed milk dissolved in PBS, Tween - PVP was added to the wells (200 µl/well), and incubated for 30 min at room temperature. The wells were emptied and blotted dry without washing.

Tissue culture fluids containing MAbs (prepared and supplied by the Virology Department) diluted 1:3 in PBS-Tween-PVP were added to the plates (100 µl/well) which were then incubated for 2.5 h at room temperature. Four of the MAbs were raised against ACMV while one was raised against the Indian Cassava Mosaic Virus (ICMV). One row of 10 wells was left without any MAb but filled with PBS - Tween - PVP buffer (used for DAS-ELISA). Washing was repeated.

Rabbit anti-mouse IgG (whole molecule) - alkaline phosphate conjugate (RAM-AP) (Sigma Chemicals, product No. A1902) diluted to 1/1000 in conjugate buffer was added to the wells (100 µl/well) except for the row of wells left for DAS-ELISA. The plate was then incubated for 2.5 h at room temperature. Washing was repeated, and bound alkaline phosphatase was detected using p-nitrophenyl phosphatase (Boehringer Corp. Ltd). There were two replicates per treatment .

Absorbance A_{405nm} was measured in a Titertek Multiscan. Measurements were taken after 1 h at room temperature and again after subsequent incubation overnight at 4°C.

2.5.2. DAS-ELISA (With Polyclonal antibody conjugate (PAC)

The procedure was adopted as described for TAS-ELISA except that at the time of MAb application, only the buffer (PBS-Tween-PVP) was applied as indicated above. Polyclonal antibody raised against ACMV was diluted at 1/1000 in conjugate buffer, and added to the row of ten wells (100 µl/well) during the stage of rabbit anti-mouse addition in the TAS-ELISA. All other steps were as described for TAS-ELISA. In this work, DAS-ELISA was done in the same microtitre plate as TAS-ELISA.

2.5.3. Scoring system for ELISA readings

The following scoring system was used for ELISA:

A_{405nm}	SCORE
> 1.80	4
1.21-1.80	3
0.61-1.20	2
0.30-0.60	1
0.15-0.3	t (trace)
< 0.15	0

2.6. Nucleic acid extraction

DNA extracts from leaves of cassava and test plants were made by method B as described by Robinson [15] except that ethanol precipitation was done with 2.5 volumes of 100% ethanol and 0.1 vol. 3 M Sodium acetate, pH 5.2 at -20°C overnight, washed with 100% ethanol, dried with a vacuum pump for 5 minutes and dissolved in 25 µl of sterile distilled water.

2.7. Polymerase Chain Reaction (PCR)

One µl each of two degenerate oligonucleotide primers with the following sequences were used in the PCR. reaction.

Primer 1 (0.68 µg/µl) - 5'--TAATATTACCKGWKGVCCSC--3' (20 nt)

Primer 2 (0.78 µg/µl) - 5'--TGGACYTTRCAWGBCCTTCACA--3' (23 nt)

(Where K = G or T, R = A or G, S = C or G, W = A or T, Y = C or T, B = C, G or T, and V = A , C or G.)

In addition to the above primers, PCR mixtures contained in a volume of 100 µl, 10 µl reaction buffer (10 mM-Tris HCl, pH 8.3, 5 mM-KCl), 10 µl (25 mM) $MgCl_2$, 2 µl (10 mM) dNTP, 2 µl of extracted nucleic acid and 74 µl of sterile distilled water. This mixture was heated at 95°C for 5 minutes and then transferred onto ice. An appropriate amount of 2 U Taq polymerase (Cambio) was then added and overlaid with 50 ul paraffin oil. The individual mixtures for the different sources of DNA extracted were put on a PCR intelligent heating machine (Cambio) and programmed for the following cycles.

1 x 2 min 94°C, 1 min 55°C, and 2 min 72° C
35 x 45 sec 94°C, 1 min 55°C, and 2 min 72° C
1 x 45 sec 94°C, 1 min 55°C, and 5 min 72°C

2.7.1. Analysis of PCR Products

10 µl samples of PCR products were analysed by electrophoresis in 10 g/l agarose gels in Tris-borate-EDTA buffer run for 1.5 h at 150 V stained with 0.5 ug/ml ethidium bromide and photographed on a UV trans-illuminator. PCR products of samples were run along side a 1 Kb ladder marker (Life Technologies).

3. RESULTS AND DISCUSSION

3.1. Selected variants

Two variants each were selected from both *in vitro* and *in vivo* populations. Both *in vitro* variants were selected from materials irradiated at 30 Gy while *in vivo* variants were from 25 and 30 Gy. Fig. 1 shows leaf samples of variants. There was a significant reduction in the chlorotic mottling symptom association with ACMV in the *in vivo* leaf sample with some reduction of chlorosis in the *in vitro* samples as compared to the control.

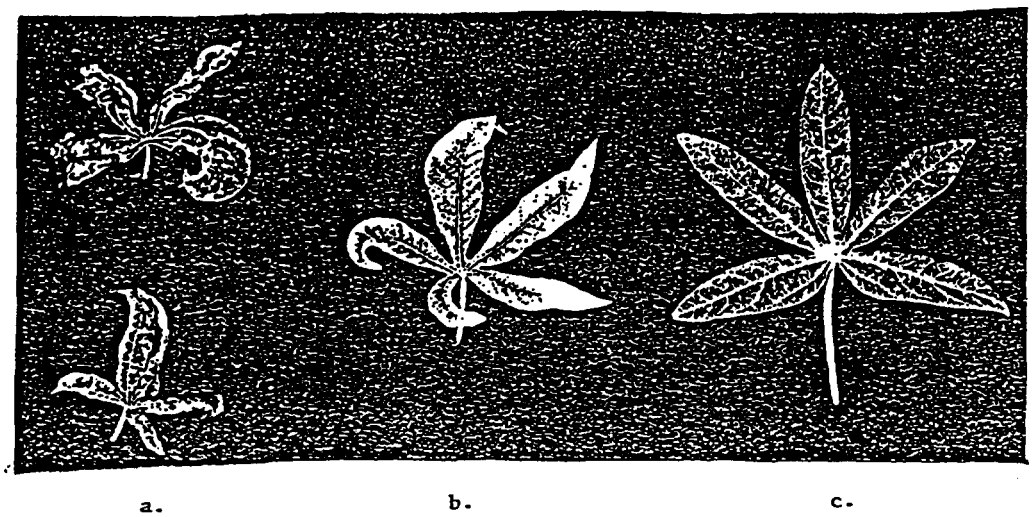

Fig. 1. Leaf samples of (a) original parent material, (b) in vitro and (c) in vivo variants.

These observations do not necessarily indicate resistance to ACMV, as it has long been reported that some cuttings taken from ACMV-infected sources grow into uninfected plants [19]. However, this feature, if consistent in the variant, could be regarded as some form of resistance.

3.2. Evaluation for resistance

3.2.1. Inoculation of Test-Plants

Fig. 2 (A-C) shows results of inoculation of cassava sap to *N. benthamiana*. There were chlorotic mottling and stunting of test-plants inoculated with sap from the original

cassava cv. 'Bosom nsia' (BN) and variants from *in vitro* mutagenesis (VT_1 and VT_2) but there were no visible signs of the disease on test-plants inoculated with sap from leaves of VV_1 and VV_2, even though slight stunting was observed with VV_1.

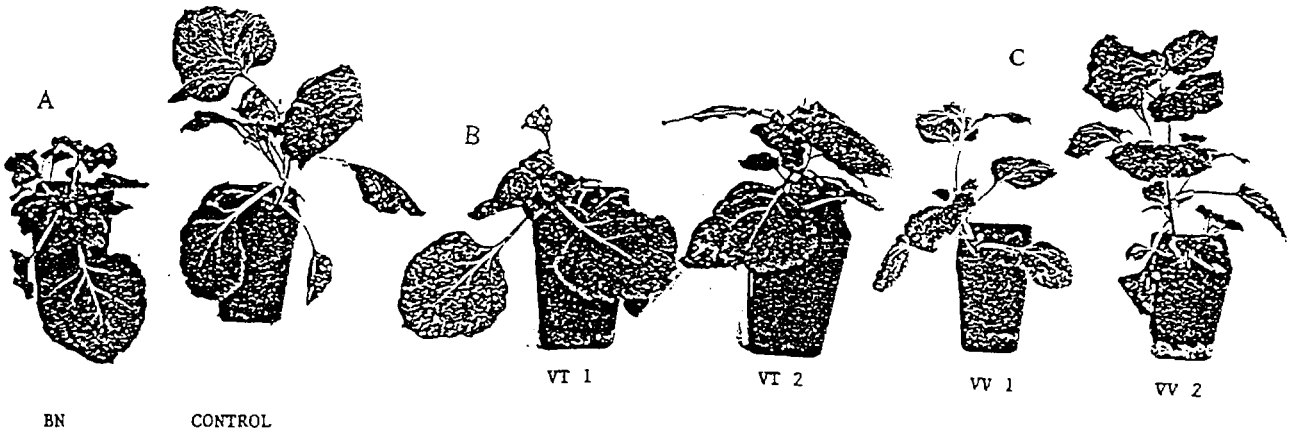

Fig. 2. Test plants inoculated with sap from original cv. BN with (A) control, (B) and (C) variants.

The absence of symptoms in the VV_1 inoculated test-plants suggests that either the virus particles in the leaves of the variants were absent or the concentration of virus particles was below that needed to inoculate the test-plants successfully. Walkey [24] stated that if a virus occurs in very low concentrations in the donor host, it is not possible to transmit it directly. In fact, it has been estimated that as many as 10^5 or more particles must be inoculated to infect a cell, and this figure may be even higher with multi-component viruses such as cowpea mosaic virus, 10^7 [23] and alfalfa mosaic virus, 10^9 [3]. Mechanical transmission to suitable host plants is extensively used as a quantitative bioassay, hence the absence of symptoms in test-plants inoculated with sap from variants VV_1 and VV_2 suggests low virus concentration or absence of virus in the sap.

3.2.2. ELISA

The reaction of five MAbs and Polyclonal antibody conjugate (PAC) to leaf-extracts of cassava is shown in Table I. There was a moderate to strong reaction with all MAbs except MAb SCR 60 which gave trace or no response. MAbs SCR 17, 18, 20, and 23 were specifically raised against ACMV, hence the positive reaction; while SCR 60 was raised against the Indian cassava mosaic virus and was used as a check for the ELISA test. Except for VV_1, there was no positive reaction with sap from the *in vivo* variants, but the *in vitro* variants and the control material (Bosom nsia) all gave positive recation with PAC .

The positive reaction with MAbs raised against ACMV conforms with other results as these are the core set of MAbs for ACMV identification [20]. The negative reaction to PAC for the *in vivo* variants indicates a probable low concentration of the virus or change in the antigenic properties of the viral coat protein. The latter is less probable since the positive response of MAbs indicate the presence of specific original epitopes on the coat protein. Low virus concentration is a much more plausible reason for the observed reaction as it confirms with the negative inoculation of test-plants with sap from the *in vivo* variants.

TABLE I. REACTION SCORES OF INFECTIVE SAP OF LEAVES OF CASSAVA VARIANTS WITH MABS AND PAC TO ACMV

	Variants of cassava cv. 'Bosom nsia'						
MAb (SCRNo.)	VV_1*	VV_1	VV_2	VT_1	VT_2	BN (Bosom nsia)	Healthy
17	2.0	2.0	3.0	3.0	2.0	2.0	0
18	2.0	3.0	3.0	3.0	3.0	3.0	0
20	2.5	2.5	3.5	3.0	3.0	2.0	t (trace)
23	3.0	3.0	3.0	3.0	3.0	3.0	0
60	0.0	0.0	0.0	t (trace)	1.0	t(trace)	0
ACMV	0	2	0	4	4	4	0

A_{405nm} values obtained in both TAS & DAS ELISA are means for duplicate wells minus values for buffer controls and were recorded after incubation with substrate for 1h at room temperature followed by overnight incubation at 4°C .
* cutting of VV_1

3.3.3. Polymerase Chain Reaction

Figs. 3a and 3b show results of PCR with DNA extracted from cassava and *N. benthamiana* leaves, respectively. There were no visible product band for 2a but 2b gave bands of similar size at about 525 bp .

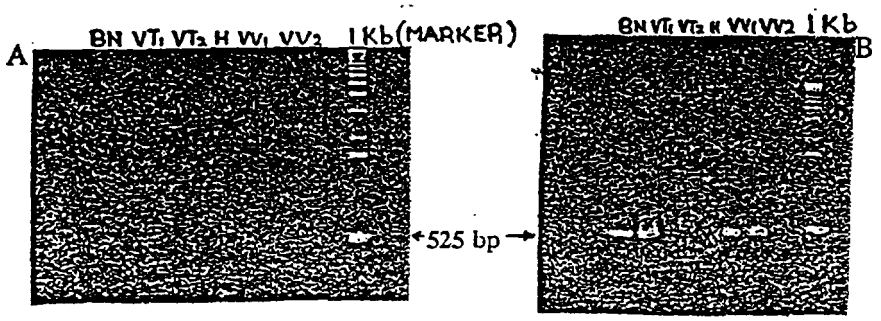

Fig. 3. Product obtained by amplification of nucleic acids from leaves of (A). variants *and (B) test plants.*

The negative results with cassava leaves is probably due to unsuccessful DNA extraction. This could be due to inhibitors in the leaves or the texture of the leaves which tend to render the process of DNA extraction inefficient. The positive results (2b) indicate the presence of virus particles in all the variants with the exception of VT_2. This again may result from an error in DNA extraction or PCR mixtures as both ELISA and inoculation tests indicated the presence of ACMV particles in VT_2.

It can be concluded from the PCR results that except for VT_2, all variants contained virus particles. The *in vitro* variants did not seem to have developed any resistance or tolerance to ACMV. The negative results of the mechanical transmission to test-plants with sap from the *in vivo* variants coupled with the lack of response with PAC suggests low virus

concentration. This suggests that these variants are tolerant to the presence of virus. The mechanism of the observed tolerance needs to be studied in order to understand the significance and importance of these variants.

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IMPROVEMENT OF CASSAVA COOKING QUALITY THROUGH MUTATION BREEDING

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Abstract

Many high-yielding cassava varieties do not have the desired cooking quality. The objective of this project was to induce mutations to produce varieties with improved cooking quality while maintaining the disease-resistance and high-yielding characteristics. A cassava mutant (ISU-W) was obtained after irradiation of a variety from IITA with gamma rays and selection. Cuttings of the mutant were grown for 12 months in a field trial and investigated for tuber yield and cooking quality. Pest and disease incidence were monitored during the entire growth period. The results showed that the mutant retained the high-yield and disease resistant characters of the parent, and had improved cooking quality based on increased smoothness, mealiness and elasticity of the flour.

1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important staple crop in many parts of Africa and other tropical regions. In Ghana, it is used in many food preparations including the following: 1. Fufu - the boiled tuber is pounded alone or with boiled plantain or cocoyam into a paste and eaten with soup. 2. Gari - the peeled tuber is grated, the moisture is squeezed out during fermentation and the dry matter is fried. 3. Ampesi - the boiled or roasted tuber is eaten with a vegetable sauce. However, there is little or no information on which the plant breeder can rely to select genotypes suited for a specific use. Hence, there is a tendency by farmers to reject an otherwise improved and high yielding variety, if it is unsuitable for their particular food usage.

The cooking quality of cassava has not received as much attention as that in potato. Many research workers have shown that the cooking quality is largely associated with mealiness, colour and flavour. Of these, mealiness is the most variable [2]. Howard [2] classified cooking quality as a broad heading, since different qualities are needed according to use of the cooked product. In potato [7] and cassava [3], a positive correlation has been reported between dry matter and mealiness, when the tubers or roots were consumed after boiling.

Worldwide, several plant breeding programmes have used induced mutations to generate variations to obtain desired mutants with agronomically important traits. In the present study, mutation techniques were used to induce genetic variation for cooking quality in cassava.

2. MATERIALS AND METHODS

Seeds of cassava varieties Isunikaniyan (ISU) and 4(2)1425 from IITA were planted in 1984. Segregants of these varieties were grown and compared with two local varieties (Akosua Tuntum and Atra) for yield, disease resistance and cooking quality characteristics upto 1987. In 1988, cuttings of the segregants from these and other varieties (30474, 60142 and 30001) were given series of irradiations after the appropriate doses (25 & 30 Gy) had

been determined. These were grown, and individual plants were examined for yield, mealiness, elasticity and smoothness of the pounded paste.

In 1991, a number of promising mutants were identified, especially in ISU-W. Much effort was subsequently concentrated on the M_1V_3 and M_1V_4 of this variety. Cuttings of this mutant were planted and examined for tuber yield and cooking quality. Only those plants were selected which had yield above 2 kg, and score of 3 or above for mealiness, smoothness and elasticity of the pounded paste. Thus, 32 plants were selected, and five cuttings each of the selected plants were grown.

In 1992, the M_1V_4 propagation was examined on the same basis as in the previous year; in addition, the dry matter content was determined and used as a selection criterion. In 1992, selected lines of the mutant ISU-W were planted on 0.2 ha. Cuttings of the mutant were given to the Department of Crop Services, Ministry of Agriculture for including in the multi-site on-farm trials. In addition, field experiments was conducted at the Faculty of Agriculture, University of Science and Technology, Kumasi, Ghana. The farm site is located at latitude 6°43'N and latitude 1°30'W in the forest belt with mean day/night temperatures of 30/22°C and relative humidity 60/95%, and has a mean annual rainfall of 1375-1625 mm. The soil was a well-drained sandy loam with pH 5.6.

The multi-site trials which included mutant ISU-W with three other improved varieties and a local variety were planted at a spacing of 1.0 x 1.0 m in 5 rows with 10 plants per row at eight sites representing similar agro-ecological zones. Planting was done at various sites during the major rainy season in May, 1994. Friedman's 2-way analysis was used to establish difference between the varieties at different locations.

Weeding was done as and when necessary, and insect pests and cassava mosaic virus disease (CMVD) incidence were rated during the entire growth period. Plants were harvested after 12 months, and examined for the number of tubers per plant, tuber yield and cooking quality. The cooking quality parameters considered were the mealiness of the cooked tuber and the elasticity and smoothness of the pounded paste. The method of Safo-Kantanka and Owusu-Nipah [5] was followed to determine the cooking quality. The scoring system for mealiness (texture), elasticity and smoothness was as follows:

Score	Mealiness	Smoothness	Elasticity
0	not mealy	not smooth	not elastic
1	moderately mealy	moderately smooth	moderately elastic
2	mealy	smooth	elastic
3	very mealy	very smooth	very elastic

3. RESULTS AND DISCUSSION

The seed progeny segregated into different lines and were grouped according to their stem colour. For example, Isunikaniyan (ISU) segregated into a white and dark brown-stemmed types, and was named ISU-white (ISU-W) and ISU-dark brown (ISU-DB), respectively. Similarly, 4(2)1425 produced the segregants, 1425-LB (Light brown), 1425-W (White), and 1425-DB (Dark brown).

Comparison of the segregants with the local varieties showed that the former had poor cooking quality, though some segregants were better than the others (Table I). The comparison of individual plants from the irradiated and local varieties showed varietal differences for mealiness (Table I). Howard [2] emphasized the importance of genotype in determining mealiness (texture). Only the local variety Akosua Tuntum consistently showed good cooking quality during all the years. After irradiation, ISU-W progeny produced tubers which on boiling were mealy, and had smooth and elastic consistency of the pounded paste. The importance of genotype in the control of mealiness (texture) was therefore confirmed. The study of physico-chemical properties of starch and dry matter content of the selected varieties of cassava showed that the mealier varieties had higher content of dry matter and starch (Table II). This indicates that when the gene (s) controlling mealiness are altered by mutagenic treatment, it is reflected in the size of starch granules. It appears that a point mutation might be operative, thus suggesting the simplicity of the genetic control of this complex trait.

TABLE I . COOKING QUALITY CLASSIFICATION OF CASSAVA VARIETIES[a]

Varieties	1987		1988		1990		
	Mealiness of cooked tuber	Consistency of pounded paste	Mealiness of cooked tuber	Consistency of pounded paste	Mealiness of cooked tuber	Smoothness of pounded paste	Elasticity of pounded paste
Akosua Tuntum[c]	M	SE	M	SE	2.2	2.8	2.4
Atra[c]	-	-	M	SE	3.0	1.8	0.3
ISU-W	NM	SE	M	SE	1.1	2.8	2.1
ISU-DB	NM	LI	NM	LI	1.4	1.8	1.8
30474-DB	M	LI	M	LI	0.6	1.0	1.3
30474-LB	M	SE	NM	SE	1.0	2.3	2.3
30001-W	M	LI	M	SE	1.5	0.5	0.3
30001-DB	M	SE	M	SE	0.1	1.1	1.2
4(2)1425-DB	M	SE	NM	SE	0.1	0.8	0.8
4(2)1425-W	NM	LI	NM	LI	-	-	-
4(2)1425-LB	NM	LI	NM	SE	-	-	-

a. Cooking quality classification: M - mealy, NM - not mealy, SE - smooth elastic paste, LI - lumpy, inelastic paste.
b. Score: 0-3 non-mealy to very mealy texture; 0-3 lumpy and inelastic paste to increasing degree of smooth and elastic paste.
c. Local varieties, the rest are segregants of varieties received from IITA. The letters after hyphen distinguish segregants by their stem colour: W - white, DB - dark brown, LB - light brown.

Selection of M_1V_3 and M_1V_4 plants showed that the mutants had good yield and cooking qualities [6]. Comparison of selected lines of ISU-W and other varieties showed improved cooking qualities of ISU-W (Table III), confirming the earlier claim of their superiority.

The results of the multi-site trials revealed significant differences in yield and mealiness of varieties at different locations (Tables IV & V). It has been reported that the genotype and environment influence mealiness of cooked potatoes [2]. Nix [4] found that the

TABLE II. MEALINESS OF COOKED PRODUCT, DRY MATTER AND STARCH CONTENT OF CASSAVA VARIETIES

Variety	Mealiness of cooked product		Dry matter content (%)	Starch content (%)
	Description	Score		
Akosua Tuntum	Mealy	2.2	38.50	31.20
Atra	Mealy	3.0	36.60	30.90
ISU-DB	Intermediate	1.4	34.20	26.50
30474-LB	Intermediate	1.0	36.00	19.60
30001-DB	Not mealy	0.1	35.20	21.60
4(2)1425-DB	Not mealy	0.1	32.60	21.90
L.S.D.(5%)			1.33	1.13

TABLE III. YIELD AND COOKING QUALITY CHARACTERISTICS OF CASSAVA VARIETIES

Variety	Mean no.of tubers/ plant	Mean tuber yield/ plant (kg)	Meali-ness	Elasti-city	Smooth-ness
ISU-W (A)	7.6	5.54	2.80	3.00	2.80
(B)	8.4	5.57	2.60	2.80	2.70
(C)	7.3	5.52	2.50	2.70	2.70
30474-DB		5.20	1.20	2.40	2.20
30001-W		4.40	1.80	2.20	2.20
60142		4.91	2.00	2.00	2.00
L.S.D.(5%)		1.01	0.57	0.62	0.60

yield of crops was a function of the genotype and its response to the environmental and cultural conditions. 'Afisiafi' and 'Gblemo Duade' varieties had good yield but showed consistently poor cooking quality characteristics across the sites. It must be emphasized that in many areas where cassava is consumed as various preparations after boiling the tuber, these varieties may be rejected in spite of their high yields. The local variety gave poor yield, but had good cooking quality. No relationship between yield and cooking quality of cassava and potatoes has been established; however, positive correlation between root dry matter and mealiness has been reported [3,7].

The low yield of the local variety may be due to its genetic make-up and high incidence of CMVD, which affects photosynthesis. 'Abasa Fitaa' and 'ISU-W' varieties produced fairly good yield, and had good cooking quality from most sites. This result is important in terms of selection criterion and adoption of varieties by farmers. Results of the multi-site trials showed that the local variety was more susceptible to CMVD than the other varieties. ISU-W was more resistant to CMVD in all regions and no serious pests were observed on its plants.

TABLE IV. YIELD, COOKING QUALITY AND DISEASE INCIDENCE OF CASSAVA VARIETIES

Variety	No. of tubers/ plant	Tuber yield/ plant	Cassava mosaic virus disease (CMVD)	Cooking quality		
				Fufu Meali-ness	Ampesi Elasti-city	Gari
Abasa Fitaa	5.3	25.1	0.8	2.3	2.3	2.9
Afisi-afi	7.4	28.8	0.8	1.0	1.0	3.0
Gblemo Duade	7.4	42.6	1.6	1.0	1.0	3.0
ISU-W	5.7	27.0	0.5	2.5	2.0	2.9
Local	4.8	15.5	2.8	2.5	2.5	2.9
C.V	16.8	27.2	23.4	30.9	36.1	8.2
L.S.D. (5%)	1.0	7.6	0.6	0.6	0.6	0.67

* Score of CMVD : 0-3, None- very heavy incidence

TABLE V. ROOT TUBER YIELD (t/ha) AT DIFFERENT LOCATIONS

Variety**	1	2	3	4	5	6	7	8
1 (ABF)	22.2	23.6	15.4	18.0	16.0	49.4	30.4	26.0
2 (GDF)	25.8	26.8	20.6	56.0	44.8	90.0	48.5	28.5
3 (AFI)	22.7	28.1	13.2	32.0	34.0	60.4	25.0	15.5
4 (ISU)	27.8	18.0	13.0	35.0	25.2	58.7	24.3	14.1
5 (LOC)	14.8	9.8	11.7	12.0	13.5	36.3	15.3	11.0

*Location: 1-Duase;2-Akomadan;3-Offinso;4-Datoyili;5-Nyeshe;6-Subinso;7-Techiman;8-Nkoranza
Variety: ABF=Abasa Fitaa;GDE=Gbemo Duade;AFI=Afisiafi;ISU=Isu-W mutant;LOC=A Local Variety.

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RADIATION INDUCED MUTATIONS FOR BREEDING OF SORGHUM

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Abstract

Several sorghum cultivars of Mali were irradiated with different doses of gamma rays and compared with the Caudatum types. Radio-sensitivity studies suggested that the local types were less sensitive to radiation than the introduced types. Whereas the local varieties survived dose of 300 Gy, in Caudatum types, seed germination and growth were significantly reduced at 200 Gy. Several agronomically important mutants were obtained among the progeny of the local types. Some of the mutants were shorter and had improved panicle characteristics. Radiation-induced variation was observed in several characters such as plant height, resistance to lodging, plant architecture, drought tolerance, panicle length and compactness, seed size and color, seed quality (viterous or floury) and protein content, glume color and structure, flowering date (early and late maturity), and tillering capacity. One mutant was drought tolerant. Promising mutants were selected and are presently under evaluation in the National List Trials to confirm their potential and future release. Selected variants have been also crossed with local types to obtain promising material.

1. INTRODUCTION

Sorghum is the second most important crop after pearl mille in Mali, and is grown on approximately 650,000 ha. The main varieties grown are local which have good grain quality and are well adpted to the climatic conditions. However, they are poor in yield (800 kg/ha), and have tall stalks (5 to 6 m) and prone to lodging. In order to combine the desired characters, several local types were crossed with an introduced Caudatum type which has short height and is high yielding. Although, all the promising progenies had good yield, they had poor grain quality. It seemed that there was a linkage between the two characters. Hence, we initiated a mutagenic program to enhace genetic variability in the local varieties, and to select desired mutants for producing varieties with impoved agronomic performance or to use them as parents in crosses to improve the local varieties. Our objective was to obtain well adapted varieties with 2.5 to 3.5 m stalk-length, with resistance to lodging, high yield (2,000 to 3,000 kg/ha), and good grain quality for making meal or 'couscous'.

2. MATERIALS AND METHODS

2.1. Material

The following varieties from different races of sorghum were irradiated: CSM388 and IPS0001 Guinea gambicum types, CSM228 Guinea margaritiferum type, Gadiaba durra type and SC a Caudatum type.

2.2. Methods

Dry seeds with about 12% moisture were sent for irradiation to IAEA Laboratory, Seibersdorf and to Lausanne Univeristy. In Seibersdorf, the seeds were irradiated with gamma ray doses of 20, 25, 30 KR at a rate of 880 rad/min; at Lausanne Univeristy, the doses were 10, 15, 20 KR at a rate of 3,000 rad/min. M_1, M_2 and M_3 progenies were grown as single plants, and self-pollinated. From M_2 onwards, each line had ca. 52 hills, and pedigree selection method was folllowed.

3. RESULTS

3.1. Radiation-sensitivity studies

The results obtained by Sanogo [1] indicated that Caudatum type (SC) was more sensitive to radiation than the local varieties and that the radiation increased seedling lethality and plant sterility in M_1 generation (Table I). Irradiation reduced stalk-length in all cases. Reduction in stalk-length of local varieties is very important for resistance to lodging and for increasing plant density in the field.

TABLE I. SOME POLYGENIC CHARACTERISTICS OF M_1 PLANTS*

Variety/Dose (kr)	Plant-stalk after 35 days (cm)	Number of plant with tillers	Number of early plant	Number of grains per panicle
CSM388 0	119.0	0	0	2569
20	119.0	16	0	2760
25	109.0	7	4	3196
30	100.5	5	0	2948
Gadiaba 0	90.0	0	0	2657
20	85.5	5	4	3382
25	80.0	3	0	3784
30	65.5	2	0	3780
CSM228 0	99.2	0	0	420
20	103.5	7	0	802
30	106.0	2	0	1309
SC 0	64.2	0	0	3779
20	58.0	8	4	3930
25	47.5	6	0	1937
30	44.5	3	0	2778

* Mean of 20 M_1 plants

3.2. Genetic variability

3.2.1. Spectrum of changes in plant characteristics

The spectrum of genetic variation observed in M_3 generation is given in Table II. It shows that mutagenesis can induce changes in many characters of sorghum including yield components [2, 3, 4]. Some times, more than two characters were changed. All the variations observed in the caudatum type were lost after four generations, and suggests unstable or environmental variation. In local varieties, a number of mutants were stable from the fifth generation; others continued to segregate in the subsequent generations.

3.2.2. Selected mutants and their potential

The selected mutants are described below:

MIG-SOR86-30-03: Several studies indicate that this mutant is drought tolerant. This mutant shows deep rooting system, high photosynthesis [6, 7] and tolerance to drought [8].

TABLE II. VARIATION OBSERVED WITH DIFFERENT DOSES OF GAMA RAYS IN M_2 GENERATION OF 4 SORGHUM VARIETIES

Variety	CSM388			GADIABA			CSM228			SC		
Dose (kr)	20	25	30	20	25	30	20	25	30	20	25	30
Variation observed												
Vigour	+	+	+	-	+	-	-	-	-	-	-	-
Tillering	+	+	+	+	+	+	-	-	-	-	-	-
Axial tillers	-	-	-	+	-	-	+	+	+	+	+	+
Sterility	+	+	-	+	+	-	-	-	-	+	+	+
Semi-sterility	+	+	-	-	-	-	-	-	-	-	-	-
Panicle length	-	-	-	-	-	+	-	-	-	-	-	-
Crooked panicle	-	+	-				-	-	-	-	-	-
Erect panicle				+	-	-						
Loose panicle				+	+	-				-	-	-
Compact panicle	+	-	-				-	-	-			
Black glumes	+	-	+	-	-	-	-	-	-			
Big grains	+	-	+	-	-	-	-	-	-	-	-	-
Grains shape	+	-	-	-	-	-	-	-	-	-	-	-
Short types	-	-	-	-	-	-	-	-	-	-	-	-
Late maturity	+	-	-	-	-	-	-	-	-	-	-	-
Early maturity	-	+	-	+	-	-	-	-	-	+	-	-
High yield	+	+	+	+	+	+	+	+	+	-	-	-

(+ = variation observed; - = variation not observed; columns with no sign indicate that the characteristic did not show variation)

Under drought MIG-SOR86-30-03 lost about 25% of yield; in contrast, the control showed 100% loss in yield. However, under normal conditions it is less productive compared to the parent; but has improved grain quality. It seems to combine for drought tolerance with grain quality.

- MIK-SOR86-25-11: This mutant is about 25% less productive than his parent, but has short duration of growth. It is more adapted than the parent to Sahelian region.

- MIK-SOR86-25-16: This mutant is lodging resistant and has high protein content. Cooking quality is lower than the parent, but is acceptable to the farmers.

- MIK-SOR86-30-41: This mutant is more lodging resistant and produces about 25% more yield than the parent CSM388. Stalk length is reduced by approximately 50 cm.

- MID-SOR88-10-01 and MID-SOR-88-10: These two mutants yield more than their parent by about 35%. The first mutant has a long panicle (about 55 cm compared to that of the parent (25 cm), and there is a change in grain structure. In the second mutants, the panicle is about 50 cm with high grain density. Grain is the same size as in the parent but is more viterous.

- MIK-SOR86-25-16: This mutant is lodging resistant and has a high protein content; cooking quality is lower than the parent, but acceptable.

- MIP-SOR90-30-23 is more lodging resistant and gives 25% more yield than the parent.

4. CONCLUSIONS

The results show that mutagenesis allows breeders to get sorghum types for African farmers [5], who like a variety which is less tall than their local types but more tall than the introduced types. The stalks are used for cooking, making houses, and fodder. The second and no less important parameter is grain cooking quality. The local varieties have good cooking quality but less productive; the caudatum type is used in America for animal feed and so grain quality is less important. The new varieties developed through mutagenesis of local types combine the advantages of medium size, good grain quality and high productivity. One of them is drought tolerant and will be used to improve the local varieties. Some of the mutants are now being tested in farmers fields.

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BREEDING FOR RESISTANCE TO GRAIN SHATTERING IN *Oryza glaberrima* Steud. THROUGH INDUCED MUTATIONS

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Abstract

Several local varieties of African rice, *O. glaberrima*, which is an important crop in the inland Niger River Delta in Mali, were irradiated with gamma rays in two different experiments. Several mutants with changed kernel colour from red to white and improved characters such as early maturity, non-shattering of grain, and reduced plant height were selected among the advanced segregating generations. Some of the selected mutants are being tested in multi-location trials.

1. INTRODUCTION

The deep water rice in Mali covers 500,000 ha [1] of which 36,000 ha are cultivated under controlled submersion in the Mopti region. The area occupied by *Oryza glaberrima* under natural submersion and semi-controlled submersion is difficult to assess but is more important than that under by *Oryza sativa*.

O. glaberrima in the inland Niger River Delta [2] is characterized by good adaptability and high resistance to drought and major diseases which makes it preferable to *O. sativa*. Under drought conditions, which occur frequently in Mali, yields of *O. sativa* are reduced and the local *O. glaberrima* varieties generally perform better. However, *O. glaberrima* is very sensitive to grain shattering at ripening; therefore, the yield of *O. sativa* is higher than that of *O. glaberrima* when the environmental conditions are good. In the market, *O. glaberrima* is not appreciated because of its red kernels. For *O. glaberrima* and *O. sativa*, the price is about 160 Fcfa/kg and 275 Fcfa/kg , respectively. Beacuse of the importance of *O. glaberrima* in Mali, an experiment was initiated in 1988 to improve its undesirable characteristics through induced mutations, with the financial and technical support of IAEA. Many mutants were obtained from this experiment, the most promising of which are being evaluated for their yield potential. The second experiment was started in 1993, and has been continued with selection in M_4 populations during 1995. This report presents the results of these two experiments.

FIRST EXPERIMENT

2. MATERIAL & METHODS

The objective of this experiment was to improve resistance to shattering and yield in *O. glaberrima* through induced mutations. Ten local varieties of *O. glaberrima* namely, 'Bougatimbo', 'Bouyadian', 'Gorbal', 'Kaka', 'Piékono', 'Pièpi', 'Simomboro', 'Tombo', 'Yélé' and 'Youssouwel' were irradiated to generate genetic variability. Seeds (100 g of each variety for each dose) were irradiated with gamma rays from a ^{60}Co source at the IAEA Laboratory, Seibersdorf, with doses of 20 and 30 krads. The M_1 plants were grown in isolation to avoid cross-pollination at a density of 33 plants/m^2.

2.1. Field trials

The yield potential of M_4 selected mutants was compared with that of their parents in field test at Mopti in the medium and shallow water zones. Selected mutants, SMMG88-8-1-1, SMMG88-9, SMMG88-13-1, SMMG88-15-1-1, SMMG88-15-2, SMMG88-20-1, SMMG88-20-2-1 and cv. 'Gorbal' were planted in the field trials with 4 replications in randomized blocks with plots 5 m x 3 m, 10 rows/plot; the distance between rows was 30 cm. Fertilizer application of 100 kg/ha ammonium phosphate was made at sowing and 50 kg/ha urea was applied when water was 5 cm deep.

3. RESULTS

3.1. Effect of gamma irradiation

In the M_1 generation, the survival in the irradiated varieties was 70% of the control except variety 'Gorbal' in which it was 35% of the control. There was no difference in plant survival with the two doses. In the M_2 generation, a high genetic variability was generated, and segregation was observed for many characteristics, such as growth duration, plant height, seed characteristics (length, size) and degree of sterility. Selection for variation in seed shattering was done at maturity by shaking 2 to 3 panicles, and evaluating the amount of grain shattered, based on the IRRI index.

In the M_3 generation, a fertile mutant of cv. 'Gorbal' segregated for grain colour and other characteristics. At this stage, fifteen mutants were retained, twelve had white kernels and three had red kernels. Finally, eight promising mutants were selected in the M_4 generation based on the following criteria: adaptability to deep water rice conditions, early maturity, white kernel and resistance to major stress.

3.2. Field trials

In the medium zone, the germination and plant density were good. With an yield of 3917 kg/ha, SMMG88-8-1-1 exceeded the grain yield of the check parent 'Gorbal' and all the other lines. Statistically, there was no difference between this variety and four other varieties (Table I).

In the shallow zone, the plant density was very low because of drought. Water retreated at the flowering stage of the mutants and before the flowering stage of the control; hence, the 1000-grain weight of mutants was low and the control did not produce any grain. The analysis of variance showed highly significant difference between the varieties. Four mutants gave similar yield (Table II), among them SMMG88-9 gave the best production with 2302 kg/ha, followed by SMMG88-8-1-1 (2271 kg/ha).

4. CONCLUSIONS

Several mutants of the local varieties of *O. glaberrima* which have production equal to or more than the introduced *O. sativa* varieties were obtained. These mutants have several advantages over *O. sativa* varieties. Under controlled submersion conditions, where only the white grain rice growing is allowed for land rent reasons, mutants with white grain can be grown. Of these, SMMG88-8-1-1, SMMG88-8-1-3, SMMG88-13-1 and SMMG88-20-2-1 can

be grown in the medium and shallow water zones whereas SMMG88-15-1-1, SMMG88-15-2 and SMMG88-20-1 in the deep water zones.

Under natural submersion and semi-controlled conditions, mutants SMMG88-15-1-1, SMMG88-15-2 and SMMG88-20-1 may be suitable for growing in the deep water zone because of their resistance to the submersion and their good elongation capacity. The other five mutants with moderate resistance to submersion are likley suitable for medium and shallow water zones.

TABLE I. AGRONOMIC CHARACTERISTICS OF MUTANTS (MEDIUM ZONE)

Variety	Days to maturity	Plant height (cm)	No. of tillers /m	No. of panicles /m	No. of grains /panicle	1000 gr weight (g)	Grain yield (kg/ha)
Parent (Gorbal)	168	176	81	65	100	29.85	3.302 abc*
SMMG88.1.1	141	145	138	99	123	23.08	3.917 a
SMMG88.9	145	144	103	74	125	26.18	3.573 ab
SMMG88.13.1	147	145	101	70	150	24.96	3.565 ab
SMMG88.15.1.1	158	200	63	49	162	24.53	2.146 c
SMMG88.15.2	158	187	55	44	206	23.80	2.136 c
SMMG88.20.1	150	195	56	49	190	27.36	2.563 bc
SMMG88.20.2.1	147	140	95	66	140	27.41	2.844 abc

F test - highly significant; CV% 19,94%
*The numbers followed by the same letters are not statistically different according to Duncan's test.
* Date of sowing: 7-07-1993
*Maximum Water level: 100 cm

TABLE II. AGRONOMIC CHARACTERISTICS OF MUTANTS (SHALLOW ZONE)

Variety	Days to maturity	Plant height (cm)	No. of tillers /m	No. of panicles /m	No. of grains /panicle	1000-grain weight (g)	Grain yield (kg/ha)
Parent (Gorbal)							
SMMG88.8.1.1	145	112	98	83	149	21.65	2.271 a*
SMMG88.9	147	113	78	65	139	25.77	2.302 a
SMMG88.13.1	147	118	67	55	158	24.35	1.917 ab
SMMG88.15.1.1	160	173	58	49	207	22.61	615 c
SMMG88.15.2	160	184	71	60	160	22.81	1.375 bc
SMMG88.20.1	155	170	61	49	170	25.73	1.177 bc
SMMG88.20.2.1	147	116	68	54	172	25.21	1.896 ab

F test- HS , CV% 21,95%
* The numbers followed by the same letter are not statistically different according to Duncan's test.
* Date of sowing: 8-07-1993
* Maximum water level: 40 cm

SECOND EXPERIMENT

1. MATERIAL & METHODS

This experiment was initiated in 1993 to improve resistance to shattering of three local *O. glaberrima* varieties. Three varieties, 'Haïra', 'Tombo' and 'Yele', prone to shattering, were irradiated with 20 krads gamma rays from a ^{60}Co source. The M_1 plants were grown in isolation to avoid cross-pollination. The plot size in each treatment was 21 m x 5 m. The sowing was done with a single grain per hill, spaced at 0.30 m x 0.20 m. Data were recorded on germination percentage, 50% flowering date, plant height at maturity, sterility and disease incidence. At maturity, the main panicle of each tuft was harvested separately.

2. RESULTS

2.1. Effect of gamma irradiation

In the M_1, seed germination was 75%, 81% and 72% respectively for 'Haïra', 'Tombo' and 'Yele'. Seed sterility at maturity was 32.6% Haïra, 36.3% Tombo and 25.8% Yele, and was the highest in the cv. 'Tombo' (15%). The irradiation did not reduce the height of the M_1 plants. In every treatment, the flowering occurred over 10 days-duration.

2.2. M_2 population

During the rainy season 1994, M_2 population of 24827 plants was grown. This consisted of 5792 plants from 'Haïra', 9055 plants from 'Tombo', and 9980 plants from 'Yele'. The population was grown by transplanting one plant per hill without any fertilizer.

A high genetic variability was generated and segregation was observed in each treatment for many characters (grain colour, awn, plant height, seed size, etc). A total of 300 mutants were identified in the M_2 populations of which only nine (Table III) from 'Haïra', were fertile, the remaining mutants had fertility of 5%. The mutants could be classed into three groups:

1. MUT93-1, MUT93-2 and MUT93-4 with red grain (kernel), similar to their parent 'Haïra'; these have good resistance to shattering and short duration if growth.

2. MUT93-3 and MUT93-5 with white grain and an acceptable resistance to shattering.

3. MUT93-6, MUT93-7, MUT93-8 and MUT93-9, with white grain, but no important differences between them and their parents except grain colour.

2.3. Generation M_3

During 1995 dry season, M_3 mutants were planted in a greenhouse (1 plant per pot). A segregation was observed only in the population MUT93-2 for plant height, growth duration, grain size, colour, awn, etc. In this segregating population, ten M_3 mutants were selected for their interesting agronomic characteristics (Table IV). In addition to their good resistance to shattering, they showed other important characters. These included: 1. Secondary

branches on the panicle, an important component of yield, while they are absent in the control. 2. A short period of dormancy (about 3 weeks), against twelve weeks for the control.

TABLE III. AGRONOMIC CHARACTERISTICS OF SELECTED MUTANTS IN M_2

Variety	Days to maturity	Plant height (cm)	Shattering (%)	Kernel colour
parent (Haïra)	165	185	35	red
MUT93-1	124	150	16	red
MUT93-2	135	160	17	red
MUT93-3	165	167	20	white
MUT93-4	165	156	22	red
MUT93-5	158	160	25	white
MUT93-6	163	180	35	white
MUT93-7	163	182	35	white
MUT93-8	165	175	34	white
MUT93-9	164	177	34	white

Date of sowing in nursery: 27-06-1994
Date of transplanting: 28-07-1994

3. They are not photosensitive while the control is photo-sensitive. All non-segregating mutants were rejected because they did not show any interesting character.

TABLE IV. AGRONOMIC CHARACTERISTICS OF MUTANTS IN M_3

Variety	Days to maturity	Plant height (cm)	Rate of shattering (%)	Kernel colour
parent (Haïra)				red
MUT93-2-1	120	120	8	white
MUT93-2-2	145	160	11	red
MUT93-2-3	145	160	18	red
MUT93-2-4	145	158	12	red
MUT93-2-5	143	163	18	red
MUT93-2-6	140	165	10	white
MUT93-2-7	140	165	14	white
MUT93-2-8	146	165	18	red
MUT93-2-9	146	165	10	white
MUT93-2-10	146	165	17	red

*Date of sowing: 2-01-1995

2.4. Generation M_4

Seeds of selected lines were sown in the nursery; 21 days after germination, the plants were transplanted in the field (1 plant per hill) with spacing 30 cm x 20 cm. The fertilizer, namely 'complex cotton' (14N-22P-12K-7B-1S), was applied at rate of 200 kg/ha.

Segregation was observed in all head lines, except MUT93-2 which was stable. The choice of mutants was done in five head lines on the basis of resistance to shattering, resistance to major stress (diseases and insects), characteristics of panicle and grain. In contrast to the control, the selected mutants showed a good improvement in resistance to grain shattering.

Concerning the height of plants, the control has a medium height; in fact it is a floating variety and the height depends on the water level. In natural submersion conditions, it can adapt up 2m of flooding. Like the control, all the mutants with a height up to 150cm are sensitive to lodging. In contrast, those which have a height of 140 and 150cm are less sensitive to lodging.

In spite of its reduced 1000 grain weight, MUT93-2-1 is very promising on the basis of the following agronomic characters: resistance to leaf blast and panicle blast under natural infection, non-lodging, dark green leaves till maturity, broad leaves, strong stems and white kernel.

TABLE V. SOME AGRONOMIC CHARACTERISTICS OF MUTANTS M_4

Variety	Days to maturity	Plant height (cm)	Shattering (%)	Grain		Paddy	Kernel colour
				length (mm)	width (mm)	1000 gr weight (g)	
Parent (Haïra)	150	150	32	8.49	3.38	24.8	red
MUT93-2-1	128	130	7	7.50	2.95	21.6	white
MUT93-2-2-1	146	150	9	10.30	3.23	25.9	red
MUT93-2-4-1	133	190	11	10.10	3.22	29.8	white
MUT93-2-4-2	138	160	9	9.38	3.28	28.8	white
MUT93-2-4-3	146	180	11	9.10	3.42	30.6	red
MUT93-2-4-4	140	170	6	10.10	3.35	28.0	white
MUT93-2-4-5	142	180	8	9.47	3.45	29.8	white
MUT93-2-4-6	143	180	11	9.68	3.26	27.9	red
MUT93-6-1	148	150	9	9.44	3.26	25.4	white
MUT93-2-7-1	140	150	8	10.40	2.76	24.2	white
MUT93-2-7-2	137	175	13	9.64	3.20	27.4	white
MUT93-2-7-3	137	180	13	9.16	3.20	26.7	white
MUT93-2-7-4	140	180	10	9.62	3.10	27.3	white
MUT93-2-9-1	138	140	10	9.23	2.73	26.0	white

*Maximum water level: 35 cm *Date of sowing in nursery: 1-07-1995 *Date of transplanting: 22-07-1995

3.4. Conclusion

The results showed that through induced mutations, it is possible to improve many characters of *O. glaberrima* varieties. However, from the results obtained, it appears that the probability of obtaining mutants which are resistant to (fertile and resistant to shattering) is very low. By obtaining resistant mutants to shattering, our main objective was reached. Evaluation for yield potential will begin in the 1996 rainy season.

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DNA ANALYSIS IN THREE POPULATIONS OF AFRICAN SPINACH (*Basella* spp.)

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Abstract

African spinach (*Basella* spp.) is an important vegetable in West Africa, and was introduced by early colonialists. Its alien origin is supported by its narrow genetic variability. Flowcytometry and RAPD polymorphism were used to investigate genetic variation in three populations of *Basella* - 'Congo native', 'Congo domesticated', and an introduced cultivar, 'Sri Lanka' from Sri Lanka. Normal spinach (*Spinacia oleracea*) cv. 'Prince F_1 Hybrid' was used to test sensitivity and to verify detection of genetic variation. Nuclei were isolated from young leaves of *Basella*, stained with DAPI and ethidium bromide, and ploidy level and total DNA content were determined by using a flowcytometer. The two sexually propagated populations, 'Congo domesticated' and 'Sri Lanka' showed very low amount of genetic variation as revealed by RAPD analysis; the third population 'Congo native' showed a limited amount of polymorphism.

1. INTRODUCTION

Breeding better crops for the future is a major task to increase food production and to meet nutritional needs. More than 3000 plant species have been used for agricultural purposes. However, most efforts have been devoted only to major crops with little or no genetic improvement of other crops which have been neglected. Human nutritional needs cannot be covered completely from cereals and root and tuber crops. The vegetable crops play a basic role to complement human diet for vitamins and macro- and micro-element requirement.

Genetic variation is essential for breeding new varieties to complement human diet and improve the rising demand for quality. It is also a pre-requisite for any adaptive changes in species that lack sufficient genetic variation and are at risk of extinction. Many humid tropical regions, although rich in genetic diversity lack native edible crops. Most of the crops presently cultivated in these regions are of recent introduction, and very often have been derived from few accessions.

African spinach (*Baselle alba* and *B. rubra*) is an important leaf vegetable in West Africa, appreciated for its organoleptic characteristics, yield potential and as source of high levels of iron and vitamins B and C [1]. The crop is a valuable income source for small farmers near the principal urban centers. However, in the Congo river basin, its production is hampered by a foliar disease, with symptoms closely resembling those of rusts (*Puccinia*) [2]. In Congo, at least three types of African spinach are known: 1. Vegetatively propagated type, named in here 'Congo native'. It has a viney habit, has anthocyanin in leaf-veins and stems, does not flower, and is resistant to the rust. 2. Seed propagated type, named in here 'Congo domesticated'. It is autogamous, has green stems and leaves but is very susceptible to *Puccinia* rust, and 3. A recent introduction from Sri Lanka, which is sexually reproducing, autogamous, and has broad green leaves and stems resembling a polyploid. In the 'Congo domesticated' type, a colour variant is known and is often considered a different taxon, *Basella rubra*. Using the classical phenotypic markers, it is not possible to detect any genetic variability among the populations of the three types because the individual plant populations

show a remarkable uniformity. Hence, the analysis of genetic variability in the Congo basin African spinach populations is required for formulating a breeding strategy. Because of the lack of morphological markers, an alternative is to use molecular markers for genetic identification of species and cultivars. Recently, the use of Random Amplified Polymorphism's (RAPDs) has been proposed and applied to a number of crops such as cassava [3], tea [4] and eggplant [5]. The aim of the present study was to characterize variability in *Basella* of the Congo area at the DNA and nuclear level.

2. MATERIAL AND METHODS

2.1. Plant material

Three *Baselle alba* populations were collected from the Congo river basin. One accession was bought in a local market in Brazaville as seed; seed sample of the 'Sri Lanka' population was provided by Prof. C. Makambila, Univ. of Brazaville, and plant material was collected from a farmers' field, 30 km from Brazaville. All plant material was potted in soil and maintained in a greenhouse. Seeds from the potted plants were collected, and replanted to analyze possible DNA polymorphism.

2.2. Flow cytometry

Young leaves from plants of *Baselle* were collected and nuclei were isolated by chopping with a sharp scalpel in 1 ml LB01 buffer containing 15 mM Tris, 2 mM Na_2 EDTA, 80 mM KCl, 20 mM NaCl, 0.5 spermine, 15 mM 2-mercaptoethanol and 0.1 Triton X-100, pH 7.5. The fluorochrome DAPI was added to the buffer at a concentration of a 2 mg/l. To estimate total DNA content, the same buffer was used, but ethidium bromide was added at a concentration of 50 mg/l. Samples were filtered through a 15 mm pore nylon filter, and incubated on ice for 5-10 minutes.

Ploidy levels were measured using a Partec CA-II flow cytometer equipped with a 100 Watt high-pressure mercury lamp and the filter combination UG1, TK420 and GG475. The gain was adjusted so that the peak of G_0 and G_1 nuclei of greenhouse grown Congo domesticated plants was on channel 50. At least 10,000 nuclei were measured and data evaluated using the Partec software package. To determine total DNA content, nuclei from pea plants (*Pisum sativum* cv. 'Lincoln') were extracted and used as a reference [6].

2.3. Chromosome counting

Actively growing root tips were obtained by placing *Basella* cuttings in water containing IAA. Roots were treated with colchicine (0.05-0.1%) at 4 C° for 24 hours. Root tips were fixed in Carnoy II solution at 4 C° for 24 hours, and transferred to 70% ethanol. After 30 min maceration in 5 M HCl, the tissue was washed, and stained in lacto-propionic orcein for 2 hours. The stained tissues were washed in distilled water, and macerated with 45% acetic acid solution for few minutes, and analyzed for chromosome number [7].

2.4. DNA extraction

Total DNA was extracted using a modified CTAB method [8], and stored at a concentration of 10 ng/microliter.

2.5. Primers

25 primers (10-mers) were used; their G+C content ranged from 60 to 80% to increase specificity. They were purchased from University of British Columbia.

2.6. PCR

All PCR reaction were performed using a reaction mixture consisting of 3.5 mM $MgCl_2$, 100 mM dNTP, 200 nM of 10-mer, 1unit Taq polymerase and 50 ng of genomic DNA and adjusted to a final volume of 25 µml. The amplification reaction was performed in a Perkin Elmer 9600 thermal cycler. After an initial denaturation step at 94 C° (2 min), 30 cycles of 93 C° (1'), 40 C° (1') and 72 C° (2') were performed, which was followed by one cycle of 6' at 72 C°. The amplified DNA fragments were resolved by electrophoresis on 1.5% agarose gels, and visualized by staining with ethidium bromide. All data were stored in computer files generated by a MWG GelPrint 2000i, and analyzed using the RFLP scan software for polymorphism. The data was then utilized to construct a dendrogram by using the NTYSYS V.I.P. program for creating relatedness tree.

3. RESULTS & DISCUSSION

3.1. Flow cytometry

Using DAPI, the sexually propagated samples, 'Sri Lanka' and 'Congo domesticated' showed the same ploidy level, ruling out the possibility that the phenotypical features of 'Sri Lanka' resulted from polyploidy. This was further confirmed from the analysis of total DNA content (Table I). All samples of these population gave similar values for total DNA content, ca 6 pg (2C). The third population, which is vegetatively propagated, showed a lower DNA content, as shown both by DAPI and ethidium bromide staining.

TABLE I . DNA CONTENT OF *Basella*

Population	DNA Content* (pg)
Baselle Congo (Domesticated)	6.83
Baselle Congo (Native)	5.73
Baselle Sri Lanka	6.83

*the mean of 5 replicates of the ratio between the median value of each sample and the median value of the reference material (*Pisum sativum* cv. Lincoln) x 9.07, the DNA (pg) of the reference material.

3.2. Chromosome counting

The chromosome numbers ranged from 2n = 48 to 36. In the vegetative propagated plants, complex mixoploidy was observed. The preliminary data suggested a complex situation and requires further investigation.

3.3. PCR

Eleven of the twenty-five primers tested produced polymorphic bands. Primer 1 and 6 revealed higher polymorphism; therefore, were used throughout this study. However, polymorphic variability within any of the three populations was not detected. Some variation was detected among the green and red plants. Of the three populations studied, the vegetatively propagated *Basella* differed the most from the other two. The similarity index are shown in Table II. No differences were detected within the progeny obtained from selected plants. The similarity measure was also utilized to construct a dendrogram (Fig. 1) by the group average strategy (UPGMA) and the program Numerical Taxonomy and Multivariate System.

The results of this study clearly showed that in the *Basella* populations collected from Congo, the genetic variation is extremely low, if not absent. Further research is in progress to characterize promising plant material and to confirm the validity of RAPDs for detecting genetic variation. More 10-mers are being tested and we have started to use oligo-nucleotide fingerprinting. Nevertheless, the results obtained with 'Congo domesticated' and 'Sri Lanka' are posing puzzling question as to the basis of the striking phenotypic differences between the two populations. Although, our results are preliminary, they suggest the lack of genetic variability in *Basella* from Congo. This could be overcome by either mutation induction or by germplasm introduction from other geographical areas. Plant breeders require genetic variability in their plant material to meet the ever-changing demand for new varieties with wide acceptance. This requires a deep knowledge of the genetic structure and variability in the plant species.

TABLE II. ESTIMATES OF GENETIC SIMILARITY IN THREE POPULATIONS OF *Basella*.

Population	Domesticated	Domesticated Red	Sri Lanka
Domesticated	1.0000		
Domesticated Red	0.8246	1.0000	
Sri Lanka	0.8495	0.9359	1.0000
Native	0.7390	0.7898	0.8671

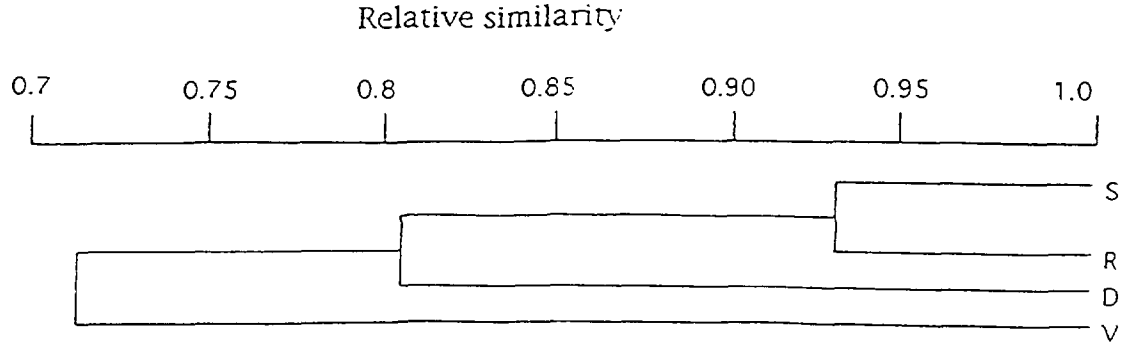

Fig. 1. Dendrogram of the Basella populations derived from RAPDs data.

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PLANT TYPE IMPROVEMENT OF INDIGENOUS RICE CULTIVARS THROUGH INDUCED MUTATIONS

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Morogoro, United Republic of Tanzania

Abstract

A high yielding, locally adapted cultivar 'Afaa Mwanza 1/159' of rice (*Oryza sativa* L.) which is tall and late in maturity, was irradiated with gamma rays at doses of 170, 210 and 250 Gy to shorten plant height and time of maturity. Twelve mutants were selected, and evaluated for yield performance in field trials from M_6 to M_9 generations. All the mutants were shorter in plant height, and gave higher mean yield than the parent. Correlation coefficient analysis showed that the number of productive tillers, number of panicles per square meter and grain filling in the panicle were important characters which influenced yield. On the other hand, panicle length had negative influence on yield. Cv. 'Supa India' and 'Salama' were also irradiated with doses of 170, 210, 240 Gy gamma rays. Analysis of M_2 populations of these cultivars indicated that mutagenesis created a lot of variation in plant height, maturity, spikelet fertility and panicle length. The induced variation shall be useful in selecting desired plant types.

1. INTRODUCTION

Rice is the staple food for approximately half of the world's population. It is predominantly a food crop in developing countries, which during 1987-89 produced and consumed 95% of the world output of rice, and accounted for nearly 80% of its trade [2]. Rice is an important crop in Tanzania. It has been estimated that about 60% of the population eat some rice [7]. Tanzania is the largest rice producer in Southern Africa Development Community (SADC) region. Yields of rice are disappointingly low in the developing tropical areas where most of the rice is grown. In Tanzania, for example average yield is estimated between 1.5 to 2.1 tons per ha [8] as compared to the average yields of more than 5 t/ha in the temperate countries, such as USA, Japan, Korea and Australia.

Since the inception of the International Rice Research Institute (IRRI) in 1962, it became evident that probably the most important single reason for the low yield of rice in the tropics was that the typical tropical rice plant is tall and leafy [1]. Plants with such characteristics are able to compete reasonably well with weeds, and produce fair grain yields under the prevailing conditions of low-inputs. However, when the modern methods of rice production, such as the use of adequate fertilizer (especially N), the adoption of good weed, insect and water control practices are applied, tropical varieties grow excessively tall, produce long droopy leaves, and tend to lodge. This led the plant breeders at IRRI to develop short-statured, high-yielding plant types with erect leaves, which resulted in a major breakthrough to increase rice yields. The plant breeders were able to shorten the plant from the traditional height of about 180 cm to 100 cm. They made the leaves short and upright, so that the water ran off quickly, and sunlight penetrated the lower leaves.

A major factor limiting rice production in Tanzania is the lack of lodging resistant, nitrogen responsive, high yielding varieties. Farmers still grow traditional varieties which are tall, and prone to lodging, when nitrogen is applied. The popularity of these cultivars lies in their superior grain quality and performance under high risk and poor management conditions. These varieties have long maturation period, and are not suitable in areas with marginal rainfall, since most of the rice grown is rainfed.

Mutagenesis is an effective tool for improving specific characters of existing varieties. Successful use of induced mutations to improve varieties has been reported by many workers [14, 11, 10]. The characters which have been successfully improved are plant height, maturity period and grain characteristics. A large number of short-statured mutants of local varieties have been produced in many countries [9, 15, 16]. Some of the semi-dwarf mutants are allelic and others are non-allelic to the semi-dwarfing gene of 'Deo-geo-woo-gen' (DGWG) [3, 10]. The mutation breeding programme at the Sokoine University of Agriculture (SUA) was aimed at reducing plant height and maturation period of the popular high yielding indigenous cultivars while maintaining the good qualities of the parents.

2. MATERIALS AND METHODS

2.1. 'Afaa Mwanza 1/159'

Dry seeds of cv. 'Afaa Mwanza 1/159' were sent for irradiation to the IAEA Laboratory, Seibersdorf (near Vienna) in 1987. The seeds were irradiated with gamma ray doses of 170, 210 and 250 Gy from a ^{60}CO source. The irradiated seeds and controls were sown in a field in Tanzania. The M_2 was raised from single M_1 plants, and grown as panicle-to-row progenies. Dwarf and semi-dwarf variants were selected among the M_2 population. In the subsequent generations, variants were selected on the basis of plant height, short growth duration and yielding ability. The selected variants were advanced to subsequent generations.

During 1992-1994, twelve mutants from M_6, M_7 and M_8 generations were evaluated for yield in replicated trials at the farm of Sokoine University of Agriculture (SUA), Morogoro, Tanzania. The design used was complete randomized block design with three replications. The plot size was 5 m x 2 m in which the plants were spaced 20 cm x 20 cm. Fertilizer was applied at a rate of 100 kg/ha in three split doses at planting, tillering and panicle initiation. M_9 generation was evaluated using the same design, spacing and cultural management at five sites at Morogoro, Dakawa, Ilonga, Ruvu and Katrin. However, the plot size was 5 m x 3 m.

Various characters, including plant height, days to 50% flowering, panicle length, number of panicles per square meter, 1000-grain weight, grain yield, grain appearance as well as biochemical properties were studied, and data were subjected to analysis of variance, according to the method of Gomez and Gomez [4].

2.2. 'Supa India' and 'Salama' Cultivars

Dry seed of the above cultivars were irradiated with 170, 210, 240 Gy gamma rays from ^{60}CO in Seibersdorf, in May, 1994. The irradiated seed and controls were sown on July 16, 1994 at SUA Crop Museum. The M_1 panicles were harvested, and planted as M_2 panicle-to-row progeny. Data on days to 50% flowering, number of tillers per plant, plant height and spikelet fertility were recorded. The M_2 plants were selected using plant height, early maturity and grain type as the selection criteria, and harvested individually.

3. RESULTS AND DISCUSSION

3.1. 'Afaa Mwanza 1/159'

The performance of the selected mutants for three years at SUA, Morogoro is presented in Tables I and II. The overall mean performance of mutants obtained from irradiation with 170 Gy and 210 Gy was higher than that of the parent for the three years of study. There was a highly significant difference between year and genotype x year interactions. During 1994, yields were higher than the other years. The low yield in 1995 likely resulted from floods when the crop was still at tillering stage.

TABLE I. GRAIN YIELD OF MUTANTS AND THEIR PARENT (SUA, MOROGORO)

Entry No.	Description	Yield (ton/ha) 1993	1994	1995	Mean
1	Afaa Mwanza 210Gy	5.45	6.24	3.44	5.04
2	"	3.74	6.18	2.59	4.17
3	"	2.45	6.36	3.05	3.95
5	"	2.86	6.08	2.22	3.72
6	"	3.15	6.14	2.25	3.85
7	"	2.29	6.09	3.17	3.85
4	Afaa Mwanza 170Gy	4.53	5.32	2.17	4.01
9	"	3.64	5.36	2.45	3.82
12	"	3.73	6.28	1.85	3.95
13	"	3.48	5.16	2.48	3.71
Control	Afaa Mwanza	3.31	4.76	2.56	3.54
CV%		4.6	14.0	16.96	

During all years, plant height of the variants was shorter than that of the parent. The mean plant height of the variants ranged from 86.5 to 95.8 cm, as compared to 140.2 cm of the parent. The mean number of days to 50% flowering were less for the mutants than the parent (Table II). The year 1994 was favourable in terms of rainfall, and the trial was planted early during the rainy season. That may explain the higher yield and taller plant growth 1994 as compared to the other years. In summary, mutagenesis reduced plant height of the parent variety. The results suggest that it was possible to select mutants that combined short culm, earliness, and high yield potential. Reduction in plant height through induced mutations has been reported [11, 15, 10]. A number of semi-dwarf rice mutants have been released in various countries during the past years [3, 12, 13].

TABLE II. PLANT HEIGHT AND DAYS TO 50% FLOWERING OF MUTANTS AND THEIR PARENT (SUA, MOROGORO)

Entry No.	Mutagen/dose	Plant Height 1993	1994	1995	Mean	Days to 50% Flowering 1993	1994	1995	Mean
1	Afaa Mwanza 210Gy	84.7	111.2	84.3	93.7	105	99	88	97
2	"	85.6	113.0	88.9	95.8	118	91	90	100
3	"	85.6	113.0	87.4	95.3	107	93	92	97
5	"	85.6	109.1	87.6	94.1	102	93	80	93
6	"	84.3	96.3	79.0	86.5	111	87	89	96
7	"	82.4	104.9	88.9	92.1	110	94	97	100
4	Afaa Mwanza 170 Gy	80.7	114.9	82.3	92.5	108	86	79	91
9	"	82.4	103.6	82.1	89.4	118	91	86	98
12	"	84.4	115.7	78.9	93.0	99	89	87	92
13	"	82.8	112.2	81.5	92.2	104	90	94	96
Control	Afaa Mwanza	115.2	183.2	122.2	140.2	115	99	96	103
CV%		4.4	5.1	5.3	3.3	2.2	4.4		

The grain yield of M_9 generation at three locations is presented in Table III. There was a highly significant variation for locations and genotype x location interaction. A number of mutants out-yielded the parent variety. Mutant No. 1 was the highest yielder followed by No 12; Mutant No. 6 yielded the lowest.

The correlation coefficient analysis for M_9 at Morogoro is presented in Table IV. Grain yield per plot was positively correlated with the number of effective tillers per plant,

TABLE III. GRAIN YIELD OF MUTANTS AND THEIR PARENT GROWN IN THREE LOCATIONS

Entry No.	Description	Yield (tons/ha) Morogoro	Dakawa	Ilonga	Mean
1	Afaa Mwanza 210Gy	3.44	4.84	3.50	3.93
2	"	2.59	4.23	2.43	3.08
3	"	3.05	4.23	1.86	3.05
5	"	2.22	4.10	3.09	3.14
6	"	2.25	4.40	3.08	3.24
7	"	3.17	3.73	3.02	3.31
4	"	2.18	3.83	3.29	2.77
9	"	2.47	3.63	2.84	2.98
12	"	1.85	4.73	3.26	3.28
13	"	2.48	4.60	2.95	3.34
Control	Afaa Mwanza	2.56	3.77	3.45	3.26
"	Supa India	2.48	2.43	2.53	2.48
Sx		±0.250	±0.333	±0.314	
CV%		16.96	14.54	11.07	

number of panicles per square meter and percentage of filled grains per plant. Grain yield was negatively correlated to panicle length. Number of panicles per unit area has been reported to be positively associated with grain yield in rice [6, 5]. The positive correlation of grain yield and number of panicles per unit area indicates that the improvement of this character in the test population would be effective in increasing yield.

TABLE IV. CORRELATION COEFFICIENT BETWEEN YIELD, YIELD COMPONENTS AND GROWTH PARAMETERS OF M_3 MUTANTS (n = 36) (SUA, MOROGORO)

	No. of tillers/ plants	Days to 50% Fl	No. of panicles /sq. m.	Plant Height	Panicle length	1000-grain wt	% filled grains	Yield/ plot
No. of tillers/plant	1.00							
Days to 50% Fl	0.395	1.00						
No. of panicles/ sq. m.	0.583	0.310	1.00					
Plant Height	-0.235	0.045	-0.281	1.000				
Panicle length	-0.378	-0.250	-0.413	0.662	1000			
1000 grain wt.	-0.582	-0.519	-0.598	0.441	0.612	1.000		
% filled grains	0.270	0.142	-0.078	0.310	0.045	0.092	1.000	
Yield/plot	0.412	0.262	0.403	0.093	-0.432	-0.294	0.296	1.000

Evaluation of grain appearance is present in Table V. Most mutants had medium and long grain, while Mutant No. 13 had extra-long kernels. All the mutants showed some amount of endosperm opacity, except Mutant No. 13, which had translucent grains.

The eating and cooking qualities were determined by amylose content, gel consistency and gelatinization temperature, and aroma. All mutants, except Mutant No. 9 and their parent had intermediate amylose content (Table VI). Variation in gel consistency, gelatinization temperature and aroma was observed. Mutant No. 13, which had extra long grains, translucent endosperm, intermediate amylose content, soft gel consistency and low to intermediate gelatinization temperature, stands a good chance of acceptance by consumers in Tanzania. Mutants Nos. 1 and 2 which had high yield potential (Tables I & II) have acceptable cooking quality but their grains have more opacity.

This study has demonstrated that semi-dwarf mutants, induced with gamma irradiation, combined high yield potential with earliness and acceptable grain quality. Preliminary multi-location trials have identified Mutant No. 1 to be higher yielding than the rest. The average performance of mutants at SUA for three seasons have indicated that Mutant Nos. 1, 2, 3, 4 and 12 have a high yield potential.

TABLE V. PHYSICAL GRAIN CHARACTERISTICS OF RICE GENOTYPES

Radiation dose	Entry No.	Variety	Length (mm)	Size Category	Length/ width	Description	Opacity Chalkiness	Description
170 Gy	4	Afaa Mwanza	7.09	Long	2.53	Medium	< 10	Small
	8	"	6.55	Medium	2.58	Medium	< 10	Small
	9	"	7.00	Long	2.80	Medium	< 10	Small
	10	"	6.58	Medium	2.63	Medium	10-20	Medium
	12	"	7.24	Long	2.60	Medium	> 20	Large
	13	"	7.73	Extra-long	3.00	Medium	no chalkiness	Trans.
210 Gy	1	"	6.27	Medium	2.26	Medium	10-20	Medium
	2	"	6.30	Medium	2.18	Medium	10-20	Medium
	3	"	6.33	Medium	2.22	Medium	> 20	Large
	5	"	6.67	Long	2.50	Medium	> 20	Large
	6	"	6.96	Long	3.03	Slender	< 10	Small
	7	"	6.40	Medium	2.41	Slender	10-20	Medium
Control		Afaa Mwanza	6.83	Long	2.72	Medium	no chalkiness	Trans.
"	Supa India		8.12	Extra Long	3.11	Slender	"	Trans.

TABLE VI. PHYSICO-CHEMICAL CHARACTERISTICS OF SOME RICE GENOTYPES

Radiation dose	Entry No.	Variety	Amylose content		Aroma	Gel		Gelatinization Temp.	
			%	Description		Consistency	Description	Temp.	Description
170 Gy	4	Afaa Mwanza	19.46	Int.	Slight	51.5	Med.	< 69	Low
	8		22.20	Int.	Mod.	50.1	Med.	70-74	Int
	9		19.36	Low	No aroma	90.0	Soft	74-75	H/Int
	10		22.08	Int.	Mod.	50.0	Med.	70-74	Int
	12		22.08	Int	Mod.	83.0	Soft	70-74	Int
	13		21.85	Int	Strong	96.0	Soft	< 69	Low
210 Gy	1		22.91	Int	No aroma	95.0	Soft	< 69	Low
	2		21.85	Int	No aroma	99.0	Soft	70-74	Int
	3		21.64	Int	Slight	99.0	Soft	70-74	Int
	5		22.67	Int	Slight	90.0	Soft	70-74	Int
	6		22.43	Int	Slight	79.0	Med/soft	70-74	Int
	7		23.07	Int	Slight	88.0	Soft	70-74	Int
Control		Afaa Mwanza	22.78	Int	Mod.	45.0	Med.	70-74	Int
		Supa India	23.93	Int	Strong	28.5	Hard	< 69	Low

3.2. 'Supa India' and 'Salama'

Primary panicles of each M_1 plant were harvested individually, and spikelet fertility was determined by dividing the total number of grains by the number of filled grains in a panicle. The results (Tables VII & VIII) indicated that there was a reduction in spikelet fertility of M_1 plants as compared to the control. There were some difference between the two cultivars. Spikelet fertility decreased as dose rate increased, except for cv. 'Supa India' which had a higher mean value with 240 Gy than at the lower doses.

TABLE VII. SPIKELET FERTILITY (%) IN M_1 GENERATION OF CV. 'SUPA INDIA' TREATED WITH GAMMA RAYS - 1994 DRY SEASON

Dose (Gy)	No. of plants	X±SD	Range
170	70	52.83±20.64	10.6 - 84.0
210	50	52.43±16.08	24.2 - 81.2
240	93	55.15±16.70	19.0 - 85.5
Control	34	66.46±16.95	31.3 - 87.6

TABLE VIII. SPIKELET FERTILITY (%) IN M_2 GENERATION OF CV. 'SALAMA' TREATED WITH GAMMA RAYS - 1994 DRY SEASON

Dose (Gy)	No. of Plants	X±SD	Range
170 Gy	79	52.40±24.78	7.4 - 91.8
210 Gy	64	50.92±18.86	13.4 - 88.0
Control	10	58.75±22.35	26.6 - 87.8

In M_2, plant height, days to 50% flowering and panicles length were studied. There was a significant reduction in plant height (Table IX). The reduction in plant height increased with the dose. However, the reduction in the progeny of cv. 'Supa India' treated with 210 Gy was more than with 240 Gy. This could be attributed to Yellow Mottle Virus disease which affected plants from treatment with 210 Gy.

TABLE IX. EFFECT OF MUTAGEN AND DOSE RATE ON PLANT HEIGHT (cm) OF M_2 POPULATIONS

		'Supa India'			'Salama'		
Mutagen	Dose rate	Range	Mean±SD	CV	Range	Mean±SD	CV
Gamma rays	170 Gy	79-113	107.8±12.9	12.0	87-172	141.7±19.9	14.1
"	210 Gy	55-118	88.4±13.9	26.3	86-167	135.2±16.2	11.9
"	240 Gy	80-158	114.1±15.4	16.5			
	Control	120-141	125.0±13.4	10.7	120-170	152.0±12.2	8.4

The number of days to flower were less in the respective control populations than in the treated materials (Table X). However, the wide variation in this trait in the M_2 population indicated that it was possible to select mutants for desired maturity period. Generally, cv. 'Salama' and its mutants flowered earlier than cv. 'Supa India' and its mutants; this could be because cv. 'Salama' is an upland cultivar and has been selected for earliness.

TABLE X. EFFECT OF GAMMA RAY DOSE ON DAYS TO 50% FLOWERING IN M_2 POPULATIONS OF RICE CULTIVARS

Dose rate	cv. 'Supa India'			cv. 'Salama'		
	Range	Mean±SD	CV	Range	Mean±SD	CV
170 Gy	107-131	121±3.0	13.9	81-111	98.2±10.6	10.8
210 Gy	114-131	119±3.1	5.8	88-113	98.6±8.2	8.3
240 Gy	110-139	123±7.4	8.0			
Control	107-123	115±5.1	3.9	80-85	83.0±3.1	13.3

The effect of irradiation on panicle length is shown in Table XI. There was no significant difference between different doses; however, the parents had higher values for this trait than the treated materials, suggesting that mutagenesis generally reduced panicle length. The effect of mutagenesis on spikelet fertility is shown in Table XII. The mean value for this trait was higher in the progeny than the parents. There was a wide variation in spikelet fertility in all treatments. Since spikelet fertility is an important yield component, it would be possible to select desirable mutants in the subsequent generations. This study has indicated that mutagenesis created a wide variation in plant height, maturity, spikelet fertility and panicle length. Therefore, plants with desired characteristics could be selected in the subsequent generations. Pronounced changes in plant height and tillering ability observed in the treated populations suggested that improved plant types are induced by mutagenic treatment.

TABLE XI. EFFECT OF MUTAGEN AND DOSE RATE ON PANICLE LENGTH (cm) OF M_2 POPULATIONS OF RICE CULTIVARS

Mutagen	Dose rate	cv. 'Supa India'			cv. 'Salama'		
		Range	Mean±SD	CV	Range	Mean±SD	CV
Gamma rays	170 Gy	15-34	21.2±3.0	13.9	17-26	22.2±1.8	8.2
"	210 Gy	13-26	20.1±2.9	5.5	17-28	22.8±2.3	10.3
"	240 Gy	14-29	22.8±2.6	2.8			
	Control	19-29	23.6±2.6	19.7	18-30	23.0±1.7	2.0

TABLE XII. EFFECT OF MUTAGEN AND DOSE RATE ON SPIKELET FERTILITY OF M_2 POPULATIONS

Mutagen	Dose rate	'Supa India'			'Salama'		
		Range	Mean±SD	CV	Range	Mean±SD	CV
Gamma rays	170Gy	7.7 - 48.6	22.7±11.2	49.6	5.9 - 61.5	30.3±12.5	41.5
"	210Gy	15.4 - 43.6	25.3±7.9	31.5	12.0 - 64.2	29.2±13.2	45.4
"	240Gy	9.1 - 43.4	21.5±7.1	32.9			
	Control	3.8 - 24.2	15.4±6.2	40.1	11.1 - 45.6	27.6±9.5	34.3

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SOMATIC EMBRYOGENESIS IN CASSAVA: A TOOL FOR MUTATION BREEDING

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Abstract

Cassava is an important food and livestock feed crop. The effect of gamma radiation on somatic embryogenesis and plant regeneration in cassava clones of African origin was investigated. Explants from young leaves of cassava were cultured on MS medium, supplemented with 18.1 mM 2,4-D and 2 mM CuSO4, solidified with 0.3% Phytagel. Compact and friable calli were observed after 10-15 days of explant culture in dark, which produced somatic embryos in all but one clone. The somatic embryos showed morphological aberrations, such as fused cotyledons, lack of meristematic tip, epicotyl elongation, and had low germination rate; desiccation of embryos increased germination. Histological study showed that the somatic embryos were of multicellular origin. Leaf explants were irradiated with doses between 4 to 38 Gy of gamma rays, and cultured on somatic embryo induction medium. In addition, somatic embryos were irradiated with gamma ray doses from 10 to 18 Gy, and analyzed for gemination. LD_{50} for embryogenic response of leaf-explants was at around 20 Gy, while that for somatic embryo germination was ca. 10 Gy.

1. *INTRODUCTION*

Cassava is a multi-purpose plant and its role as a staple food and livestock feed is well established. Traditional cross-breeding is somewhat constrained by the sporadic flowering and the relatively low number of seeds produced. The vegetative propagation of cassava allows retention of desirable traits obtained through sexual breeding. In many vegetatively propagated plants, cross-breeding takes long time, and spontaneous mutations have been valuable as a source of variation to obtain new cultivars. Somatic mutations that occurred spontaneously among the clones of cassava may have played an important role in its domestication. Vegetative propagation of cassava permits maintenance of high level of heterozygosity. Mutagenic treatment may uncover recessive alleles by mutating or deleting corresponding dominant alleles. In vegetatively propagated species, a mutation can arise either by the direct effect of a mutagen on DNA or by disruption of a chimeric situation. A meristem is composed of three histogenic layers (L1, L2, L3). Following mutagenic treatment, it is possible that these layers undergo a restructuring, and genes previously masked in one layer may express after mutagenesis in the another layer.

Mutation induction optimizes genetic variation with minimal genome disruption [1]. In cassava, mutagenesis has been successfully used to enhance the limited genetic variation in mealiness and cooking quality [2]. However, the treatment of multicellular structures such as buds leads to chimeric tissues. Only after shoot growth and a number of cycles of vegetative propagation, can a stable periclinal chimera or a solid mutated tissue be obtained. To bypass this problem, several systems have been proposed, e.g. by mutagenic treatment of single cells. This may not necessarily require treatment of isolated cells or protoplast. It may also include treatment of differentiated tissues coupled with tissue culture on defined media to induce regeneration from single cells, e.g. through adventitious bud formation or somatic embryos [3].

The objective of the present research was to study the effect of gamma radiation on somatic embryogenesis and plant regeneration in several cassava clones of African origin.

2. MATERIAL AND METHODS

2.1. Plant material

In vitro cultures of Mcol 22 and Mcol 1505 plants were obtained from CIAT (Centro Internacional de Agricoltura Tropical), Cali, Colombia. All other clones (see Table I) were obtained from NRCRI, Umuahia, Nigeria. The plants were propagated on GA7 medium, consisting of MS medium [4], supplemented with 3% sucrose, 1 mg/l thiamin, 100 mg/l myo-inositol, 0.1 mM BAP, 0.01 mM NAA, 0.1 mM GA3, pH 5.8 and maintained on a gyrator shaker at 60 rpm at 28 C, under continuous lighting of 270 mW dm^2.

2.2. Somatic embryogenesis

For somatic embryogenesis, young leaf lobes, 4-6 mm long, were inoculated on MS medium, pH 5.8, containing 18.1 mM 2,4-D and 2 mM CuSO4, and solidified with 0.3% Phytagel, according the protocol of Matthews et al. [5]. The explants were incubated in dark at 25°C. The same medium but with half the amount of 2,4-D was used to induce somatic embryos in liquid medium. Small pieces of embryogenic callus were cultured in 100 ml flasks, placed on a gyrator shaker (120 rpm), and observed at weekly intervals for somatic embryo development.

2.2.1. Somatic embryo maturation

Primary, late-globular stage somatic embryos were transferred to MS medium containing 10 mg/l thiamine HCl, 1.5 mg/l pyridoxine HCl, 1.5 mg/l nicotinic acid, 2 mg/l glycine and 100 mg/l inositol, 3% sucrose, 0.5 charcoal and 3% Phytagel.

2.2.2. Somatic embryo desiccation

Mature embryos, ca. 10 mm long with well developed green cotyledons were placed in a Petri-dish, sealed with parafilm and kept under dim light at 25°C. The duration needed to achieve ca. 20% weight loss was determined in each case.

2.2.3. Germination

Desiccated embryos were transferred to a medium containing half-strength MS major salts, full-strength minor MS salts, 10 mg/l thiamine HCl, 1.5 mg/l pyridoxine HCl, 1.5 mg/l nicotinic acid, 2 mg/l glycine, 100 mg/l inositol, 2% sucrose, and 2% Phytagel.

2.3. Histology

Samples were fixed in FAA solution, dehydrated gradually with ethanol-toluene series, embedded in paraplast and cut in 10 μm sections with a sliding microtome. Sections were fixed on glass slides, and triple stained with hematoxylin, fast green and safranin.

2.4. Gamma irradiation

Two experiments were performed: 1) Radio-sensitivity of the explants, i.e. young leaf lobes, and 2) of somatic embryos. Leaf lobes were irradiated using a ^{60}Co source with doses of 0, 4, 8, 12, 16, 20, 24, 28, 32, 36, 38 Gy, and cultured on somatic embryo induction medium. After 10-15 days, the explants were screened for somatic embryo formation.

For the treatment of somatic embryos, explants of MC_0l22 were sorted according to their developmental stage, i.e. according to the cotyledonary development, and irradiated with gamma rays (0, 10, 12, 14, 16, 18 Gy). After treatment, all the plant material was plated on medium for somatic embryogenesis, and analyzed for gemination.

TABLE I. RESPONSE OF CASSAVA CLONES TO FORM SOMATIC EMBRYOS

Clone	Embryogenic explants (%)	Explants with more than 10 pro-embryos (%)	Mature embryos harvested/ explant	Mature embryos harvested after 40 days	Embryos germinated into plants (%)
MCol22	90-100	60-70	4.8	26	45.55
MCol1505	90-100	50-70	0	0	0
TMS-71173	15-20	15-20	6.7	94	23.5
TMS-30211	0	0	0	0	0
TMS-4488	20-30	15-20	0	0	0
ANKRA	20-30	20-30	1	3	0
TMS-01935	10-15	0-10	2	3	0
TMS-00942	60-70	40-50	0	0	0
TMS-30572	0-20	0-20	0	0	0
TMS-91934	10-20	5-15	0	0	0
U41044	30-40	20-35	0	0	0

3. RESULTS

3.1. *In vitro* somatic embryogenesis

Compact and friable calli were observed after 10-15 days of explant culture on the induction media in dark. Induction of somatic embryos was achieved in all but one clone tested, and ranged from 0 to 60%, e.g. 14% in TMS71173, 8% in TMS 4488, 22% in Ankra, 4% in TMS 01935, and 0 in TMS30211. TMS30572 with 14%, and TMS00942 with 54% gave the best response. A comparable response was obtained in liquid culture; however, no cyclic embryogenesis was achieved in liquid medium. It is noteworthy that embryos were also produced by friable callus. To our knowledge, this is the first report of somatic embryogenesis in cassava from friable callus. However, in all cases, the somatic embryos obtained showed a number of morphological aberrations, such as fused cotyledons, lack of meristematic tip, epicotyl elongation, and low germination rate. When transferred to the regeneration medium, these abnormal explants did not germinate. A higher germination percentage was achieved after desiccation of somatic embryos, ca. 5 mm length, for a variable time ranging from 48 to 288 hrs on Petri dishes lined with filter paper (Table II).

TABLE II. EFFECT OF DESICCATION TIME ON SOMATIC EMBRYO GERMINATION OF CASSAVA (M.COL 22)

Desiccation time (hours)	Germination (%)
0	3
48	12
72	11
144	19

3.2. Histology

Somatic embryogenesis in relation to mutation breeding allows the possibility to produce large populations of vegetative propagules, and of irradiating somatic tissues capable of regenerating plants from single cells, thus overcoming the undesirable phenomenon of chimerism. Freshly isolated leaf lobes were composed of small, regularly spaced mesophyll cells with densely staining nuclei and poorly staining vascular tissues. After 3-4 days of culture, cells within the leaf-lobe lamina stained even more heavily and the abaxial explant surface become slightly swollen and glossy. By day seven, divisions were observed in some cells near to the abaxial surface and mid-vein of the explants. After three weeks of culture, cell division continued and globular somatic embryos developed from multiple cell budding, the abaxial cell layer forming the embryo protoderm. After four weeks in culture, longitudinal sections revealed the presence of bipolar structures formed by root and shoot meristems, cotyledons and procambium. However, the normal embryogenic pathway consisting of a well defined morphogenetic (globular, heart, torpedo and cotyledonary stage embryos) events was seldom observed. In particular, the system used in our laboratory produced abnormal torpedo-stage embryos without heart-stage formation. These abnormal torpedo finally developed in "horn-shape" embryos, probably from cotyledon fusion. Longitudinal sections were not able to differentiate this structure from normal dicotyledonary embryos. However, cross sections revealed a circular procambial strand. Our histological study conducted at different times during the embryo formation points out the multicellular origin of the newly formed pro-embryogenic structures.

3. 3. Radio-sensitivity

Irradiated leaf lobes showed higher resistance to gamma rays than the somatic embryos (Fig.1). A fifty percent reduction in embryogenic response from leaf-lobes was found at around 20 Gy while for somatic embryos the same reduction was observed at doses of ca. 10 Gy. For the radio-sensitivity test, somatic embryos were divided into three class according to their size and roughly to their developmental stage, i.e. small (< 5 mm), medium (< 5-10 mm) and large (> 10 mm). According to our results, the somatic embryo response seemed to be concentrated in a very limited dose range, i.e. 5-10 Gy. This is a much lower dose than recommended for *in vitro* cuttings.

4. DISCUSSION

Asexual multiplication is a unique process by which plants give rise to new individuals identical to the parental plant. Somatic embryogenesis combines many advantages over classical vegetative propagation, providing a framework for genetic manipulation and an efficient method for storage and distribution of otherwise bulky planting material in vegetatively propagated crops. Beside these, somatic embryos could be used to support mutation breeding in plants where cross breeding is hampered either by high heterozygosity or apomixis. However, mutagenic treatment of asexual propagules results in chimera formation and lengthy procedures for the cleaning up of desired mutation.

Traditionally, mutation induction in cassava has been performed using cuttings or *in vitro* plants. Both systems have the advantage of being cheap and easy to handle. The formation of chimeras is unavoidable in multicellular target such as plant meristems. Further, a mutation is a random event that occurs with a certain frequency. Hence, it is essential to irradiate an adequate number of cells or vegetative primordia to ensure the success of the breeding programme. Plant mutation breeding relies on an efficient system of induction, recovery and genetic confirmation. Hence, the number of regenerant population should be high, compatible with the final goal, with efficient mutant recovery and cleaning to overcome the chimera problem. Such a system should be cost effective in terms of time and labour. Our results with somatic embryogenesis in cassava and specially with the African varieties showed that only the first pre-requisite was fulfilled, i.e. the possibility to produce a large population for mutagenic treatment. The percentage of regenerants ranged from 54 to 8% and each explants was able to sustain somatic embryos formation for more then 2 month. However, the high ratio of aberrant/normal embryos, and the consequent low germination and the long time needed to complete the cycle from explant to potted plant suggest caution with respect to the recovery and cleaning of mutations. The same caution should be applied to the other two pre-requisites.

The histological data from the present studies do not support the single cell origin of the somatic embryos in cassava. This fact suggests prudence and need of deeper investigation before applying this system in breeding programme. Nevertheless, our findings, i.e. the possibility to induce somatic embryogenesis in different African cassava clones could be considered a first step toward a better system for mutation induction. It is necessary to define the factors that are responsible for the blockage of the genetic and metabolic pathways leading to the correct development of somatic embryos in order to refine the technology. Further studies are also needed to establish a reliable protocol for induction of somatic embryos from single cell cultures initiated from friable callus.

In conclusion, while the induction of somatic embryos does not represent a problem in African cassava clones, the efficiency of plant regeneration is still a limiting factor. It is possible to reculture primary somatic embryos as a source for plant regeneration and irradiation. Abnormal embryo structures and shoot elongation is still a primary problem in our system. The use of a desiccation step could increase and improve plant regeneration but still at a low frequency, specially in the African clones. The origin of abnormal somatic embryos has been discussed by Stamp and Henshaw [6], suggesting that leaf-like embryos are the result of partial expression of morphogenetic competence. Liu et al. [7] reported that auxin polar transport is essential for the establishment of bilateral symmetry during early embryogenesis. If for some reason, auxin transport is blocked in the top of the globular embryos, this growth factor must diffuse from the meristematic region towards the peripheral cells, thus stimulating the formation of a collar like cotyledon, which ultimately forms a horn like structure.

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CREATION OF VARIATION IN *Basella* FOR RUST RESISTANCE THROUGH MUTAGENESIS

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Abstract

African spinach, basella is grown as a leafy vegetable in Central Africa. Basella cultivars belong to the species *Basella alba* and *B. rubra* which are seed propagated and are likely Asiatic in origin. *Basella alba* seeds were irradiated with doses of 50, 100, 200, 300 and 500 Gy to create variation for rust resistance which is caused by the fungus, *Uromyces basellae* Sidow. The effects of irradiation were investigated on seed germination, plant mortality and height. Seed germination varied from 97% for those irradiated with 50 Gy to 39% with 500 Gy, and LD_{50} for seed germination was between 300 to 400 Gy. Doses between 50 and 150 Gy did not cause any mortality of plants obtained from irradiated seeds; however, doses between 200 to 500 Gy caused high mortality among such plants. Irradiation with 150 Gy inhibited plant growth by 48% in relation to the growth of control plants. Based on the results, radiation doses above 150 and up to 400 Gy were used for the production of desired variation.

1. INTRODUCTION

In Congo, plant biotechnology is being used for the last ten years to improve the leafy vegetables, baselle (*Basella alba* and *B. rubra*) for resistance to baselle rust caused by the fungus, *Uromyces basellae* Sidow. In Central Africa, two groups of baselle or African spinach are grown. The first group is represented by the species *Basella alba* and *B. rubra*, which are commonly used by people in Central Africa; they are seed propagated, but are susceptible to rust. *B. alba* is characterized by green stems, and *B. rubra* by purplish stems. Both seem to have an Asiatic origin. The second group is represented by an unidentified *Basella sp.* This variety has dark green leaves and stems, and is propagated vegetatively but is resistance to baselle rust. Hence, it is not possible to use conventional methods to produce hybrids which combine good quality with disease-resistance. Therefore, the following two strategies were used to produce the desired variants: 1. somaclonal variation among tissue cultures initiated from meristematic cells of susceptible varieties of *Basella alba* and *B. Rubra*. The use of such techniques has been reported in literature [1,3,6]. 2. the use of induced mutations to obtain disease resistant plants [2,4]. Mutagenesis of cell cultures and seeds of susceptible varieties sensitive to a given disease with gamma rays has been reported [7, 8, 9, 10]. For this purpose, germplasm collections of different varieties of *Basella* were made and cultured *in vitro*, and studies were undertaken on the characterization of the pathogen and the disease. The objective of these studies was to produce and select variants which combined good quality with resistance to rust.

2. MATERIALS AND METHODS

2.1. Irradiation of seeds

Mutagenic treatment was given with Cesium 137 irradiation source of LISA I type with dose delivery of 2.5 Gy/minute. *Basella alba* seeds in lots of 246 were irradiated with doses of 50, 100, 200, 300 and 500 Gy. A control lot of 246 non-irradiated seeds was included. After the treatment, the seeds were sown and the following parameters were investigated to evaluate the effects of irradiation: 1. the rate of germination, particularly the emergence of cotyledons above ground level, 2. plant height, and 3. plant mortality during the first 24 days.

2.2. Identification of the pathogen

The pathogen was identified from samples sent to Netherlands. Scanning and transmission electron microscopic studies were carried out to describe the pathogen.

2.3. Research on pathogen control

In addition to mutagenesis and somaclonal variation, research on other methods such as chemotherapy and amelioration of cultural practices were carried out. For this purpose, some fungicides were tested on *in vitro* germination of ecidioconidia and *in vivo* by spraying plots. baselle plots were compared by growing either under plastic shelter or in open, and the percentages of plants showing damage were determined in plots after 20, 25 and 30 days.

3. RESULTS

3.1. Disease description

Baselle rust is characterized by the formation of oval to circular, yellow orange spots on the lower and upper surface of the leaves (Figs. 1-3). The leaf-spots represent ecidioconidia from the fungal fructification. Electron microscopy studies showed presence of many spores under the surface of each ecidioconidia. The pathogen produced only one type of ecidioconidia. Infectious cycle includes the following stages: 1. infection phase of leaves by ecidioconidia, 2. penetration phase, and 3. reproduction phase or fructification followed by dissemination of ecidioconidia.

Basella alba and *Basella rubra* are the main hosts of *Uromyces basellae.* Other plants, notably, *Hibiscus sabdaripha* and *Solanum nigrum*, also constitute secondary hosts. Recently, the disease has also been observed on an epiphyte that could produce an epiphytic conservation of *Uromyces basella.* The studies on different varieties of *Basella* can be summarized as follows: 1. Varieties of baselle, which are propagated sexually (*B. rubra, B. alba*), are all sensitive to the pathogen, 2. *Basella sp.*, which is propagated from vegetative parts, is resistant to the pathogen, and 3. Some plants of *Basella alba* and *Basella rubra* showed containment of disease symptoms; these plants might be hypersensitive to infection.

3.2. Pathogen

In the previous studies, based on the cup-shaped characteristics of ecidioconidia bags, the disease causing fungus was identified as *Puccinia sp.* [11]. However, a new identification was made in The Netherlands from the samples sent by us, which established the pathogen causing baselle rust to be *Uromyces basella* Sidow.

The observations made with light, scanning and transmission electron microscopy can be summarized as follows: 1. The pathogen produces only one type of ecidioconidia, 2. The germination of ecidioconidia produces a germinal filament that penetrates in foliar tissues through stomata, 3. The external morphology of ecidioconidia shows characteristic morphology by which these can be distinguished from other types, and 4. Ultrastructural studies suggest a mono-nuclear structure.

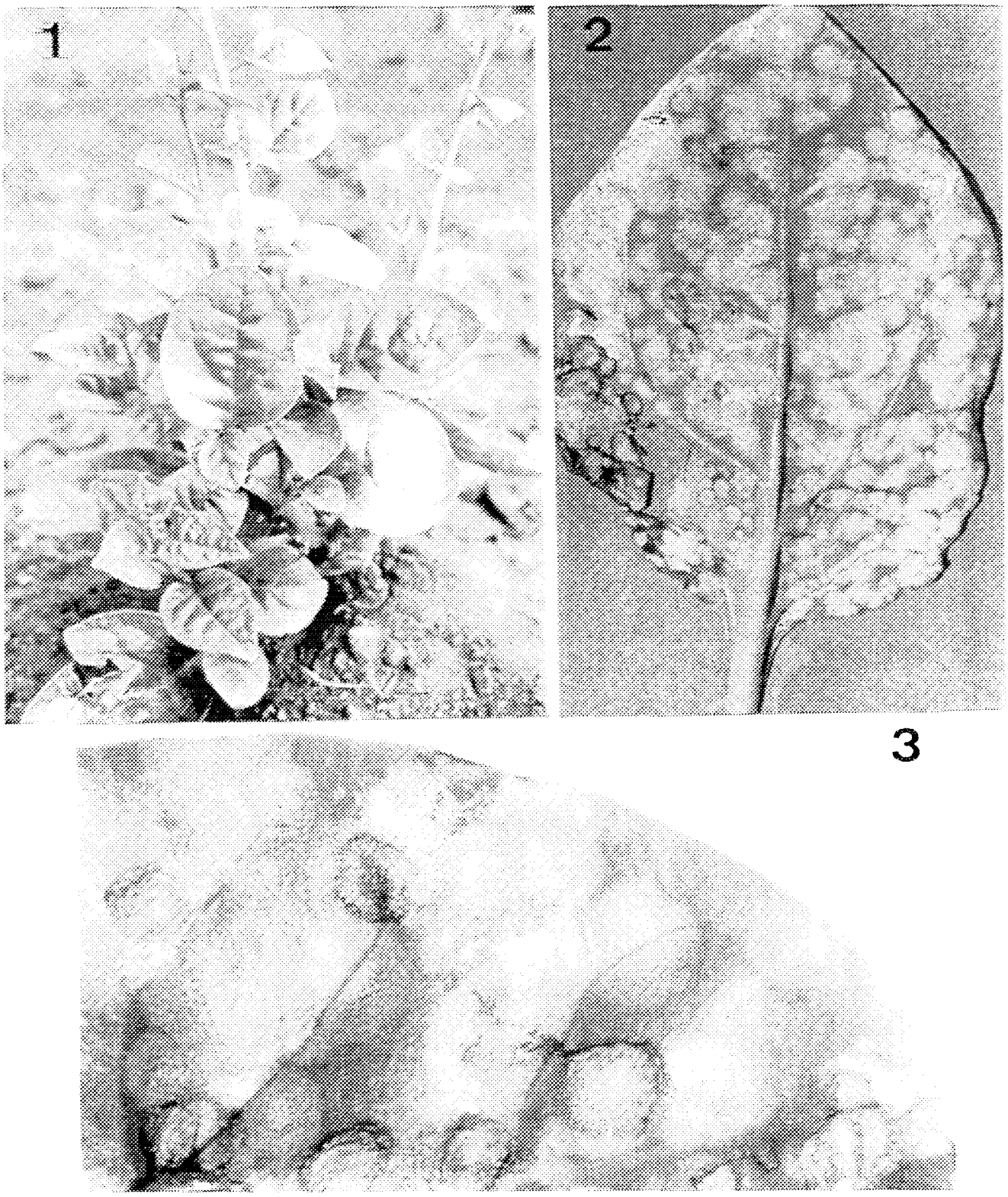

Fig. 1-3. 1. Basella alba plant 2. Baselle leaf with symptoms of rust 3. Ecidioconidia on leaf

3.3. Mutagenesis of *Basella alba* seeds

The first objective was to determine the irradiation doses which produced a 50% reduction in various plant characters. The following results were obtained by studying different parameters.

Germination rate of irradiated seeds: Seed germination varied from 97.3% for seeds irradiated with 50 Gy to 36.8% with 500 Gy (Fig.4). Considering these results, inhibition of germination of irradiated seeds ranged between 2.7% at 50 Gy and 63.2% at 500 Gy (Fig.5).

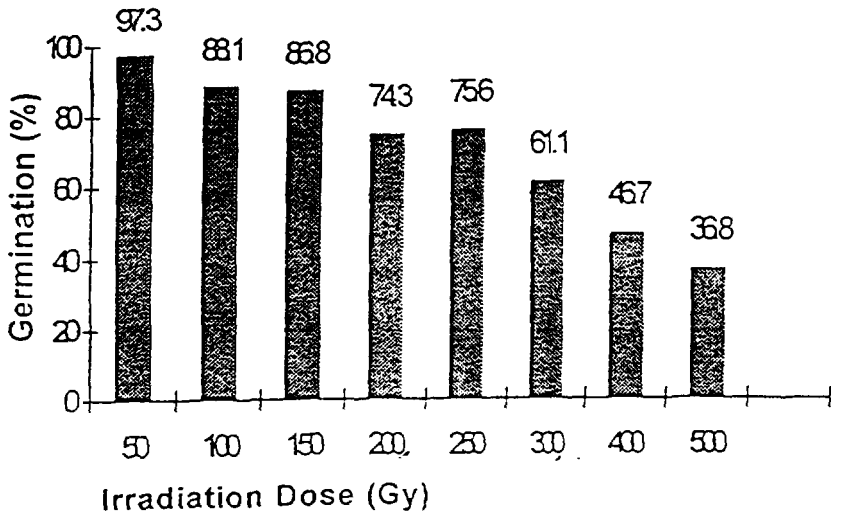

Fig. 4. Effect of irradiation on seed germination.

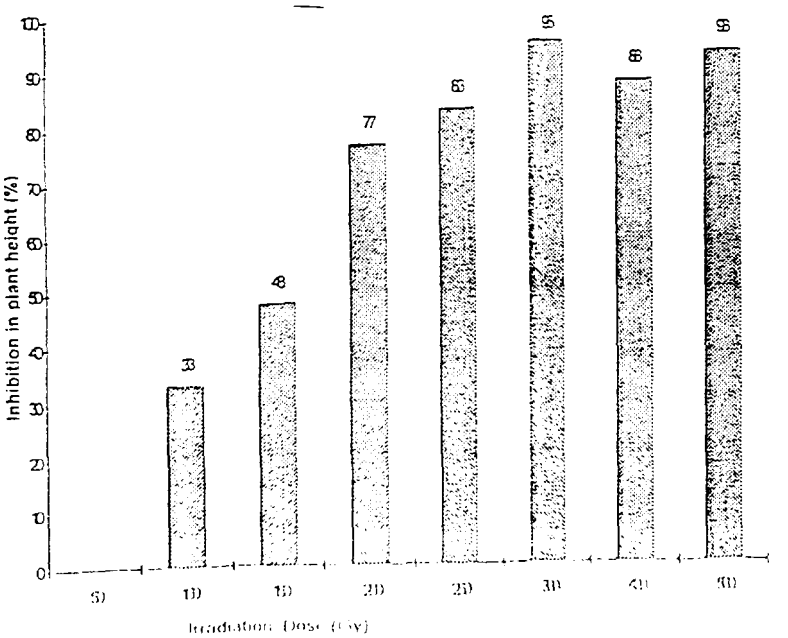

Fig. 5. Effect of irradiation on the inhibition of growth of plants obtained from irradiated seeds.

***Evaluation of plants* growth:** Height of plants obtained from irradiated seeds were measured after 24 days (Fig. 6). Mean height ranged from 45 cm for plants obtained from seeds irradiated with 50 Gy to 3 cm of those irradiated with 500 Gy. The results showed that the irradiation with 100 Gy inhibited vegetative growth of plants by 33.5% and with 500 Gy 98.5% (Fig. 7).

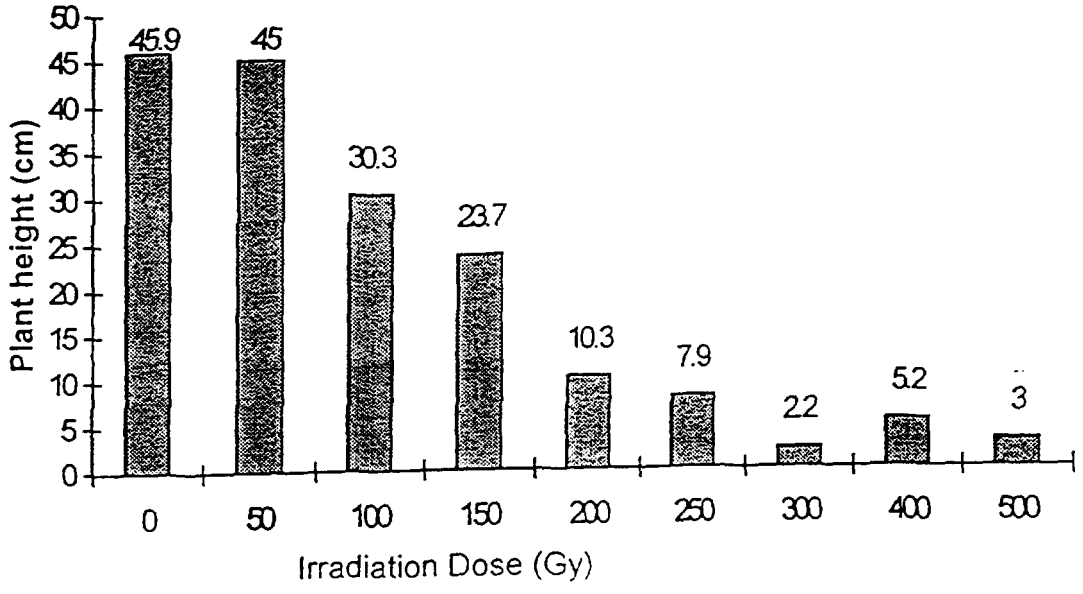

Fig. 6. Effect of irradiation on height of plants produced from irradiated seeds.

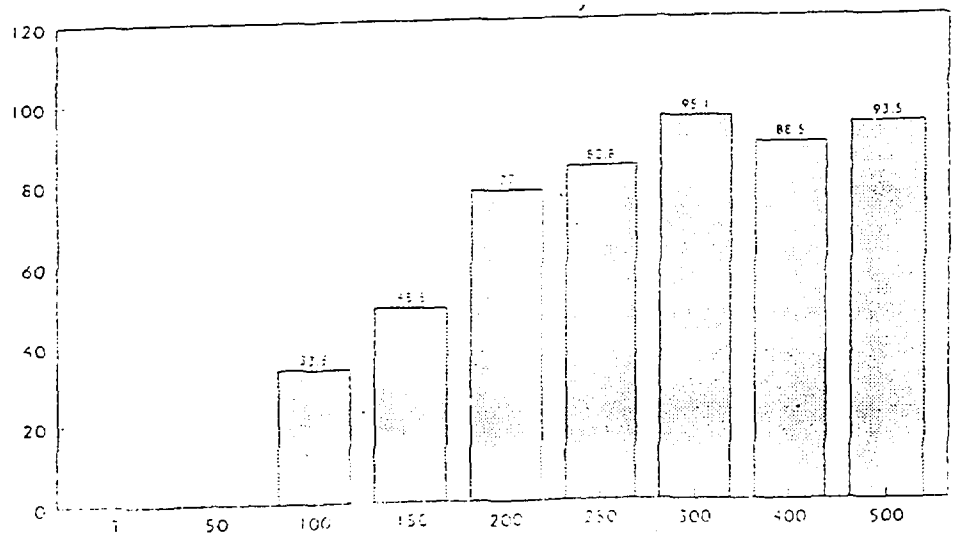

Fig. 7. Effect of irradiation on inhibitation of plant height.

Mortality among plants obtained from irradiated seeds: Plants were scored for survival after 24 days of seed irradiation. The results showed that the doses between 50 and 150 Gy did not lead to mortality of plants obtained from irradiated seeds (Fig.8); however, doses between 200 and 500 Gy caused between 66 to 84% mortality among such plants (Fig. 9).

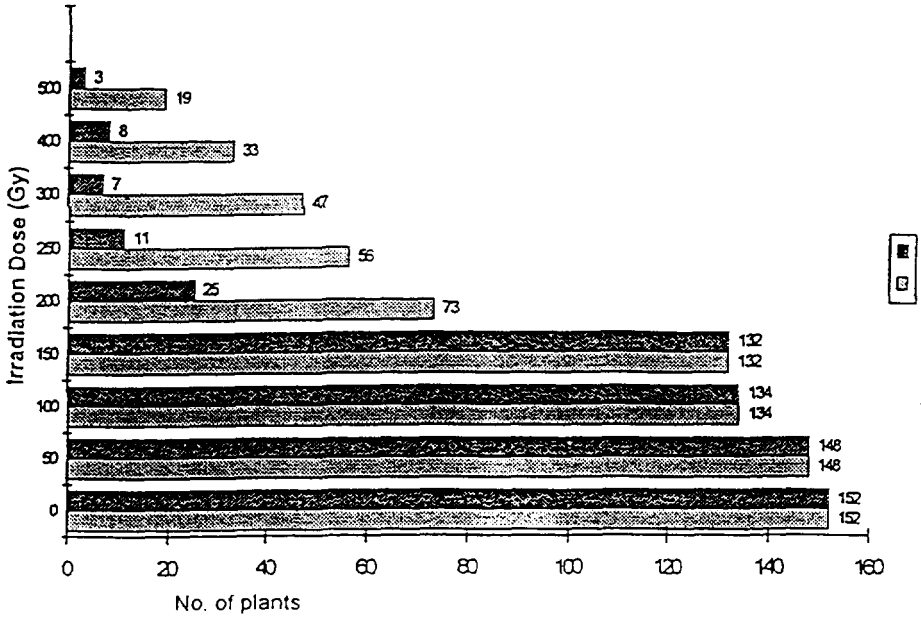

Fig. 8. Survival of plants obtained from irradiated seeds.

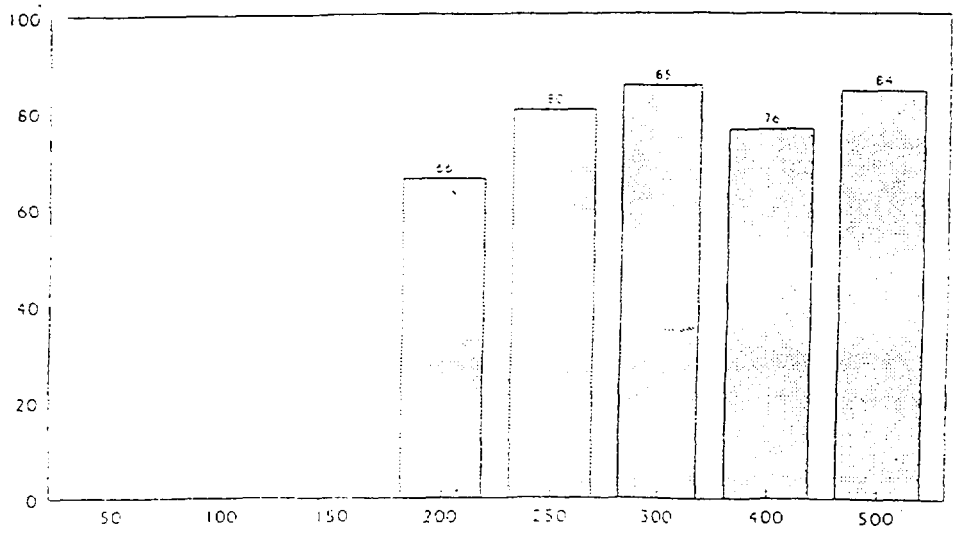

Fig. 9. Effect of irradiation on plant survival.

4. CONCLUSIONS AND DISCUSSION

In the present study, the rust disease in *Basella* was investigated, and the effects of radiation on seed germination and plant mortality were studied to induce variation for disease resistance.

Seeds of *Basella alba* and *B. rubra* varieties susceptible to *Basella* rust were irradiated with different doses of gamma rays between 50 and 500 Gy. Considering the germination percentages of irradiated seeds, doses of 300 and 400 Gy inhibited seed germination from 39% with 300 Gy to 53% with 400 Gy. Considering the size of plants obtained from irradiated seeds, the irradiation dose of 150 Gy inhibited plant growth by 48% in relation to the growth of control plants. Hence, during the subsequent experiments, doses of 150 Gy to 400 Gy shall be used. Irradiation of large seeds lots with these doses would allow us to generate sufficiently large M_1 population, and to select plants in the subsequent generation for resistance to rust. Other manipulations, such as the mutagenic treatment of tissues cultures, belonging to *Basella alba* and *B. rubra* would be pursued with the same aim. The cultivars known as *Basella alba* and *B. rubra* respond well to *in vitro* culture and the desired variants could also be selected among somaclonal variants. The use of chemical substances with mutagenic effects in culture media should allow to reach these goals.

Other agronomic measures can also be combined to reduce the losses from the rust disease. Maneb 80 as a fungicide can be used to control baselle rust. The reduction in the vegetative life cycle should allow to obtain plants with less infection than those with a longer vegetative duration. Results obtained have also shown that the culture of baselle under plastic shelter reduces the development of disease significantly. All aspects of this research could be used to develop a strategy to control the incidence of rust in basella.

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REGENERATION AND GENETIC TRANSFORMATION OF COWPEA (*Vigna unguiculata* Walp.)

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Abstract

Regeneration of cowpea (*Vigna unguiculata* Walp.) was achieved through massive bud formation induced in apical and lateral meristems by the herbicide Thidiazuron (TDZ). The effect of TDZ (5, 10, or 20 μM) was tested *in vitro* on four different cowpea genotypes. Thidiazuron, even at the highest concentration, had no effect on seed germination. After one month of culture, multiple bud cluster formation was observed in all genotypes tested; about 80% of shoot apices regenerated multiple buds, whilst only 34% of cotyledonary nodes behaved in the same way. Histology of regenerating multiple bud clusters revealed that regeneration initiated from pre-existing meristems in the apex and cotyledonary node. Thidiazuron at 10 μM appeared to be the best concentration to produce clusters with high number of buds, ranging from 5 to 10. Shoot elongation occurred only on MS medium without TDZ. On the same medium, 75% of elongated shoots rooted. For genetic transformation of cowpea, a direct DNA transfer methods *in planta* under *in vivo* conditions was tested by electroporation of plasmid DNA into the nodal meristematic cells. Some transformed plants were obtained, and produced T_1 transformed progenies; their transgenic nature was confirmed by Southern analysis.

1. INTRODUCTION

Cowpea (*Vigna unguiculata* Walp.) is affected by several insect pests in field and during the storage [1]. Since the wild relatives of cowpeas, which are disease resistant and few, are cross-incompatible with the cultivated types [2], researchers are trying to introduce new genes into cowpea genome by genetic transformation to obtain improved genotypes tolerant to insects.

Plant genetic manipulation is conventionally based on the formation of shoots from tissues cultured *in vitro*. Both direct (e.g. gene gun, electroporation) or indirect (e.g. *Agrobacterium*-mediated) DNA introduction methods into plant cell genome require the development of buds formed from transformed tissues. *In vitro,* this phenomenon occurs mainly as bud differentiation from undifferentiated cells or bud regeneration from already pre-existent meristems. *In vitro* regeneration has been obtained using the herbicide Thidiazuron in several grain legumes, e.g. in pea, chickpea, and lentil through organogenesis [3] or through somatic embryogenesis as in peanut [4]. Here, we report results on the effect of this growth regulator on multiple bud proliferation in cowpea and their origin through regeneration. Since it is difficult to achieve stable transfer of DNA into cowpea meristematic cells using standard procedures, a direct method was tested *in planta* under *in vivo* conditions. Plasmid DNA electroporation was tried into nodal meristematic cells, as reported for some other grain legumes [8].

2. MATERIALS AND METHODS

2.1. Materials

Four genotypes were used for *in vitro* culture. These were the Italian cv. 'Cornetto', one IITA selected line, TVu9062, and two breeding lines, IFOB89/44A2 (OAU-10) and

IFOB89/256 (OAU-3) developed at the Department of Plant Science, Obafemi Awolowo University, Lie-ife, Nigeria, supplied by Dr. I.O. Obisesan. For transformation, cowpea cv. 'Cornetto' was used in the experiments.

2.2. Methods

2.2.1. Regeneration studies

Seeds of each genotype were sterilized as reported previously [5], and cultured on MS medium containing 3% sucrose and supplemented with TDZ 0 (control), 5, 10, and 20 μM. Gelrite 0.25% was used as gelling agent. Seed cultures were kept in a growth chamber in darkness at 24°C for one month. Seed germination was scored after 15 days of culture. Multiple bud proliferation from shoot apices and cotyledonary nodes was scored after 30 days of culture for the presence of more than three buds arising from the apex and/or from the cotyledonary node. Clusters were classified on the basis of well developed buds as follows: 0 - no multiple buds; 1 - one to five buds; 2 - six to ten buds; 3 - more than ten buds.

2.2.2. Shoot and root formation

The multiple bud clusters were removed from the embryo axis, and cultured on the above medium without TDZ and kept at 24°C, under 16/8 hr light/dark. Elongated shoots were transferred to the same medium, and maintained under the same conditions for rooting stage. The rooted shoots were transplanted in soil.

2. 3. Histology

Histological studies on regeneration process was carried out on cotyledonary nodes and apices of all genotypes after 6, 11, 15, and 21 days of *in vitro* culture. Explants were fixed in FAA (90 ml 96% ethanol, 5 ml 40% formaldehyde, 5 ml glacial acetic acid) for 24 to 48 hrs. Samples were dehydrated and embedded into paraffin wax as reported by Johansen [6]. Transverse sections (12 μm thick) of cotyledonary nodes and apices were stained in Schiff's Reagent, and counter stained in Fast Green [6]. Analysis and microphotography were carried out using a light microscope (20X and 80X).

2.4. Plasmid vectors

Plasmid pML112 carrying reporter gene *gus,* marker gene *nptII* and useful gene α-amylase inhibitor (supplied by Prof. M. Chrispeels, University of California, San Diego, USA) and PGPT 1.0 (EcoR 1 /Hind III fragment of the plasmid p35SGUSINT [7], carrying the *gus* gene interrupted by an intron cloned into pUC8 vector [8], were used in the electroporation experiments. Plasmids were amplified in *Escherichia coli* DH5α strain, and isolated using the Quiagen Maxi Preparation Kit™. Purified DNA was re-suspended in TE buffer (1 mM Tris 0.1 mM Na_2EDTA, pH 7.8).

2. 5. *In planta* DNA electroporation

Electroporation was carried out according to the procedure of Chowra et al. [8]. The day before electroporation, plants were de-budded, leaving only the top axillary bud underneath the terminal one. The next day, 2 ml of the electroporation buffer (200-300 μg of plasmid DNA dissolved in 150 RI of lipofectin, suspended into 2 ml of sterilized Murashige

& Skoog salt solution) were injected in the axillary bud using a Hamilton syringe. The bud was then dipped into the electroporation buffer containing in a circular electrode,18 mm diameter (Hoefer Scientific). Two pulses of 99 msec each (square wave) were delivered at 100 V using the electroporation system Progenetor™ (Hoefer Scientific). The electroporated buds on the plants were allowed to grow and set seeds. Seeds were collected from each plant.

2.6. GUS assay

Two to three weeks after electroporation, leaves from branches that grew out of the treated buds were assayed for GUS activity according to the histochemical staining procedure described by Jefferson [9]. The same assay was performed on the primary leaves taken from the T_1 seedlings. Only well stained tissues were scored as GUS positive.

2.7. DNA isolation and Southern Blot Analysis

Leaf samples (1-2 g) from GUS-positive T_1 plants were used for DNA isolation according to the CTAB protocol of Doyle & Doyle [10]. Approximately 25 µg of genomic DNA was restricted with EcoR I and with EcoR I/ Hind III. Undigested and digested DNA was subjected to electrophoresis on 0.8% agarose and blotted on Bio-Rad Zeta Probe Membrane. The target DNA was fixed by baking at 80°C for 2 hours. The Sspi/Sacl (about 2.4 kbp) fragment from PGPT 1.0 was used as probe. The Genius™ kit (Boehringer Mannheim), involving the non-radioactive method of detection, was adopted. Labeling, hybridization, and detection were done according to the manufacturer's protocol.

3. RESULTS

3.1 Cowpea regeneration

After 15 days of *in vitro* culture on regeneration media, seeds were scored for germination. Only seeds showing a very well growing root and a healthy plumule, i.e. without yellow or greenish spots, were considered fully viable and, hence, potential producers of regenerants. Percentages of viable seeds are shown in Table I. No statistical differences were noticed in the germination frequency with different TDZ concentration in each genotype and control, whilst differences were found between genotypes. On average, germination frequency of treated seeds was high for cv. 'Cornetto' (96%) and low for the Nigerian breeding line OAU-3 (47%).

After one month of *in vitro* culture, seeds were scored for regenerantion. All controls were green and did not show any regeneration, but the seeds cultured on media containing TDZ showed regeneration. They showed clusters of many buds replacing the original shoot apex and cotyledonary node buds. The regeneration frequency was significantly higher for apices in all genotypes compared to that from cotyledonary nodes (Table II). On average, more than 80% of apices showed multiple bud proliferation, whereas only 34% of cotyledonary nodes produced bud clusters.

Based on the number of buds per cluster the best concentration of Thidiazuron was 10 µM which induced formation of at least 5 buds per cluster. Apices produced more buds per cluster in all genotypes than cotyledons; the best result was obtained in TVu9062, with more than 20% of clusters bearing six to ten buds (Fig. 1). However, cv. 'Cornetto' showed that about 80% of clusters formed had between one to five buds. OAU-10 genotype performed

better than OAU-3; the former when cultured on medium with 10 μM TDZ produced 15% apices with six to ten buds. In TVu9062, an increase in TDZ concentration strongly inhibited the number of regenerated buds per cluster in cotyledonary nodes, whereas data for other genotypes did not show any specific trend.

TABLE I. GERMINATION OF FOUR COWPEA GENOTYPES AT THREE TDZ LEVELS SCORED AFTER 15 DAYS OF *IN VITRO* CULTURE

	Genotype			
	Cornetto	TVu9062O	OAU-1O	OAU-3
TDZ (mM)	germ. (%)	germ. (%)	germ. (%)	germ. (%)
0	100.0	95.4	88.9	58.0
5	96.9	93.6	75.0	36.5
10	100.0	90.0	75.0	48.1
20	91.7	94.1	90.0	55.8

TABLE II. REGENERATION OF MULTIPLE BUD CLUSTERS FROM APICES AND COTYLEDONARY NODES OF COWPEA AFTER ONE MONTH *IN VITRO* CULTURE IN THE PRESENCE OF TDZ

	GENOTYPE							
	CORNETTO		TVU9062		OAU-L		OAU-3	
TDZ μM	APEX (%)	COTYL. (%)	APEX (%)	COTYL. (%)	APEX (%)	COTYL. (%)	APEX (%)	COTYL. (%)
5	89	31	91	81	83	17	48	30
10	90	21	91	47	95	28	81	29
20	82	23	74	38	99	27	57	33

Histology of controls showed an initial development of buds from cotyledonary node, which lasted almost two weeks, followed by degeneration of meristematic tissues. No shoot development or multiple bud formation was observed in the controls after one month of *in vitro* culture. On apices and cotyledonary nodes isolated from plantlets of all genotype grown in presence of 5, 10, and 20 μM TDZ, the histological analysis did not reveal any substantial differences either among genotypes or TDZ concentration. Distinct regeneration sites were observed in 15 day-old cultured meristems, isolated both from apices and from cotyledonary nodes. At the end of bud induction, the size of the apex and the cotyledonary node buds increased 4 to 5 times compared with the controls.

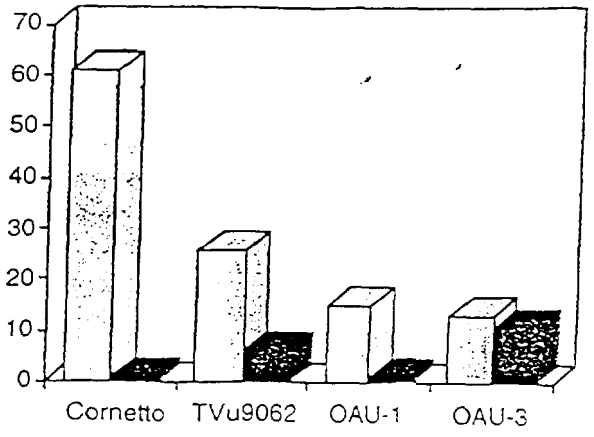

Fig. 1. Percentage of bud clusters regenerated (6 to 10 buds per cluster) from cowpea apices (open area) and cotyledonary nodes (dotted bar) after one month of in vitro culture in the presence of 10 μm TDZ.

Shoot elongation occurred only when apical and cotyledonary node-derived bud clusters were detached from the plantlet and transferred to the elongation medium. The average number of elongated shoots per each bud cluster was different for each genotype. In general, the apical multiple buds, regenerated in presence of 10 or 20 μM TDZ, were more efficient to produce many healthy elongated shoots. The cv. 'Cornetto' and the IITA genotype TVu9062 performed better than the Nigerian lines OAU-10 and OAU-3, with 3.7 and 3.0 shoots per cluster, respectively. More than 75% of elongated shoots, taken from clusters, rooted in about three weeks when transferred to fresh medium without TDZ.

3.2. Cowpea genetic transformation

When cowpea plants were electroporated using the vector containing *gus, nptII* and α-amylase inhibitor genes, 6.4 % of tested T_1 seeds were positive for both GUS expression and resistance to kanamycine (Table III). More than 5300 cowpea putative transformed seeds are now under biological test, i.e., being fed to larvae of *Callosobruchus maculatus.*

TABLE III. *IN VIVO* NODAL MERISTEM ELECTROPORATION OF COWPEA CV. 'CORNETTO'

	Produced T_1	Tested T_1	Phenotypic Response		
Plants[#] (No.)	seeds (No.)	plantlets (No.)	kan+ (%)	GUS+ (%)	kan+/GUS+ (%)
Electroporated					
294	5373	373	10.4	22.3	6.4
Controls					
60	1151	41	12.1	31.7	2.4

[#] Number of treated plants and putative T_1 seeds produced, and the frequency of putative T_1 plantlets showing the transgene phenotype. Plantlets producing secondary roots in presence of kanamycin 200 mg/l were scored as kan+; plantlets showing *gus* positive tissues after histochemical assay of leaflets were scored as gus+.

In another experiment, carried out with the same genotype, the plasmid PGPT 1.0, harboring the *gus* gene interrupted by an intron was electroporated into cowpea nodal meristem of 40 plants. Nine *gus* positive T_1 plants were obtained. On the basis of their progenies, we can assume that some of the T_0 plants were chimeric for the transgene. Two T_1 plants were analyzed for the stable integration of the transgene; the resulting Southern blot is presented in Figure 2. The presence of high molecular signals in undigested genomic DNA and hybridization at the expected molecular weight in digested genomic DNA definitively confirmed the transgenic nature of these progenies, and hence the stable integration of the *gus* gene for one generation .

4. DISCUSSION

4.1. Cowpea regeneration

Thidiazuron induced massive bud proliferation in cowpea through regeneration of new meristems from the ones already present. The results obtained were in good agreement with those reported in bean [11]. Among genotypes, TVu9062 ranked the first while VITA 4 was the worst, suggesting that there is enough genetic variability in cowpea for "regeneration". However, Thidiazuron had detrimental effect in inducing multiple bud proliferation, even at the highest concentration tested.

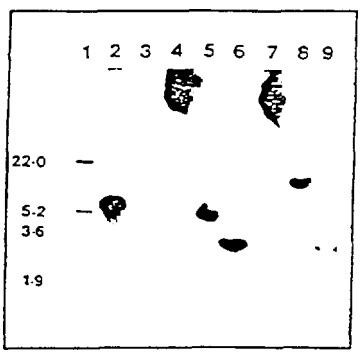

Fig. 2. Southern blot of two T_1 cowpea plants. Legend: Lane 1. Size marker (λ EcoR 1/Hind III); Lane 2. PGPT 1.0 Linear. Lane 3. Cowpea genomic DNA Control. Lanes 4 and 7. PT 10.2 and PT 15.21, Undigested DNA. Lanes 5 and 8. PT 10.2 and PT 15.21, digested with EcoR 1. Lanes 6 and 9. PT 10.2 and PT 15.21 digested with EcoR I/ Hind III.

The enlargement of apical and lateral buds was mainly due to the pre-existing meristematic cell divisions and the histological analysis clearly showed that bud clusters were initiated from these cells; moreover, new vascular tissues were regenerated. In our experiments, differentiation of new buds from undifferentiated cells or somatic embryogenesis induced by TDZ was not observed; however, we cannot exclude its occurrence at very low frequency, as reported in *Nicotiana* [12] and peanut [4].

Thidiazuron had a detrimental effect on the elongation of regenerated shoots. In a preliminary experiment on cv. 'Cornetto' (data not shown), bud clusters derived from both apices and cotyledonary node buds were cultured on the elongation medium without and with 10 μM TDZ. Shoots coming from bud clusters in the absence of TDZ elongated, while those

from TDZ medium did not grow. The cytokinin-like activity of Thidiazuron [13], a phenylurea derivative, inhibited shoot elongation.

Rooting was not affected either by bud origin or TDZ concentration in the induction medium (data not shown). This latter result is not in agreement with the those obtained on *Miscanthus sinensis* by Nielsen et al. [14], and on *Cucumis melo* by Leshen et al. [15].

4.2. Cowpea transformation

Genetic transformation of grain legumes, except soybean, is still difficult by *in vitro* techniques widely used in other species, i.e., Solanaceae. This is mainly due to the lack of a reliable protocol of regeneration of shoots from leaves, stems, roots, etc. Recently, genetic transformation of important grain legumes has been obtained, e.g. in pea *(Pisum sativum* L.) [16,17] through *in vitro* co-culture of embryos with *Agrobacterium tumefaciens,* and bean *(Phaseolus vulgaris* L.) [18] through micro-projectile bombardment. Both methods rely on DNA transfer into meristems, thus giving stable transformed progenies without *in vitro* differentiation, but by regeneration of new meristems from those already present. The bud regeneration pathway through meristematic cell division highly reduces the chance of inducing somaclonal variation, but increases the frequency of chimeric shoot formation in contrast to other transformation methods based on differentiation.

Genetic transformation of cowpeas has been attempted by several researchers in recent years. It has been definitely demonstrated that this species is susceptible to *Agrobacterium tumefaciens* [19]; thus, chimeric tissues, expressing both *nptII and gus* genes [20], were obtained. However, no transformed progenies were obtained from these tissues, because of the lack of *in vitro* differentiation protocols. This bottle-neck forced us to develop a genetic transformation system based on highly morphogenic tissues, like meristems, already present in the apex and nodal buds. In this context, direct (i.e. electroporation-mediated) DNA transfer method has been applied to the plants, in order to avoid all the problems related to *in vitro* techniques, and to make the overall protocol easier, which can be followed in the absence of *in vitro* culture facilities.

DNA electroporation in cowpea mature embryo cells has been reported by Akelia & Lurquin [21], resulting in the expression of *gus* reporter gene. However, no transformed plants were obtained from somatic tissues, although all cell layers in the apical dome of germinated shoots were positive for GUS assay. The present results suggest the possibility to transfer DNA directly *in planta,* by electroporating intact nodal meristems without *in vitro* tissue culture. This protocol was used in pea, lentil, and soybean plants. Some T_0 and T_1 transgenic plants were obtained; their transgenic origin was confirmed from *gus* expression [8]. In our experiments, the presence of strong molecular signals in undigested genomic DNA and on hybridization with the expected molecular weight in digested genomic DNA, definitively confirmed the transgenic nature of the progeny, and hence the stable integration of the *gus* gene for one generation.

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DIFFERENTIAL RESPONSE OF BANANA CULTIVARS TO *F. oxysporum* f. sp. *cubense* INFECTION FOR CHITINASE ACTIVITY

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Abstract

Six banana clones with varying levels of resistance were inoculated with conidial suspension of races 1 and 4 of *Fusarium oxysporum* f. sp. *cubense*. Chitinase activity in the corm and root tissues was monitored before and after infection to relate with the field resistance or susceptibility of banana cultivars. Resistant clones showed high constitutive chitinase activity in roots and a rapid response to infection. The results suggest that chitinase could be considered as part of a complex mechanism leading to disease resistance.

1. INTRODUCTION

Bananas (*Musa* spp.) are important crops, providing carbohydrate-rich food to large populations in the tropics and subtropics. In South and Central America, bananas are also important export crops. Protection against fungal diseases represents a major challenge to banana production. In many areas of the world, losses from diseases reduce income and pose a threat to the economic survival of small-holding farmers. At present, disease control is based mainly on three strategies: application of pesticides, breeding for disease resistance and improvement of agronomic practices. However, chemical control of *Fusarium* wilt is not yet available. Banana breeding is time consuming, expensive, and constrained by sterility of most cultivated bananas. Thus, a better understanding of the resistance mechanisms is required to develop a reliable early screening method.

Like many other species, bananas employ a diverse array of defence mechanisms to resist pathogen attack. For example, callose deposition and phenolic compounds have been associated with resistance to *Fusarium oxysporum* f. sp. *cubense* (FOC) (1,5), but little is known about the physiology of the defence response.

Chitinases hydrolyze chitin, a homopolymer of β-1-4 linked N-acetyl-D-glucosamine, which is a major component of the cell wall of most fungi. Chitinases have been isolated and purified from bacteria, fungi and plants, and are able to inhibit fungal growth by degrading the newly synthesized chitin at the hyphal tips. Chitinases are synthesized in low amount in many plants species and crops. Most evidence for the role of chitinases in the plant defense response is based on data from *in vitro* system or from complementation test involving plant transformation and scoring of resistance. Purified enzymes have been shown to hydrolyse isolated fungal cell walls and inhibit the growth of pathogen in culture.

The present investigations were undertaken to study the chitinase response of bananas clones with different ploidy levels and resistance to *Fusarium oxysporum.* Previous studies showed that in several banana clones, chitinase was expressed constitutively. In this paper, we report evidence on the induction of chitinase following infection of banana plants with FOC conidial culture.

2. MATERIAL AND METHODS

2.1. Plants, pathogen and inoculation

Fusarium oxysporum f. sp. *cubense* (FOC) race 1 strain 2264 (VCG 0125) and race 4 strain Yo 604 (VCG 01290) were kindly provided by Dr. K. Pegg, Queensland Department of Primary Industries (QDPI), Indoroopilly, Queensland (Australia). The strains were cultivated on Czapek-Dox (Difco) for one week. Actively growing hyphae were removed, transferred on Potato Dextrose Agar (PDA), and incubated at 28°C under constant light to promote conidia formation. Conidial suspension in distilled water were collected by scratching the mycelial surface.

The following banana clones which differ in genome, ploidy, and susceptibility to *Fusarium oxysporum* f. sp. *cubense* race 1 and 4 were investigated: SH-3362 (AA) resistant to race 1 and 4; 'Grand Naine' (AAA), 'Pisang Mas' (AA), SH-3142 (AA), 'Dwarf Parfitt' (AAA), susceptible to race 4 and resistant to race 1, 'Highgate' (AAA) susceptible to both races. Plants were multiplied *in vitro* and rooted as described elsewhere [4]. After one month, rooted plantlets were obtained and agar was washed with tap water, the roots were trimmed and the plants were inoculated by dipping the roots in spore suspension of 10^5 conidia/ml for 10 min under air flow to facilitate the mechanical uptake of conidia in root vessels. The controls were treated in the same manner but inoculated only with distilled water. Immediately after inoculation, the plants were transferred to pots in a greenhouse and samples were taken at intervals for chitinase activity.

2.2. Chitinase analysis

Plantlets were sampled at random and the corm tissue collected under cold water by trimming the roots and the pseudostem. The tissue was weighed and ground with a mortar and pestle in liquid nitrogen. Phosphate buffer pH 6.8 was added to the resulting powder, and after homogenisation incubated for 30 min at 4°C. The homogenate was collected with a micropipette, centrifuged for 15 min at 14,000 rpm at 4°C, and the supernatant was assayed immediately for protein content according to method of Bradford [2]. Extracts with equal amount of protein were incubated in 100 ml glycol chitin, variable amounts of distilled water were added to bring the incubation mixture to 200 ml and incubated overnight at 40°C. The final reaction mixture was brought to 1 ml and read at 510 nm using a spectrophotometer, and expressed as unit protein content.

2.3. Isoelectrofocusing

Equal amounts of protein were applied on 4.6% polyacrylamide gel containing 5% of Pharmalyte as carrier ampholyte. Running conditions were as follows: 8 Watts fixed, mA from 33 to 12, voltage 2500. After running, the gel was washed, and the bands were differentiated using an overlay gel (7.5%) with 1.1 ml of 3M NaAc buffer pH 5.0, 1.3 ml glycol chitin in a final volume of 33 ml.

3. RESULTS

3.1. Growth of inoculated plants

No wilting symptoms were observed in the inoculated material up to 28 days of culture. However, internal symptoms, i.e. tissue discoloration, were observed on dissection of the susceptible clones inoculated with both FOC races.

3.2. Peroxidase activity

The clones were analysed for constitutive chitinase activity before fungal inoculation. No differences were observed between susceptible and resistant clones in constitutive chitinase.

3.2.1. Corm analysis

After inoculation with a conidial suspension of FOC race 1, all clones except 'Highgate' showed increased chitinase activity but during different time course (Fig. 1). The clone 'Dwarf Parfitt' showed a rapid and sustained increase in enzymatic activity during the first two weeks, while in 'Highgate' chitinase did not increase. The clone 'Pisang Mas' exhibited a slow increase in enzymatic activity with time. When the same banana clones were incubated with FOC race 4, clone SH3142 showed a higher increase in chitinase activity (Fig. 2). Increase in chitinase activity was particularly evident in 'Pisang Mas' with a peak activity after 5 weeks. Clone SH3142 showed a different pattern in enzymatic activity with a burst induction after 48 h, followed by another peak 5 weeks after infection (Figs. 3 and 4).

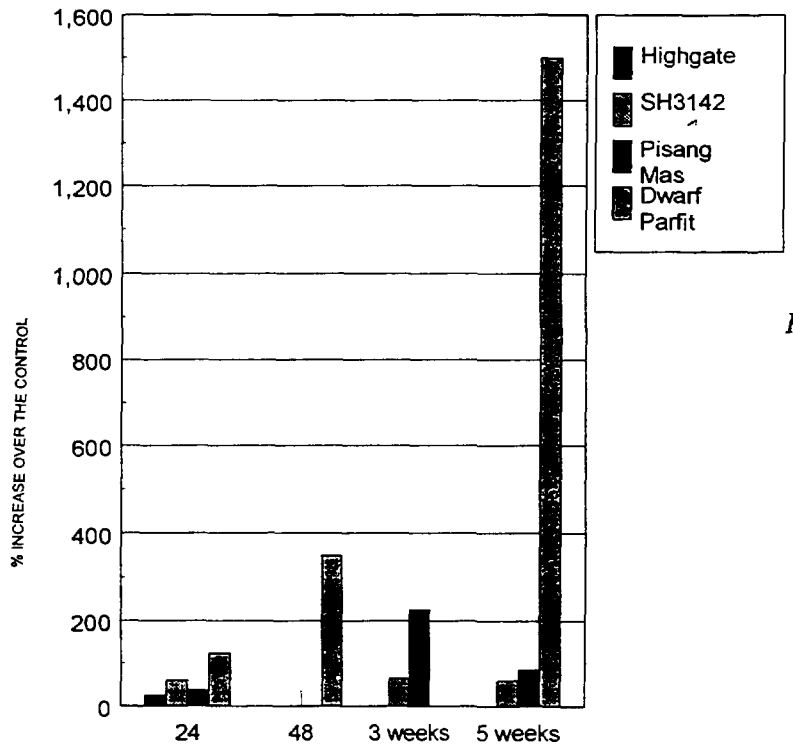

Fig. 1. Differential response of banana clones to inoculation with Fusarium oxysporum f.sp. cubense race 1 measured as chitinase activity (A_{470}) increase over the control. Relative activity is described in terms of the change of absorbance per min per 5 µl of extract. Each point represents the average of three replicates and two independent experiments.

3.2.2. Root analysis

The resistant clone SH3142, infected with race 1 and 4 showed root chitinase activity comparable to corm tissue (Figs. 5 and 6). However, in plants inoculated with race 4, there was a three to four fold increase when compared with plants inoculated with race 1. Moreover, root chitinase activity was already evident 24 hr after infection, while in corm tissue the activity increase was evident after 48 hr (Figs. 7 and 8). In 'Pisang Mas', the enzymatic activity increased both after infection with race 1 and 4 but in a transient manner, i.e. after 48 hr when infected with race 1 and after 5 weeks when inoculated with race 4; however, the increase was not statistically significant. As far as the response to Race 1 is concerned, a four fold increase over the control was observed but only at a single point. In the highly susceptible clone 'Highgate', the chitinase activity did not increase at all. The clone SH 3142 showed a more prompt response than the other clones when inoculated with race 4.

3.2.3. Isoelectrofocusing

At least 12 isochitinases have been recorded in diploid bananas [3]. To determine which isozymes contribute to the rise in total activity, IEF was used to separate the isochitinases extracted from the banana clones. At least four bands, two cationic and two anionic, concomitantly increased in the incompatible reaction between race 4 and SH-3362. However, this increase was not observed in the incompatible reaction of the same clone with race 1. The activity of most of the other isozymes remained unchanged with time (results not shown).

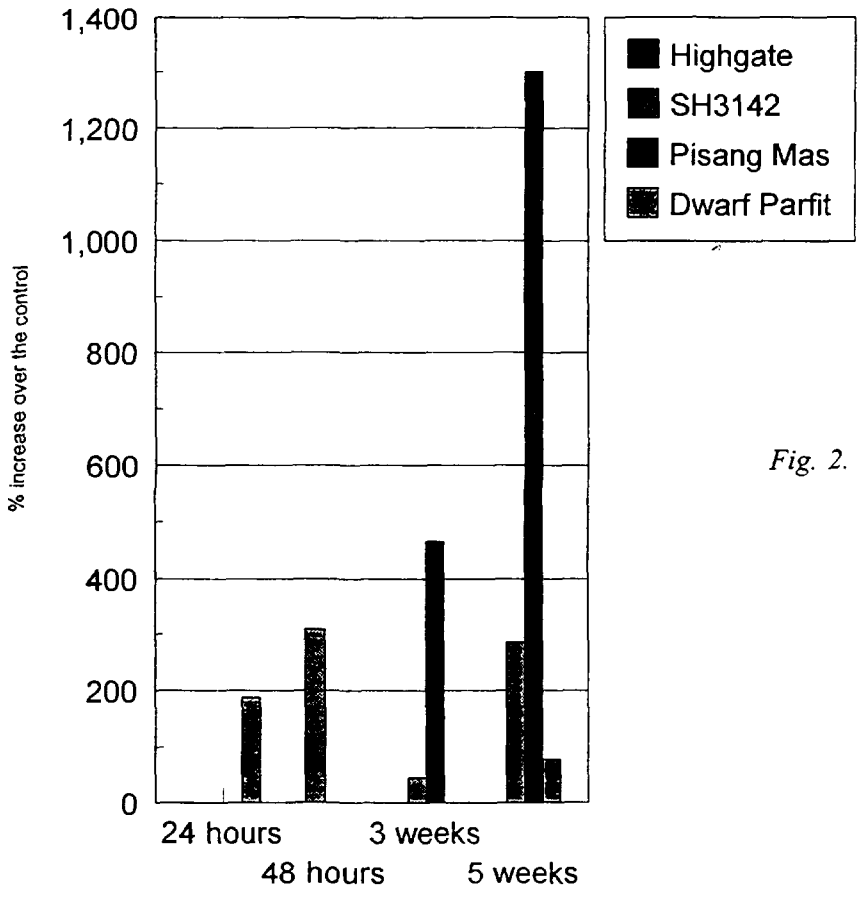

Fig. 2. Differential response of banana clones to inoculation with Fusarium oxysporum f. sp. cubense race 4 measured as chitinase activity (A_{470}) increase over the control. Relative activity is described in terms of the change of absorbance per min per 5 µl of extract. Each point represents the average of three replicates and two independent experiments.

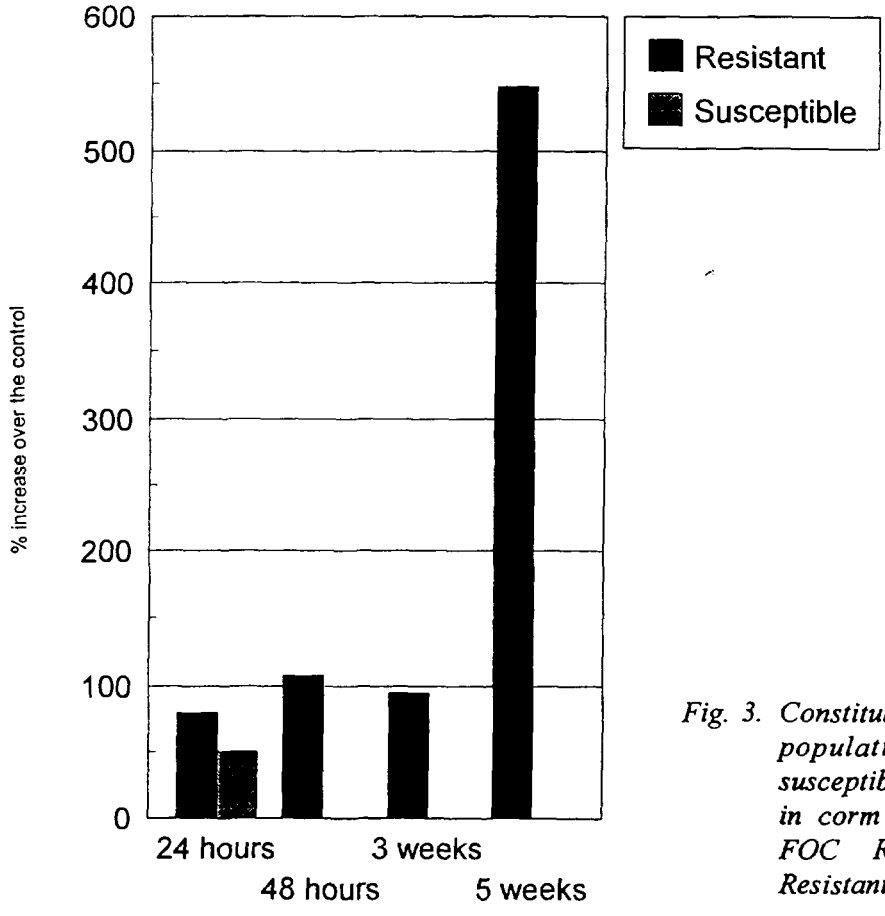

Fig. 3. Constitutive chitinase activity in two population of resistant and susceptible bananas chitinase activity in corm tissue after infection with FOC Race 1 (Susceptible vs. Resistant).

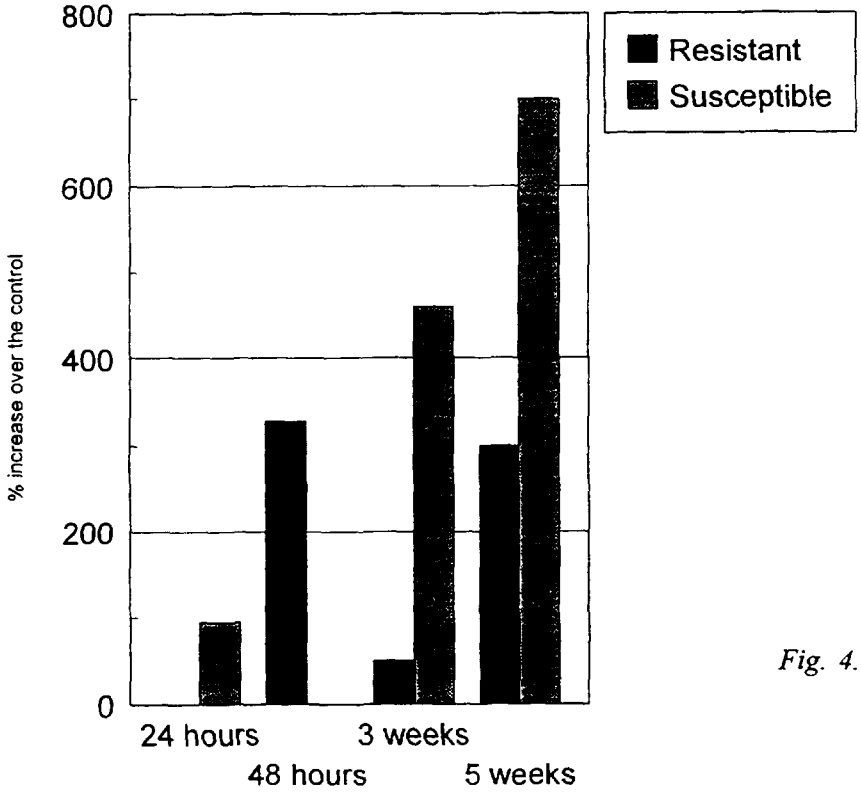

Fig. 4. Chitinase activity in corm tissue after infection with FOC Race 4 (Susceptible vs. Resistant).

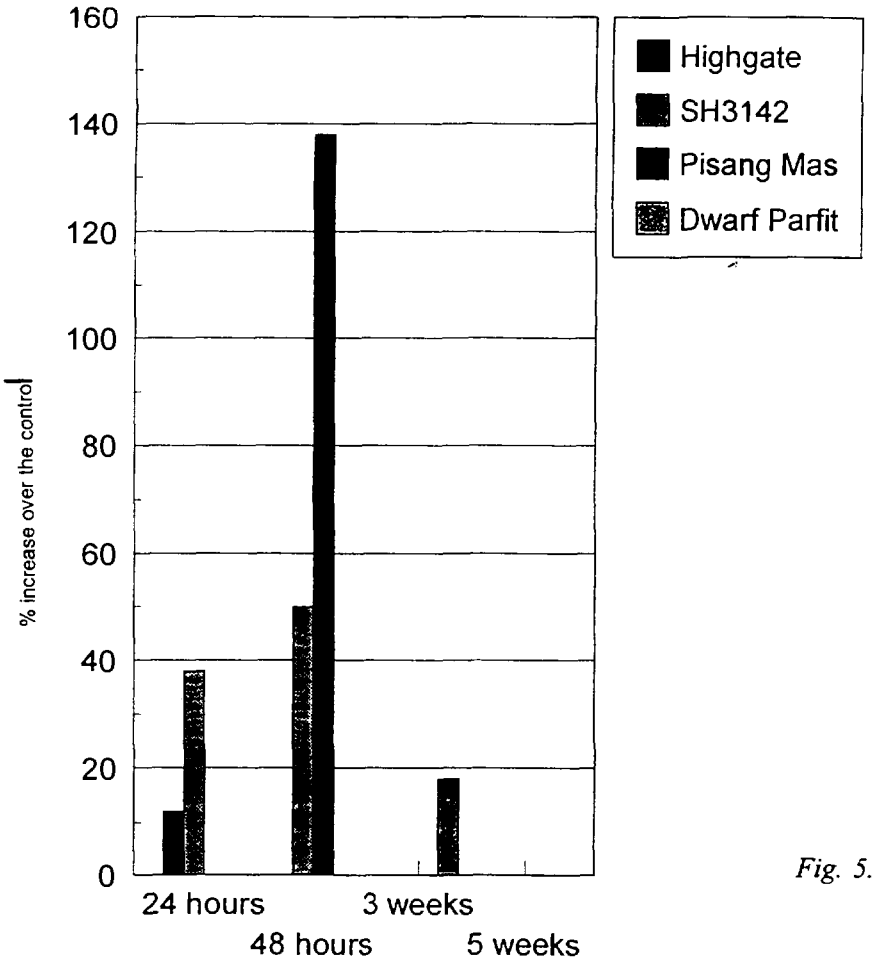

Fig. 5. Chitinase activity in root tissue after infection with Race 1

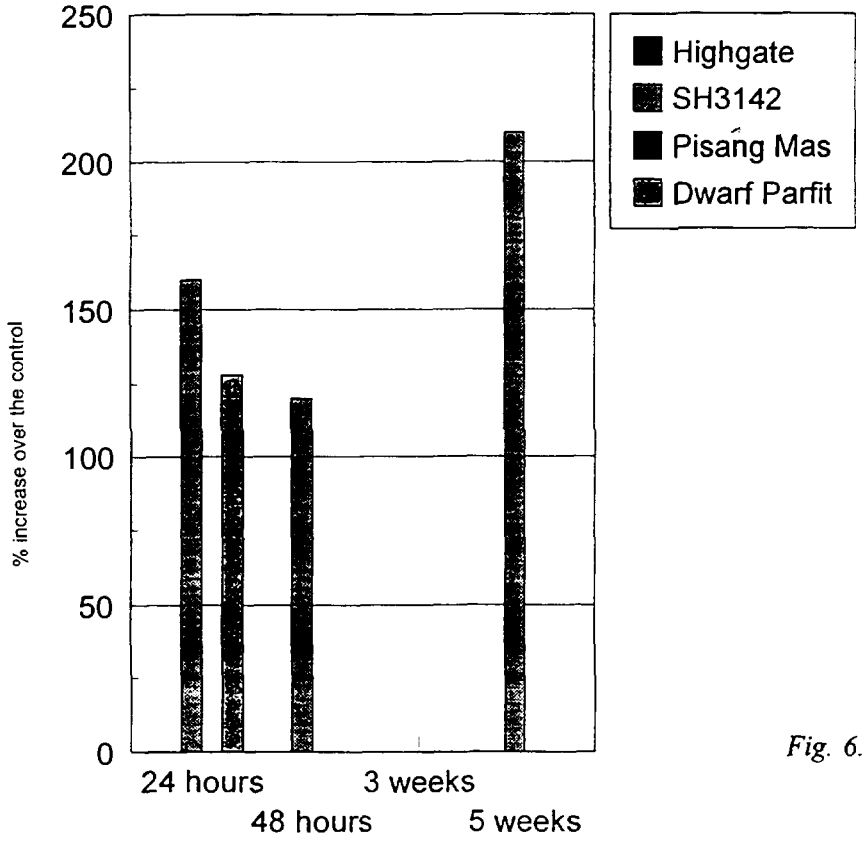

Fig. 6. Chitinase activity in root tissue after infection with Race 4.

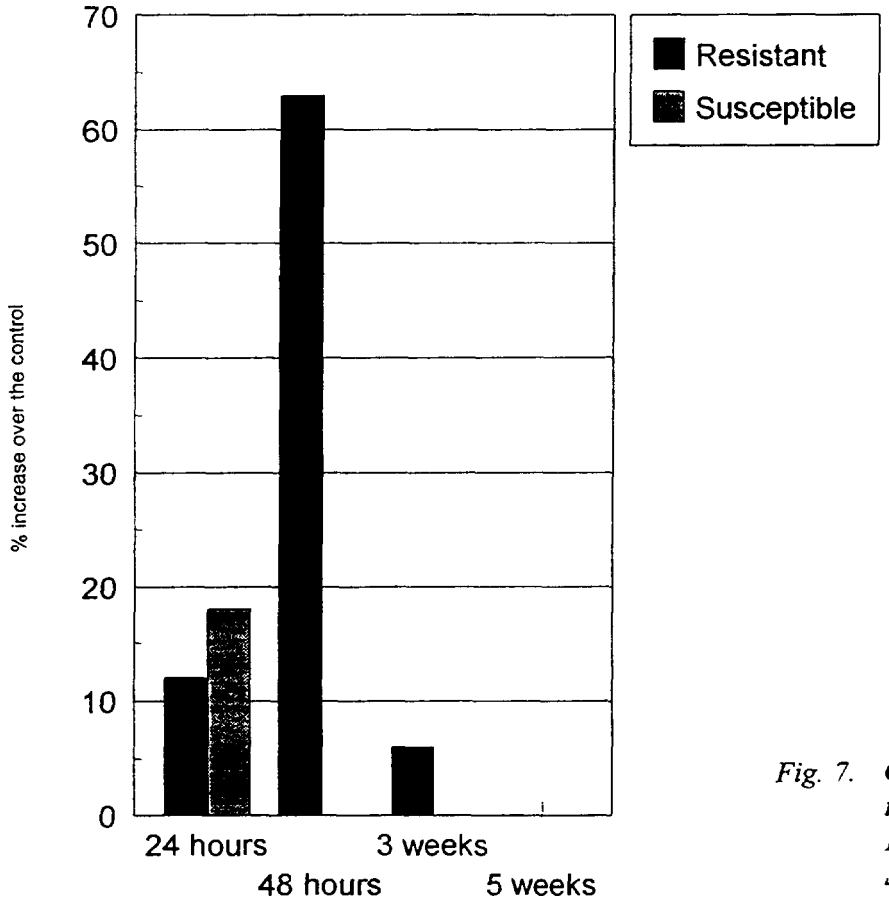

Fig. 7. Chitanase activity in root tissue after infection with FOC Race 1 (Resistant vs. Susceptible)

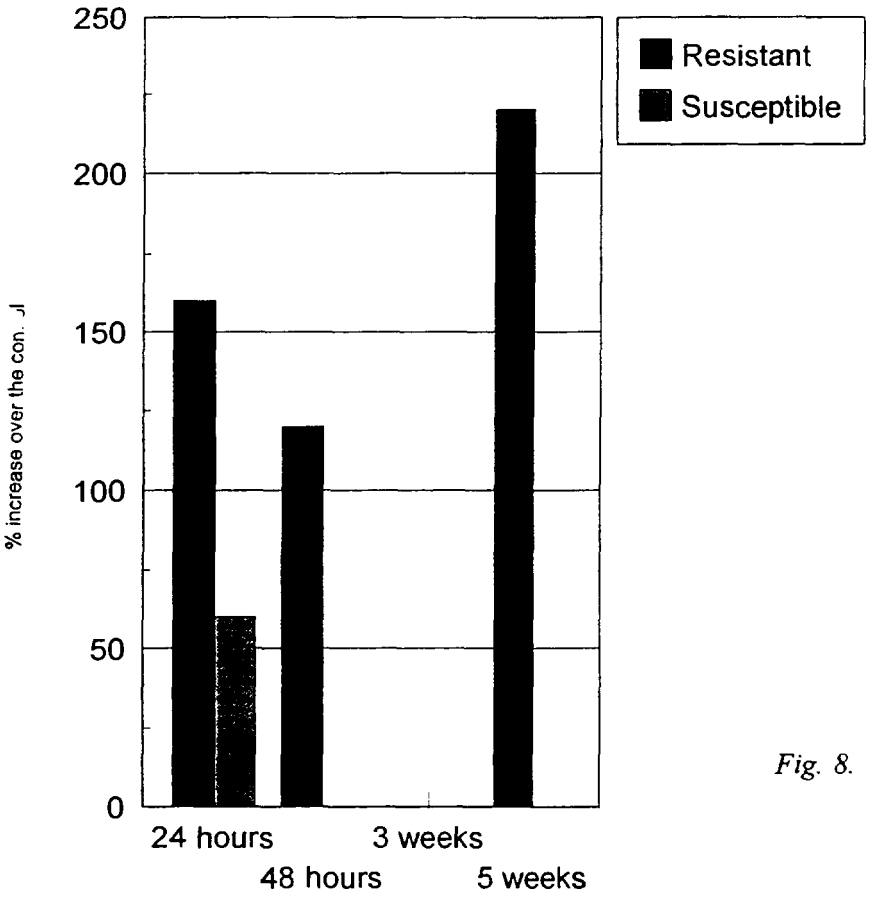

Fig. 8. Chitinase activity in root tissue after infection with FOC Race 4 (Susceptible vs. Resistant).

4. DISCUSSION

The current model on chitinase based defence mechanism considers that the enzyme acts as an anti-fungal compound as well as producer of chitin fragments, which act as elicitors or messengers to activate other metabolic compounds for defence against pathogen. The results presented in this paper confirm that the resistant clones of banana respond actively to infection [3] with a dramatic increase in the chitinase activity, but this responses is more tissue specific than the previously studied peroxidase. However, total chitinase activity by itself should not be considered an absolute parameter. The rate of production appear to be more important, i.e. the activation response of the host to the infection. The speed and magnitude for the activation of mechanism appears to be critical for expression of resistance.

Resistance of plants to invasion by potential pathogens is the result of a multiple defence reaction comprising both constitutive and inducible mechanisms. The use of biochemical markers to select for resistance may expedite breeding programmes by reducing the number of field trials. The main criteria for practical use of such markers are reliability in predicting resistance and the ease to handle the assay. This would have a comparative advantage over traditional field screening. For any marker, a number of pre-requisites must be fulfilled. Firstly, it should positively correlate with the level of resistance or susceptibility of the breeding population. Secondly, it should be able to distinguish between F_2 segregants or between individuals among a mutagenised population. Thirdly, it should be applicable to a large breeding population.

The results of this study showed that the plant reaction to pathogens can be estimated by the inducible chitinase activity during the time course of active chitinase production after infection. However, to follow the enzymatic activity over a long time involves intensive work and careful control of environmental conditions. Thus, predicting plant response on the basis of inducible chitinase becomes a long procedure.

Based on the present study, it was possible to divide the experimental population in two broad classes of resistant and susceptible sub-populations. On the basis of chitinase activity in root tissues, it would be possible to identify suitable parents for breeding for resistance to *Fusarium*. The differential response based on chitinase activity confirms our previous results [3] obtained with peroxidases, and suggests that such an approach could be useful in screening for variation among the cultivars, wild species and sub-species to identify sources of resistance.

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BANANA RESEARCH IN THE FAO/IAEA AGRICULTURE AND BIOTECHNOLOGY LABORATORY

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Abstract

The primary activity of the Agriculture and Biotechnology Laboratory on banana has been to develop and transfer mutation techniques using nuclear and related biotechnology, provide training and mutagen treatment services and technical advice to the Member States. The complex genetic nature and lack of seed formation do not allow conventional breeding of *Musa* varieties. The FAO/IAEA laboratory has developed *in vitro* techniques to induce mutations, minimize chimerisms, and rapid propagation of banana. The most commonly used method of propagation is rapid proliferation of axillary and adventitious buds from meristem tip culture. Somatic embryogenesis has been induced in clones with different genomic constitution; however, the low germination rate of somatic embryos is still a major constraint. Investigations have been carried out on enzymes associated with resistance to *Fusarium oxisporum* f. sp. *cubense*. Molecular methods based on DNA oligonucleotide and DNA amplification fingerprinting are being developed for genomic characterisation of species, cultivars and mutant clones.

1. INTRODUCTION

The Plant Breeding Unit of the FAO/IAEA Agriculture and Biotechnology Laboratory, in co-operation with the Plant Breeding and Genetics Section, Joint FAO/IAEA Division, provides scientific and technical backstopping in several areas of plant breeding. The special emphasis is on the development and transfer of mutation breeding techniques to Member States. The work aims at technology development and transfer through the following activities: i) research and development of mutation techniques using nuclear and related biotechnological methods, ii) training of scientists, and iii) provision of mutagen treatment services and technical advice.

The difficulties encountered in the improvement of vegetatively propagated crops through traditional cross-breeding require the development of alternatives methods. In this context, mutation breeding in combination with related biotechnology can plays a major role. The complexity of *Musa* genetics illustrates the need for a more sophisticated system to support both conventional cross-breeding and mutation induction programmes. This report provides a general survey of the activities of the Plant Breeding Unit in the development of an integrated breeding approach for the improvement of bananas and plantains. Most of the work and achievement presented in here should be considered the result of co-operative efforts between a number of laboratories in the developing and developed countries. Therefore, a due acknowledgement and appreciation of the joint efforts toward a common goal is recognized.

2. RESEARCH ACTIVITY IN THE LABORATORY

Bananas and plantains (*Musa* spp.) are among the world's most important crops. They are staple food for millions of people throughout the developing world and an essential source of income in some of them. The complex genetic background, lack of seed formation and distortion of segregation in diploids and triploids are some of the factors that hamper the

development of new *Musa* varieties with the required resistance and quality characteristics. Studies on the development of banana and plantains were initiated at the FAO/IAEA Laboratory in 1985 as part of Joint FAO/IAEA Division programme.

2.1. Mutation Breeding and *In Vitro* Techniques

Any breeding programme relies on the availability of genetic variation, well characterised parental material, and the possibility to rapidly screen traits among recombinants or in large mutagenised populations. The mutation breeding system developed at the FAO/IAEA laboratory is based on *in vitro* techniques for inducing mutations and avoiding genetic chimerisms, and propagation of the desired variants. The most commonly used method of propagation is rapid proliferation of axillary and adventitious buds from meristem-tip culture. This also allows the checking of viral contamination. The laboratory has now capability to check for the presence of the common viral diseases by using ELISA techniques. The material received from counterparts are tested for the presence of pathogens.

The development of somatic embryogenesis, using somatic tissue explants was a breakthrough in the development of a novel method. It opened the possibility to induce somatic embryogenesis in clones with different genomic constitution. However, a major constraint is the low germination rate of somatic embryos. Generally, somatic embryos have a strong tendency to form roots. Thus, there is a need to fine tune the germination medium and to identify the possible blockage in the somatic embryogenesis pathway. Nevertheless, this method offers new opportunities of breeding new cultivars. The system allows production of embryogenic suspension cultures for mutation induction. The unicellular system enables selection for various stresses both abiotic and biotic, and could be used in breeding procedures for *in vitro* selection and polyploidy induction.

2.2. Screening for resistance to *Fusarium* wilt

Breeding for disease resistance is a major goal in *Musa* improvement. In spite of the recent success in the development of resistant clones through cross-breeding in Honduras and somaclonal variation in Taiwan, the development of methods based on easily detectable markers should be considered a priority to shorten the lengthy field screening procedures. During the past five years, a major effort in the Plant Breeding Unit has been to work out a system for early selection of disease resistance in banana, and to identify biochemical markers linked to this trait.

Shoot tips cultures from banana clones susceptible or resistant to *Fusarium oxisporum* f. sp. *cubense* (FOC) Race 1 and 4 were grown *in vitro* in the presence of different concentration of fusaric acid and fungal crude filtrates or inoculated with conidial suspension of FOC to assess correlation between *in vivo* or *in vitro* behaviour. Explants were susceptible to both the filtrate and fusaric acid irrespective to their known field resistance/susceptibility response. No clear linkage between *in vivo* and *in vitro* behaviour was observed. The results suggested that the use of crude filtrate or non-host specific toxin to screen for resistance to FOC in *Musa* was not feasible. When peroxidase activity was used as a parameter to discriminate between susceptibility and tolerance, results were in good agreement with the field response of host plants to pathogens. Early enzymatic activity increased in the incompatible host-pathogen interaction but not in the compatible interaction. Studies were carried on other enzymatic systems involved in plant resistance, especially on chitinase. Forty-two clones were screened for chitinase activity. Further studies were conducted on the

behaviour of this enzyme; the laboratory is now trying to clarify the role of chitinase in banana resistance.

2.3. Genetic markers and molecular biology

The characterisation of breeding material and its stability represents a major concern in any improvement programme. DNA oligonucleotide and DNA amplification fingerprinting (DAF) were used for genomic characterisation of different species, cultivars and a mutant clone. Both the oligonucleotide and DAF were somatically stable, i.e., did not exhibit any difference between tissues of the same plant and between individuals of the same clone. A unique fingerprinting was found for each clone, and therefore could be used to discriminate between accessions or to characterise the parental material and mutants. The use of Random Amplified DNA (RAPDs) is being investigated for the analysis of homogeneity among near isogenic lines to identify genes for resistance. For this, PCR cloning of specific genes is being pursued to clarify their specific function in the host-parasite interactions. Genetic transformation of *Musa* represent yet another opportunity to develop new genotypes. Transgenic 'Grand Naine' were obtained by the *Agrobacterium*-mediated and high-velocity microparticle bombardment transformation. Breeding of banana and plantains is thus being complemented through the development of techniques that would enhance breeding efficiency. The Plant Breeding Unit in collaboration with several institutes is refining the technology and its transfer through training.

IMPROVEMENT OF CASSAVA FOR HIGH DRY MATTER, STARCH AND LOW CYANOGENIC GLUCOSIDE CONTENT BY MUTATION INDUCTION

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Abstract

Cassava (*Manihot esculenta* Crantz) is an important food in Nigeria. One drawback in its use as a staple food is the presence of cyanogenic glucosides which on hydrolysis produce the very toxic hydrogen cyanide (HCN). To reduce the cyanogenic levels by mutation induction, three locally adopted and high yielding varieties of cassava, TMS 30572, NR 8817 and NR 84111 were irradiated with 20, 25 and 30 Gy gamma rays. There were a wide variation in HCN, dry matter and starch content of irradiated cassava plants, screened in the MV_2 propagation. Fourteen cassavavariant lines were selected for low HCN content, and 22 lines for high dry matter content. These will be further tested for yield in replicated field trials.

1. INTRODUCTION

Cassava, *Manihot esculenta* Crantz, ranks high as a major source of cheap energy food in Nigeria. According to FAO [2], during 1985 the area under cassava cultivation in Nigeria was about 2500 x 10^3 ha compared with 1550 x 10^3 ha for yams. Production figures also show that cassava cultivation is on the increase [5]. Cassava has thus become a premier crop, a position which for several decades was occupied by yams. Among the several reasons contributing to this situation is the cultural management of cassava crop which can thrive well on marginal soils where other crops invariably fail.

With the present socio-economic changes in Nigeria, the local farmers are demanding cassava lines that are high yielding, and have high dry matter and starch, but are low in HCN content. In this paper, we report the use of mutations to induce genetic changes for high dry matter and starch content and low cyanogenic traits in the locally adopted varieties.

2. MATERIALS AND METHOD

2.1. Mutation induction

Three cassava varieties, TMS 30572, NR 8817 and NR 84111 were used in the present study. During 1993/94 cropping season, batches of 250 stem-cuttings, about 10 cm long and with 5 nodes each, from each variety were irradiated with 20, 25 and 30 Gy gamma rays from a Cobalt 60 source located at the Center for Energy Research and Development, Obafemi Awolowo University, Ile-Ife, Nigeria. During the 1994/95 season, the surviving MV_1 clones were advanced to MV_2 populations using ca. 10 cuttings per plant. The MV_2 populations were screened for hydrogen cyanide, dry matter (DM) and starch content on individual plant basis.

2.2. HCN determination

The HCN content of lines was determined using methods to estimate cyanide with alkaline picrate as suggested by Almazam [1] and Williams and Edwards [7].

2.3. DM determination

Dry matter was determined by slicing 100 g samples of cassava tubers, dried to a constant weight at 80°C in an oven.

2.4. Starch Determination

Peeled cassava tubers 100 g each were macerated and passed through 15 μM pore size sieve, using tap water. The extracted starch in water was allowed to stand for 6-12 hr, after which excess water was decanted, and the starch was dried to a constant weight in an oven at 80°C.

3. RESULTS

There was a wide variation in HCN, dry matter and starch content of the screened MV_2 cassava plants to allow meaningful selection for these traits (Table I). Based on these results, 14 cassava variant lines were selected for low HCN content (Table II). Of these, 9 lines were isolated from irradiated TMS 30572, 3 from NR 8817 and 2 from NR 84111. Of the variant lines selected for high DM content, 5 were isolated from TMS 30572, one from NR 8817 and 16 from NR 84111 (Table III).

4. DISCUSSION

Cassava is an important food in Nigeria. However, a major drawback in the use of cassava is the cyanogenic glucosides which upon hydrolysis produce toxic hydrogen cyanide. Consumption of improperly processed cassava food may lead to goitre and cretinism [6] from the ingestion and accumulation of HCN in the body. Hence, selection of cassava varieties that are acyanogenic or low in cyanogenic content is important. Hahn, et al, [3] reported that low HCN content in cassava in controlled by minor recessive gene complex, which probably accounts for the wide range in HCN content of screened MV_2 cassava plant populations, and the large number of recoverable low cyanogenic variant lines, in the present study. Moh [4] reported a wide range in HCN levels of irradiated cassava cuttings when MV_1 leaves were screened for HCN levels.

Yield of cassava root tubers is related to tuber volume and dry matter content. Yield therefore can be improved by increasing dry matter content. In cassava products such as 'gari', recovery largely depends on the dry matter content of the tubers; thus it important to have high dry matter, since such food products are marketed in dry form. The results suggest that the dry matter and starch content of cassava root tubers and their cyanogenic content can be improved through mutagenesis. The isolated variant lines will be further tested for yield in field trials.

TABLE I. HCN, DRY MATTER AND STARCH CONTENT OF MV_2 POPULATIONS OF THREE CASSAVA VARIETIES

Variety	HCN score			Dry matter content (%)			Starch content (%)		
	X±S.E	Range	CV (%)	S±S.E	Range	CV (%)	X±S.E	Range	CV (%)
TMS 30572	6.2±0.13	3 - 9	23.0	32.2±0.65	14 - 42	18.8	20.3±0.32	12 - 28	16.2
NR 8817	6.6±0.16	3 - 9	21.5	24.8±0.59	10 - 40	16.9	14.1±0.70	7 - 20	32.7
NR 84111	5.0±0.15	3 - 9	32.2	34.7±0.45	20 - 35	13.4	19.9±0.26	11 - 24	13.5

TABLE II. CASSAVA MUTANT LINES SELECTED FOR LOW CYANOGENIC GLUCOSIDE CONTENT IN THE ROOT TUBERS

Mutant Line	HCN Score	HCN Content (ppm)	Dry Matter (%)	Starch Content (%)
30572/30/002(2)	4	25 - 40	31.1	19.3
30572/30/004(1)	3	15 - 25	31.2	25.0
30572/30/005(5)	4	25 - 40	30.0	20.8
30572/30/007(4)	3	15 - 25	37.0	24.1
30572/30/007(8)	3	15 - 25	34.0	19.3
30572/30/007(6)	3	15 - 25	40.0	15.0
30572/30/008(8)	3	15 - 25	33.8	22.3
30572/20/010(1)	3	15 - 25	15.4	15.2
30572/20/010(2)	4	25 - 40	32.4	12.2
30572/Control			35.0	22.2
8817/30/004(2)	3	15 - 25	34.4	21.3
8817/25/009(3)	4	25 - 40	24.5	11.6
8817/20/003(5)	3	15 - 25	28.5	13.5
8817/Control			28.0	23.6
84111/25/002(6)	2	10 - 15	34.0	20.5
84111/10/003(1)	2	10 - 15	36.3	22.3
84111/Control			36.1	21.6

TABLE III. CASSAVA LINES SELECTED FOR HIGH DRY MATTER CONTENT

Mutant Line	DM (%)	Starch Content (%)	HCN Rating
30572/30/001(6)	42.0	28.0	M
30572/30/003(3)	40.0	23.6	M
30572/30/007(7)	40.0	15.0	L
30572/25/004(3)	40.0	22.0	M
30572/25/005(3)	40.4	20.6	H
30572/Control	35.0	22.0	
8817/25/002(3)	31.9	18.3	M
8817/Control	28.0	16.3	
84111/30/008(2)	40.0	17.5	M
84111/25/001(1)	40.8	20.0	M
84111/20/001(7)	40.0	24.6	M
84111/20/003(7)	40.0	21.3	M
84111/20/006(4)	40.0	24.6	L
84111/20/006(9)	40.3	19.9	H
84111/20/007(8)	40.4	24.3	M
84111/20/009(1)	42.3	22.6	M
84111/20/009(4)	40.3	20.0	H
84111/20/010(2)	41.3	23.2	M
84111/20/010(3)	41.3	21.3	M
84111/20/011(1)	41.3	21.5	M
84111/20/011(5)	40.8	21.3	M
84111/20/011(6)	40.9	23.0	H
84111/20/011(8)	40.8	20.1	M
84111/20/011(10)	42.7	22.7	M
84111/Control	36.1	23.6	

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PLANT REGENERATION FROM ORGAN CULTURE IN WHITE GUINEA YAM

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Abstract

Explants from leaves, leaf segments, petioles and internodal stem of *in vitro* grown seedlings of white guinea yam, *Dioscorea rotundata* Poir, cv. 'Obiaoturugo' were cultured on defined media. NAA at concentrations of 0.5-1.0 mg/l induced shoot regeneration from petiolar and inter-nodal stem pieces, and rooting occurred with little or no callusing from whole leaves or leaf segments. With concentration of 3.0-10.0 mg/l NAA, explants from petioles, inter-nodal stem, whole leaves and leaf segments formed callus which produced roots. These explants developed plantlets when subcultured on MS medium supplemented with 2.0 mg/l BAP and 0.1 mg/l NAA.

1. INTRODUCTION

The white guinea yam, *Dioscorea rotundata*, Poir, belongs to the edible species of *Dioscoreacea*. It is widely consumed in West Africa especially, in Nigeria, where over 70% of the World's yam is produced [1]. This starchy food crop is believed to have originated from the rain forests of West Africa [2]. However, yam crop has low yield, and not much research has been done on its genetic improvement [2]. Yams are difficult to breed through conventional crossing because the crop is a polyploid and highly heterozygous. Low and inconsistent flowering, poor fruit and seed set also hamper hybridization. Therefore, mutation induction has been recommended as a useful method in the genetic improvement of yams [3]. In mutation induction, the chances of isolating mutants from irradiated *in vitro* grown plants depend on several factors such as source and type of explant and age of plant before irradiation. In potato, selection of apical buds for growth after gamma irradiation gave more mutants than axillary buds [4] and among plants regenerated from leaf, stem and petiole explants [5]. A protocol for plant regeneration in yam was developed by using different organs, such as whole leaves, leaf segments, petioles and stem pieces taken from *in vitro* grown irradiated plants.

2. MATERIALS AND METHODS

Seeds of white guinea yam, *Dioscorea rotundata* Poir, cv. 'Obiaoturugo' were surface sterilized with 70% (v/v) ethanol for 1 minute and then immersed in 80% commercial bleach (Parozone) containing 1% Tween-20 for 20 minutes under aseptic conditions. These were washed 5 times with sterile distilled water. The sterile seeds were cultured on half-strength MS [6] medium salts, solidified with 0.8% agar.

The seeds germinated after two weeks. After six weeks, plants with two to five expanded leaves were aseptically removed under an air-flow cabinet, and placed in sterile Petri-dishes containing approximately 5 ml sterile distilled water. The plants were cut into leaves, petioles and stems. The petioles and stems were subdivided into segments about 3 mm long and placed in sterile Petri-dishes containing sterile distilled water. Half the number of leaves were divided into ca. 3x3 mm segments, and placed in Petri-dishes as above, and cultured on two media.

Medium I consisted of MS macro- and micro-salts, vitamins and glycine, 2% (w/v) sucrose, 0.7% (w/v) agar, supplemented with 0.5, 1.0, 3.0, 5.0, 7.0 and 10.0 mg/1 NAA (naphthalene acetic acid). Medium II was similar to Medium I but was supplemented with 0.1 mg/1 NAA and 2 mg/1 benzylaminopurine (BAP). The pH of the media was adjusted to 5.8 with NaOH or HCl before dispensing into 8 ounce baby food jars (25 ml/jar). These were autoclaved at 15 psi and 121°C for 15 minutes. Four explants each of whole leaves, leaf segments, petioles and internodal stem pieces were inoculated on Media I, and replicated five times. The leaf explants were placed with their ventral surface in contact with the medium. They were kept in culture room at 27± 1°C, under 16 hr photoperiod and light intensity of 1200 to 1400 lux. They were subcultured after 4 weeks on fresh medium. After eight weeks, the explants were transferred singly to Medium II.

3. RESULTS AND DISCUSSION

At low concentration, (0.5-1.0 mg/1), NAA induced adventitious shoot formation with roots on only petiolar and inter-nodal stem pieces (20-40%) and rooting with little or no callusing from whole leaf and leaf segment explants. However rooting was more profuse on whole leaves with some petiolar tissue than leaf segments, which developed callus tissues with passage of time. At higher concentrations, 3.0-10.0 mg/1, explants from petiolar, inter-nodal stem pieces, whole leaf and leaf segments formed callus that rooted excessively. These explants developed plantlets when subcultured on Medium II containing BAP and NAA (Table I).

NAA alone or in combination with cytokinin such as BAP has been used to induce callus and plant regeneration in yams [7, 8, 9]. However, maintaining genetic fidelity through organ regeneration via callus tissues is difficult [10,11]. The present study suggests that NAA at low concentrations could be used for micropropagation of the white guinea yam using appropriate explants. However, the protocol developed is recommended as an aid to *in vitro* mutation induction and isolation of mutants. Somaclonal variants that arise from callogenesis may be an additional source of variation for selection.

The protocol for plant regeneration via callus systems in the white guinea yam should include a basal medium supplemented with 3.0 - 10.0 mg/1 NAA, and a regeneration medium containing 2.0 mg/1 BAP and 0.1 mg/1 NAA. The chances of isolating mutants from plants exposed to gamma rays *in vitro* would increase if plants were multiplied from explants which originate from different parts of the irradiated plants. Such a protocol would be of value in the generation of mutants in yams.

TABLE I. PLANT REGENERATION FROM EXPLANTS OF WHOLE LEAVES, LEAF SEGMENTS, PETIOLES AND INTER-NODAL STEM PIECES SIX WEEKS AFTER *IN VITRO* CULTURE

Types of Explant	NAA (mg/l)	No. of Explant cultured	formed plants	rooted without callus	callused	showed no response
whole leaf	0.5	20	-	20 (100)*	-	-
	1.0	20	-	20 (100)	-	-
	3.0	20	-	10 (50)	10 (50)	-
	5.0	20	-	12 (60)	8 (40)	-
	7.0	20	-	-	20 (100)	-
	10.0	20	-	-	20 (100)	-
leaf segments	0.5	20	-	18 (90)	-	2 (10)
	1.0	20	-	16 (80)	4 (20)	-
	3.0	20	-	-	20 (100)	-
	5.0	20	-	8 (40)	12 (60)	-
	7.0	20	-	-	20 (100)	-
	10.0	20	-	-	20 (100)	-
petioles	0.5	20	8 (40)	8 (40)	4 (20)	-
	1.0	20	8 (40)	-	12 (60)	-
	3.0	20	-	-	20 (100)	-
	5.0	20	-	-	20 (100)	-
	7.0	20	-	-	20 (100)	-
	10.0	20	-	-	20 (100)	-
inter-nodal stem pieces	0.5	20	4 (20)	7 (35)	9 (45)	-
	1.0	20	8 (40)	-	12 (60)	-
	3.0	20	-	-	20 (100)	-
	5.0	20	-	-	20 (100)	-
	7.0	20	-	-	20 (100)	-
	10.0	20	-	-	20 (100)	-

*values in parentheses represent percentage.

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IMPROVEMENT OF PIGEONPEA AND COWPEA FOR DROUGHT, DISEASE AND INSECT PEST TOLERANCE THROUGH INDUCED MUTATIONS

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Abstract

Pigeonpea and cowpea are widely grown in the semi-arid and arid regions of Kenya by small scale farmers. The average yields are usually low due to insect pests, diseases and long growth duration of the local land races. Little success has been achieved through conventional breeding methods for tolerance to insect pests and diseases despite the development of high yielding and early maturing lines. Therefore, mutation induction was initiated to widen the genetic variability in the improved lines. Seeds of three promising pigeonpea cultivars KAT 60/8, KAT 777 and KAT E31/4 and of cowpea KAT 419, K8O and M66 were subjected to three doses of gamma rays; 80, 120 and 150 Gy for pigeonpea and 160, 200 and 250 Gy for cowpea. In M_1 generation, doses of 150 Gy and 250 Gy reduced emergence by about 50% and increased seedling deformities in both crops. In M_2 generation of KAT 60/8, high yielding mutants with oval shaped seeds (T_1 P_{58}) and branching (T_3 P_{28}) were identified. Two progenies of KAT 777 (T_1 P_7 and T_1 P_{11}) had small slender leaves. Selected plant progenies in M_3, M_4 and M_5 generation gave some promising high yielding variants. Although, the difference in days to flower and maturity of mutant progenies and untreated bulk were small, some mutant progenies of KAT 777 and KAT 60/8 showed tolerance to *Fusarium* wilt. None of the progenies of KAT E31/4 gave better score for *Cercospora* leaf-spot compared to the check.

1. INTRODUCTION

Pigeonpea, *(Cajanus cajan)* and cowpea, *(Vigna unguiculata)* are grown in Kenya over 164,000 ha and 252,000 ha, respectively. Although, this represent almost over half of the total area under pulses, the yield of these two crops (average 300-500 kg/ha) are low. The low yields are mainly attributed to insect pests, diseases and drought. Through conventional breeding, several early maturing (drought escape) and high yielding varieties of cowpea and pigeonpea have been developed at the National Dryland Farming Research Center, Katumani. In the experimental fields, the yield potential (2.0 tons/ha) of these improved varieties can be achieved only when crop protection measures against insect pests are ensured. The situation is more serious in the farmers fields because very few of them can purchase chemicals to protect their crops against insect pests. Conventional breeding methods for resistant to insect pests and diseases have met with little success. Mutation induction was, therefore, initiated during 1989 to complement the conventional breeding methods to improve the promising pigeonpea and cowpea varieties against major insect pests (pod borers and pod suckers) and diseases (wilt and leaf spots), and to develop early maturing, high yielding and drought tolerant/escape pigeonpea cultivars.

2. MATERIALS AND METHODS

Three promising pigeonpea and cowpea lines each, representing early, medium, and late maturity groups, were selected for the improvement of different characters (Table I). Open-pollinated seeds of each variety were subjected to three doses of gamma rays after determination of LD_{50}. The doses ranged between 80 to 150 Gy for pigeonpea, and 150 to 250

Gy for cowpea varieties [1]. Approximately, 600 seeds of each cultivar were treated with different doses at IAEA Laboratory, Seibersdorf during December, 1989.

TABLE I. AGRONOMIC CHARACTERS AND CONSTRAINTS OF PROMISING PIGEONPEA AND COWPEA VARIETIES.

Line	Maturity (days)	Potential Yield (t/ha)	Disadvantage
Pigeonpea			
KAT 60/8	150	1.0-1.5	Prone to insect pest damage.
KAT 777	180	1.5-2.0	Less tolerant to drought and wilt.
KAT E31/4	220	2.0-2.5	Late, susceptible to *Cercospora* leaf spot
Cowpeas			Late, leaf spots, rust and susceptible to insect pests.
M66	85	1.3-1.5	
K80	80	1.3-1.5	Susceptible to leaf spots and rust. Resistant to aphid but susceptible to maruca.
KAT 419	75	0.8-1.2	Resistant to leaf spot, small leaves, susceptible to insect pests damage.

The M_1 and untreated bulk seeds of pigeonpea were grown at Katumani and those of cowpea at Kibwezi during March, 1990. Observations were recorded on seedling emergence, deformity, sterility and days to flower [1]. M_1 plants were individually harvested. From each plant, 10 pods from different branches were bulked to obtain single plant progenies. These M_2 seeds were sown as single plant progenies in selected environments to expose them to drought, insect pests and diseases.

In June, 1990, all M_2 cowpea progenies were planted at Kibwezi, and 300 progenies of pigeonpea cv. KAT 60/8 at Kiboko to screen for earliness and tolerance to common insect pests under natural conditions. One hundred and twenty four M_2 progenies of KAT 777 were advanced to M_3 during October-November, 1990 at Katumani. The resulting M_3 plant progenies were evaluated in a wilt-sick plot during 1991-92 at Katumani.

For all the genotypes, the M_2 plant progenies were visually evaluated for insect pest damage, drought (wilting due to moisture stress), diseases and other genetic variability at flowering time, plant height and leaf characteristics within and amongst the progenies [2]. Desirable M_3 plant progenies were selected and evaluated at Kiboko for yield and other agronomic characters in randomized block design, replicated thrice. These were advanced to M_4 and M_5, and promising plant progenies were evaluated for wilt in the wilt-sick plot.

3. RESULTS

Due to limitation of land, cowpea progenies were only observed upto M_2 generation (Omanga, 1991). Of the 300 M_2 plant progenies of pigeonpea cv. KAT 60/8, planted at a relatively more dry location of Kiboko, only 30 were selected for further evaluation. These maintained green leaves (tolerated drought) up to harvest. In the remaining progenies, a high percent of leaves withered, and fell before harvesting. Since one of the objectives was to screen for insect-pest tolerance, only one spray was given. This was to ensure at least some harvest. However, in the M_3 generation, the 30 plant progenies were evaluated under both sprayed and unsprayed conditions.

Results from the sprayed condition revealed no remarkable differences amongst the progenies at time of 50% flowering (range 79 to 92 days), days to maturity (143 to 148 days) and grain yield (1.3 to 1.5 t/ha). Under unsprayed condition, significant differences were observed in grain yield (range 0.21 to 0.74 t/ha) but not for days to 50% flowering and maturity. Based on the data from the unsprayed plots, 15 high-yielding progenies were advanced to M_4 generation and evaluated together with the untreated check. The result of the preliminary evaluation of the M_5 progenies is shown in Table II. Yield ranged between 0.11 to 0.92 per hectare. Only two variants (T_1 P_{58} and T_3 P_{28}) had significantly higher grain yield than the check variety. Among these mutants, T_1 P_{28} has oval shaped seeds compared to the round ones of the parent cultivar, and T_3 P_{28} showed relatively more branching.

Mutation induction had been initiated in KAT E31/4 to improve its resistance to *Cercospora* leaf-spot and reduce the duration of maturity. The M_2 plant progenies were visually scored for leaf withering and fall (drought stress), susceptibility to leaf spots and maturity duration on a scale of 0-9 [2]. Twenty progenies were advanced to M_3 and evaluated for yield at Katumani. The results showed that only one plant progeny (T_2 P_{12}) gave better yield

TABLE II. TIME TO 50% FLOWER, 100-SEED WEIGHT AND GRAIN YIELD OF EIGHT OUT OF 15 MOST PROMISING KAT 60/8 MUTANTS IN M_5 GENERATION UNDER UNSPRAYED CONDITION.

Mutant lines	Days to 50% flower	100-seed weight (g)	Grain yield (t/ha)
T3 P25	97	8.7	0.69
T1 P28	98	12.5	0.67
T3 P11	101	11.3	0.86
T3 P28	99	11.0	0.70
T2 P107	99	11.6	0.66
T1 P95	98	11.5	0.92
T1 P58	100	10.8	0.63
T2 P100	98	11.8	0.72
KAT 60/8	98	13.6	0.68
Mean	98	11.6	508
CV %	1.4	6.8	0.46

compared with the untreated check (Table III). Two progenies (T_2 P_{12} and T_2 P_{27}) gave a score of 4 for *Cercospora* leaf-spot; however, none of the progenies was earlier than the check cv. KAT E31/4.

The number of progenies evaluated for wilt in the wilt-sick plot are presented in Table IV. During 1991-92, only 245 M_3 plants out of 10,040 of KAT 777 survived the wilt pathogen. In M_4, only 34 plants were harvested from different progenies, and were planted as plant to row progenies. Of these 34 plant progenies, seeds were obtained only from 4 plants. Over 80 plant progenies of KAT 60/8 were screened for wilt, and only in 3 progenies some plants showed wilt tolerance. These will be evaluated further to confirm their resistance to wilt.

TABLE III. TIME TO 50% FLOWER, PLANT HEIGHT, GRAIN YIELD AND CERCOSPORA LEAF SCORE FOR M_3 GENERATION OF KAT E31/4.

Entry	Days to 50% flower	Plant height (cm)	Grain Yield (t/ha)	Cercors-pora score
T2 P12	170	143	2.13	4
T3 P1	160	135	1.67	5
T2 P27	173	129	1.68	4
T1 P21	170	125	1.79	5
T1 P7	172	120	1.41	6
T2 P1	177	98	1.41	6
Check E31/4	175	120	1.73	6
Mean	170	134	1.35	6
CV %	3.6	19.3	27.40	27.4

4. CONCLUSIONS

The above results have clearly shown the possibility to obtain high yielding pigeonpea lines through selection from irradiated material. It has been also possible to identify wilt tolerant lines among the advanced generations. However, since out-crossing in pigeonpea sometimes exceeds 40%, a lot of care is required to ensure that the advanced progenies are genetically pure.

TABLE IV. NUMBER OF PROGENIES EVALUATED FOR WILT DURING 1991-94 AND PLANTS HARVESTED.

Year	Cultivar	KAT 777	Cultivar	KAT60/8
	No. Evaluated	No. Harvested	No. Evaluated	No. Harvested
1991-92	1004	245		
1992-93	245	34	80	27
1993-94	34	4	27	3

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CHARACTERIZATION OF STARCH AND OTHER COMPONENTS FROM AFRICAN CROPS AND QUALITY EVALUATION OF DERIVED PRODUCTS

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Abstract

Research was carried out on African staple foods on characterization of components of cereals and tubers, and quality evaluation of foods manufactured from composite flours. Cereal starch, alimentary fiber and minerals from cassava were investigated. Starch was isolated under conditions of minimum damage from seeds of three sorghum and two fonio cultivars, and its physico-chemical properties were compared with commercial wheat starch. Fiber, ash and mineral content of samples of genetically improved varieties of cassava from Ghana were determined to understand the role of factors that influence texture of cooked products. Bread and pasta were produced from either triticale alone or in combination with different amounts of cassava flour, and by varying the amount of wheat flour. The organoleptic quality of the raw materials and final products were determined.

1. INTRODUCTION

Within the framework of the FAO/IAEA Programme on "Improvement of Basic Food Crop in Africa through Plant Breeding, Including the Use of Induced Mutations" the Unit of Studies on Cereals, Rome has been carrying on research on African staple foods in two areas: i. characterization of components of cereals and tubers, and ii. quality evaluation of foods manufactured with composite flours.

Within the first line of research, we investigated on starch from cereals, and alimentary fiber and mineral content of cassava, sorghum and fonio which are traditional cereals of Africa. Several efforts have been made to explore new food uses of African cereals either alone or after mixing with other cereal flour to enhance their use and diversify utilization. Therefore, a better understanding of their components is required. In particular, very little information is available on fonio seeds and starch. The present study was undertaken to obtain basic information on the nature and functional behaviour of purified starches of these African cereals. Commercial wheat starch was used for comparison. Starch is the major constituent of cereal endosperm and plays a key role in the development of the plant. It is essential in human diet and health, and its importance in determining the property of food products is well recognized. The texture of cooked African starchy products such as those from cassava roots may also be influenced by components other than starch. Therefore, fiber, ash and mineral content of samples of genetically improved varieties of cassava from were also determined.

In the second area of research, we assessed the quality of foods manufactured with triticale, a cross between wheat and rye, whose hardiness and vigour make it suitable to grow in marginal and acidic soils where cultivation of wheat would not be possible. Therefore, triticale was used for the preparation of bread and pasta traditionally manufactured with wheat.

Since many African countries produce substantial amounts of root crops such as cassava, we substituted part of the cereal flour with cassava flour to utilize traditional crops for the preparation of new nutritional, convenient and attractive foods.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Characterization of components of cereals and tubers

Starch was extracted from two cultivars of fonio 'Hotia' and 'Koulli', and three cultivars of white sorghum 'Dwarf White Milo', 'Dabar' and 'Sorghum 100'. Fonio cultivars were grown in Ethiopia, 'Dwarf white Milo' and 'Dabar' in Sudan, and 'Sorghum 100' was grown in Italy. Wheat flour was obtained as commercial starch from BDH Ltd, Poole, England. Cassava varieties were grown in Ghana.

2.1.2. Isolation and purification of starch

Fonio and sorghum starch was isolated under conditions of minimum damage according to a wet milling procedure as reported by Carcea et al. [1].

2.1.3. Chemical and physico-chemical methods

Moisture, protein and ash content of isolated starches was determined in duplicate by AOAC methods [2]. Fonio starch lipids were extracted with water-saturated butan-1-ol (WSB) at room temperature [3] whereas sorghum lipids were extracted by n-propanol/water (3:1 v/v) at 100°C. Amylose content was determined in duplicate by the method of McCready and Hassid [4]. Water binding capacity was measured by the method of Medcalf and Gilles [5]. Swelling power and solubility determinations were carried out in triplicate between 50-90°C range at 5°C intervals following the procedure of Leach et al. [6].

Pasting properties were determined in duplicate by means of the Brabender Visco-Amylograph using the carboxymethyl cellulose (CMC) technique as described by Medcalf and Gilles [5]. Two starch concentrations (3 and 5% d.b.) were studied for fonio and three starch concentrations (2.2, 3.3, 4.4% d.b.) for sorghum. A blank curve using only CMC (Sigma Chemical Co. St. Louis, Mo) was subtracted from the starch-CMC curves to obtain the corrected starch curve.

Fiber content of cassava tubers was determined according to the AOAC method [2]. For ash determination, samples were put in a furnace at 500 °C overnight. Ash dissolved in conc. HCl was analyzed by Atomic Absorption to determine Calcium and Magnesium. Results were expressed as percentage on wet basis.

Data were analyzed by ANOVA and Duncan's multiple range test for significant differences.

2.2. Chemical and rheological methods

2.2.1. Quality evaluation of foods manufactured with composite flours

Triticale flour was produced from grain of two Italian varieties 'Rigel' and 'Mizar' dehydrated at 15% moisture for 24 h and milled in a experimental mill Buhler MLU 202. The extraction rate of the flour was 72%. Commercial bread wheat flour and durum wheat flour of average quality were purchased from the market. Commercial cassava starch from Thailand was purchased from the market. Commercial compressed yeast was used, and the fat was commercial margarine. Proximate composition of flours, loaves and pasta was determined according to official AOAC methods [2]. Farinograms where determined according to the ICC standard method No.115, alveograms by No.121 and falling number by No.107 [7].

2.2.2. Baking Experiments

Flour blends for baking were prepared as follows:

Sample	1	100:00	bread wheat (control)
	2	100:00	triticale
	3	50:50	triticale/cassava
	4	45:45:10	triticale/cassava/wheat
	5	40:40:20	triticale/cassava/wheat

The loaves were scaled 250 g each, 3 loaves per batch and baked following the conventional straight-dough method to produce white pan bread.

The baking formula used was as follows:

Ingredients	Parts
Flour	100
Salt	2
Compressed yeast	5
Fat	2 (when used)
Glycerylmonostearate	1 (when used)
Water	Variable (70-79)

Bread volume was measured after 24 h by rapeseed displacement. The bread loaves were rated according to Pyler [8] as follows: volume 15; colour and nature of crust 5; symmetry of form 5; uniformity of bake 5; texture 15; colour of crumb 10; grain 10; aroma 15; taste 20; maximum score 100.

A group of nine persons who regularly serve on tasting panels evaluated the bread characteristics and its shelf-life. Presentation of the bread samples was completely randomized, and three samples were presented to panelists during each session.

2.2.3. Pasta making experiments

The following flour blends were used in the production of spaghetti and short pasta (macaroni type):

Pasta format	Materials	
Spaghetti	100:00	triticale
"	90:00	triticale/pregelatinized triticale
Short pasta	100:00	triticale
"	60:40	triticale/cassava
"	65:35	triticale/cassava
"	65:30:05	triticale/cassava/pregelatinized cassava
"	65:25:10	triticale/cassava/pregelatinized cassava

Pre-gelatinized triticale flour was prepared in a G 20 Mapimpianti single screw extruder whereas pre-gelatinized cassava flour was prepared in the laboratory by cooking one part of cassava flour in 4 parts of boiling water for about 10 min. The pre-gelatinized cassava was left to cool, and then added to the pasta blend.

The pasta samples were processed in a pilot plant used for making pasta from durum wheat semolina (extruder namad S1, Roma, Italy and dryer Tornati Forni, Pesaro, Italy). Two drying cycles, high (HT) and low (LT) temperatures were used for drying spaghetti, whereas short pasta was dried at low temperature as follows:

Operating conditions	HT	LT
Mixing time (min)	15	15
Temperature added water (°C)	40	40
Extrusion pressure (Bar)	120	120
Minimum drying temperature (°C)	85 (1hr)	50 (7hrs 30min)
Maximum drying temperature (°C)	90 (2hr)	60 (30min)
Total drying time (h)	3	8

The amino acid composition of the pasta samples was determined according to the method of Spackman et al. [9]. The pasta samples cooked for 13 minutes were judged by a three member panel of experts who were required to evaluate, using appropriate adjectives, the samples for firmness, stickiness and bulkiness according to the method reported by Cubadda [10]. Total organic matter (TOM) present in washing water of cooked pasta was also determined according to the ICC method No. 153 [7].

3. RESULTS AND DISCUSSION

3.1. Characterization of components of cereals and tubers

Purified starch is generally contaminated with proteins and lipids of different categories. Chemical analysis of isolated starches (Table I) indicated a general high purity even when significant differences were sometimes noticed in the extent of contamination of different samples.

TABLE I. PHYSICO-CHEMICAL CHARACTERISTICS OF FONIO, SORGHUM AND WHEAT STARCH(*)

Starch	Moisture (%)	Ash (% d.m.)	Protein(x) (% d.m.)	Lipids(y) (% d.m.)	Amylose (% d.m.)	Water Binding Capacity (%)
Fonio						
Hothia	15a	0.8a	0.3c	2.8a	22.6c	88b
Koulli	13c	0.4b	0.7a	2.9a	26.1a	80c
Wheat	14b	0.2c	0.4b	2.6b	25.0b	100a
				(z)		
Sorghum						
Dabar	15.14f	0.16f	0.33h	0.75h	27.8f	96f
Milo	14.82g	0.11g	0.36h	1.45g	27.7f	79g
'100'	14.48h	0.13f,g	0.53f	1.32g	22.0g	73h
Wheat	12.44i	0.15f,g	0.43g	1.66f	24.0f,g	100

(*)All values are the means of duplicate or triplicate (A, WBC) determinations. Means within columns with different letters are significantly different ($p<0.05$). Duncan's test.

(x) N x 5.70.

(y) *Cold extraction with water-saturated-butanol.*

(z) Extraction by n-propanol/water (3:1 v/v) at 100°C

Lipids were extracted from sorghum and fonio starch and the respective wheat controls according to two different procedures. The water saturated-butanol extraction used for fonio gave higher lipid content due to varying extent of internal lipid extraction. Significant differences in the amylose content of fonio and sorghum cultivars were observed even though values ranged only between 22.0 to 27.8%.

Starches showed a lower cold water binding capacity than wheat starch due to different proportions of crystalline and amorphous regions within the granules. In particular 'Koulli' fonio starch had less ability to bind water than that of 'Hothia'; Milo and '100 sorghum' had similar w.b.c. (79% and 73%, respectively) whereas 'Dabar' showed the highest value (96%), which was positive, whenever a dough was prepared.

The study of swelling power and solubility behaviour of starches, which indicates the associative forces within the granules, showed that swelling produced disintegration of granular structure. Significant differences were observed in the swelling power and solubility of wheat, fonio and sorghum starches (Tables II and III). Wheat starch swelled gradually from 50°C to 65°C whereas fonio and sorghum starches behaved differently. Up to 65°C, sorghum and fonio starches had lower swelling power values than wheat starch thus indicating stronger bonding forces within their granules. On the contrary their swelling capacity greatly increased from 70°C. At all temperatures, less solutes were released from fonio starch than wheat. This behaviour suggests the existence of strong bonding forces in fonio. Up to 70°C the three sorghum starches were less soluble than wheat starch; Milo cultivar showed the lowest values for both swelling power and solubility.

Differences in the strength of associative forces within the granules were confirmed by the paste characteristics of starches measured by the viscoamylograph. Both fonio and sorghum

starches showed a single step-curve while wheat showed a typical two-step curve at all concentrations. 'Hotia' fonio starch had a higher peak viscosity than 'Koulli' starch.

TABLE II. SWELLING POWER (A) AND SOLUBILITY (B) OF FONIO AND WHEAT STARCHES(°)

(A)	Temperature (°C)								
Starch	50	55	60	65	70	75	80	85	90
Fonio									
Hothia	2.1b	2.1b	2.2c	3.3b	8.9a	12.7b	13.9b	19.5b	25.0b
Koulli	1.8c	1.9c	2.3b	3.0c	8.1b	16.1a	25.0a	31.1a	35.9a
Wheat	3.7a	6.3a	7.5a	8.3a	9.0a	9.6c	10.6c	12.3c	16.0c

(B)	Temperature (°C)								
Starch	50	55	60	65	70	75	80	85	90
Fonio									
Hothia	0.5b	1.0b	1.2b	1.7b	3.4c	4.5c	7.0c	10.5b	12.9c
Koulli	0.1c	0.3c	0.8c	1.0c	5.2a	8.9a	9.7a	11.2a	15.1b
Wheat	0.9a	1.4a	2.9a	4.0a	4.2b	4.8b	7.4b	10.2c	16.7a

(°)All values are the means of triplicate determinations. Means within columns with different letters are significantly different (p-0.05).

TABLE III. SWELLING POWER (A) AND SOLUBILITY (B) OF SORGHUM AND WHEAT STARCHES(°)

(A)	Temperature (°C)								
Starch	50	55	60	65	70	75	80	85	90
Sorghum									
Dabar	1.9	1.9	1.9	1.9	4.4	9.8	13.0	16.6	22.6
Milo	1.8	1.8	1.9	1.9	3.2	9.3	11.2	14.4	20.2
"100"	1.9	2.0	2.1	3.4	7.9	10.1	11.6	15.2	24.1
Wheat	3.2	5.2	6.4	7.1	7.5	7.8	8.6	10.2	13.2

(B)	Temperature (°C)								
Starch	50	55	60	65	70	75	80	85	90
Sorghum									
Dabar	0.4	0.4	0.8	1.0	2.7	7.3	9.3	11.9	16.5
Milo	0.1	0.1	0.8	1.0	1.8	6.2	8.3	11.0	13.2
"100"	0.5	0.5	0.6	1.0	4.3	6.0	7.1	9.7	16.0
Wheat	1.2	2.8	4.2	5.0	6.3	6.5	7.3	9.9	15.7

(°) All values are the means of triplicate determinations.

Peak viscosity at any concentration is a specific feature of a particular starch. 'Hothia' starch exhibited the highest peak viscosity followed by wheat. 'Koulli' starch gave the lowest value. This behaviour could be related to the higher swelling power of 'Koulli' starch at temperatures above 70°C (see Table II). The swollen granules are fragile and disintegrate during continuous stirring in the Brabender bowl, affecting negatively the viscosity of the slurry. Sorghum starches had similar shapes and temperature of initial gelatinization (65°C) but '100' seemed to have a more compact granule structure with a more uniform set of binding forces. Both fonio and sorghum starches showed a high retrogradation tendency on cooling from 95°C to 50°C and a good stability at 50°C.

Fiber content of cassava cultivars studied is reported in Table IV. Data showed high variability between cultivars. However, these differences did not seem to be related to their cooking performance.

TABLE IV. FIBRE CONTENT (% m. b.) OF CASSAVA VARIETIES

Variety	Quality	Duplicates		Mean
ANKRA	Good	4.4	4.2	4.3
AK-T	Good	4.1	3.8	3.9
60142	Bad	4.0	4.1	4.0
ISU-W	Bad	4.5	4.7	4.6
ATRA	Good	6.1	6.2	6.1
518-DB	Bad	5.3	5.6	5.4
30001-W	Bad	5.5	4.8	5.1
ISU-DB	Bad	5.4	5.8	5.6
1425-DB	Bad	5.5	5.7	5.6

In Table V ash and mineral content (Ca, Mg) of tubers and starch samples are listed. Calcium content showed a negative correlation with the tuber quality. In fact the lowest calcium was found in cv. 'Ankra' with good cooking characteristics. However, this hypothesis needs further investigation.

TABLE V. ASH AND MINERAL CONTENT OF CASSAVA VARIETIES

Variety	Techn. Quality	Ash (%)		Ca mg/100g		Mg mg/100g	
		Tuber	Starch	Tuber	Starch	Tuber	Starch
30001W	Bad	2.11	0.36	77.1	34.9	66.8	8.9
ANKRA	Good	1.67	0.30	37.4	16.2	59.2	6.6
91934	Bad	1.35	0.27	40.8	12.8	96.5	18.8
30474	Bad	1.75	0.36	74.8	43.6	91.0	17.2
60142	Bad	2.11	0.30	48.4	14.7	60.7	7.9
ISUW	Bad	1.87	0.33	43.1	20.2	52.0	9.7

3.2. Quality evaluation of foods manufactured with composite flours

The proximate chemical composition of the flours used in the preparation of bread and pasta samples is presented in Table VI.

TABLE VI. PROXIMATE COMPOSITION OF FLOURS AND SEMOLINA USED IN THE PREPARATION OF BREAD AND PASTA[a]

	Triticale Rigel	Bread wheat	Triticale Mizar	Durum wheat	Cassava
Moisture	13.5	13.8	14.3	13.3	14.4
Protein (N x 5.70)	10.9	10.8	7.8	12.5	0.1
Lipids	1.1	1.2	1.1	0.9	0.2
Ash	0.7	0.5	0.7	0.8	0.1
Carbohydrates	73.8	73.7	76.1	72.5	85.2

[a] All values are the means of duplicate determinations

Rheological and functional properties of triticale flour compared to durum wheat semolina and bread wheat flour are listed in Table VII.

TABLE VII. COMPARISON OF RHEOLOGICAL AND FUNCTIONAL PROPERTIES OF TRITICALE FLOURS, DURUM WHEAT SEMOLINA AND BREAD WHEAT FLOUR

	Triticale Rigel	Bread wheat	Triticale Mizar	Durum wheat
Alveograph:				
W ($x10^{-4}$joules)	93.00	134	62.00	169
P/L (mm)	0.84	0.70	1.38	0.98
G (cc)	17.15	21.95	13.50	15.80
Farinograph:				
H_2O absorption %	52.80	58.10	55.20	54.40
Dough development time (min)	1.15	1.301	2.15	
Stability (min)	6.30	10.00	1.15	6.15
Falling number (sec)	169	282	140	360

As far as chemical composition was concerned, cv. 'Rigel' did not show any significant differences from the control bread wheat, although cv. 'Mizar' had a somewhat lower protein content. The cv. 'Rigel' was released by ENEA, Italy in 1987 and is better than cv. 'Mizar'.

Protein content and quality have been reported as the major factors which determine dough strength and loaf volume of triticale varieties. In general, triticale flour is reported to have inferior dough strength than wheat, and its high amylolitic activity has been suggested as one of the reasons for its poor baking performance.

The alveograph results showed 'Rigel' flour was less extensible than bread wheat and both varieties had in general inferior quality than the controls. The comparison of Farinograph results showed that 'Rigel' and 'Mizar' had similar percentage water absorption, dough development time, but 'Rigel' had a somewhat higher value of stability, and was closer to both durum and bread wheat values, indicating good gluten quality. Triticale falling number was considerably lower in both varieties than durum and bread wheat.

From the initial baking trials, a formula was developed to produce triticale and composite flour bread of completely acceptable quality and volume similar to the control. Pan bread was particularly suitable from composite flours with high levels of starchy flour such as cassava. In the latter case, the bread dough used was more similar to a cake batter than to a proper bread dough. The composition and baking qualities of bread is given in Table VIII. Triticale bread compared to the control gave a very high score, being inferior to wheat in volume and colour of crumb only. The panelists noted a greyish tone in the triticale crumb.

TABLE VIII. CHEMICAL CMPOSITION AND BAKING QUALITY OF TRITICALE AND COMPOSITE FLOUR BREAD

Sample	Moisture[b] (%)	Protein[b,c] (%)	Ash[b,c] (%)	Absorption (%)	Loaf volume[d] (cc)	Total score (100 max.)
Control	35.3	7.2	1.7	70	1030	100
100:0	37.7	7.2	1.7	70	975	95
50:50 T+C	37.2	4.0	2.0	75	846	82
45:45:10 T+C+W	37.8	4.1	1.4	75	864	86
40:40:20 T+C+W	37.5	4.2	1.4	75	874	88

[a] T = Triticale, C = cassava, W = bread wheat; [b] Means of duplicate determinations
[c] Values on original moisture basis; [d] Means of triplicates from the same batch.

Amongst the loaves produced, the 50:50 triticale/cassava bread had the lowest total score. Texture was harsher than in 100% triticale bread, grain was less uniform; volume, aroma and taste were negatively affected, and the crumb colour was whiter than in the control. However, the panelists considered this bread to be highly acceptable. As expected, the triticale/cassava bread had the lowest protein content. In order to improve the nutritional qualities of the bread together with volume and texture, blends of triticale/cassava/wheat with 10-20% wheat were tried. The addition of wheat flour produced only a modest increase in protein content and bread volume compared to the triticale cassava bread. Addition of defatted soy flour up to 5% was more effective in restoring the volume and quality characteristics comparable to that of the control and to improve protein content and balance of essential amino acids (data not shown here).

Due to the inferior quality of triticale for pasta making, the influence of novel technologies such as HT drying or addition of pre-gelatinized flour on the cooking quality of

the triticale and triticale-cassava pasta was investigated. In preliminary experiments, we established that spaghetti could be produced with triticale flour only, whereas 35% cassava was the maximum that could be added to the triticale flour.

The composition of triticale and durum wheat spaghetti (Table IX) reflects the starting raw material, durum wheat spaghetti having higher proteins than triticale spaghetti. The amino acid composition of triticale spaghetti showed significantly higher arginine, aspartic acid and lysine compared with durum wheat spaghetti (data not shown here). The larger quantity of lysine in particular is relevant to the nutritional quality of triticale.

TABLE IX. CHEMICAL COMPOSITION OF SPAGHETTI FROM TRITICALE AND DURUM WHEAT SEMOLINA[a]

Components	Durum wheat	Triticale
Moisture (%)	11.1	10.9
Protein (N x 5.70) (%)	12.4	10.8
Available glucides (%)[b]	71.4	73.3
Fat (%)	1.0	1.3
Ash (%)	0.8	0.6
Dietary fibre (%)	3.3	3.1

[a] All the values are the means of duplicate determinations
[b] Obtained by difference

The cooking properties of the pasta samples are summarized in Table X. All samples where cooked in boiling water for 13 min and then judged by a panel of experts. The TOM present in washing water of cooked pasta was also determined. Spaghetti made from 100% triticale flour dried at low temperature gave a low score and a poor quality, while the 100% triticale flour dried at high temperature had the lowest TOM, suggesting aa improved cooking quality. The use of high temperature during drying seemed to improve the cooking quality of triticale spaghetti.

Triticale appeared to be more suitable for the production of short pasta. The pasta from 100% triticale dried at low temperature gave a high score, and was judged good by the panelists. The use of 35% cassava increased the pasta stickiness, lowered the overall rating and reduced quality, but when 5% pre-gelatinized cassava was added, the overall quality improved. However, the use of 10% pre-gelatinized cassava did not result in any positive effect on the cooking quality of pasta.

TABLE X. COOKING QUALITY OF PASTA FROM TRITICALE AND TRITICALE-CASSAVA FLOUR

Sample	TOM*	Firmness	Stickiness	Bulkiness	Overall Rating	Quality verdict
Spaghetti[a]						
LT 100:00 T	4.4	Rare	Very high	High	27	Poor
LT, 90:10 T+PT	4.7	Rare	Very high	Total	13	Poor
HT, 100:00 T	1.7	Insufficient	High	High	40	Mediocre
HT, 90:10 T+PT	4.5	Rare	Very high	Total	13	Poor
Short pasta[a]						
LT, 100:00 T	0.8	Good	Alm. absent	Rare	70	Good
LT, 65:35 T+C	1.9	Insufficient	High	Rare	37	Mediocre
LT, 65:30:05 T+C+PC	0.8	Insufficient	Rare	Rare	53	Fair
LT, 65:25:10 T+C+PC	1.5	Rare	Very high	Very high	20	Poor

[a] LT= low temperature, HT= high temperature, T= triticale, C= cassava, P= pre-gelatinized
[b] g/100g pasta, mean of duplicate determinations.
* TOM= Total organic matter.

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CONTRIBUTION TO THE UNDERSTANDING OF THE COOKING QUALITY OF CASSAVA (*Manihot esculenta* L. Crantz)

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Abstract

Cassava cooking quality was investigated from the mealiness of the cooked roots, and elasticity and freedom from lumpiness of the pounded paste. Microscopic study of the cells of raw and cooked roots showed that the cooking quality was related to the size of the starch granules, and the difference between varieties could be explained on the basis of cell disorganization. Dry matter and starch content were related to the differences in mealiness, and amylose content of the starch to the elasticity and smoothness of the pounded paste. Fibre content was negatively correlated with cooking quality. The loss in cooking quality during rainy season was due more to the reduction of dry matter than starch content. Changes in the gelatinization properties of the starch were also related to the loss in cooking quality. Addition of common salt to fufu paste reduced the retrogradation tendency of the starch and made fufu acceptable for consumption long after its preparation. Application of mulch during the dry season minimized soil temperature fluctuations, maintained high soil moisture, which in turn reduced changes in tuber composition.

1. INTRODUCTION

Cassava is an important starchy staple food in many parts of Africa and other tropical regions. In Ghana, the fresh roots are cooked or pounded into a paste known as 'fufu' and eaten with soup. For the Ghanaian consumer, the mealiness of the cooked roots and the elasticity and smoothness or freedom from lumps of the pounded paste are important cooking quality parameters. Local cassava cultivars, which have the desired cooking quality, are often low yielding and susceptible to many diseases and pests. Some high yielding and improved varieties, developed by the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, were found to lack the cooking quality preferred by the Ghanaian consumer. The main objective of this research was to improve the cooking quality of the IITA lines through mutation breeding. In addition, investigations were carried out to understand the cooking quality of cassava starch to develop simple selection criteria.

2. MATERIALS AND METHODS

Three M_1V_2 populations and a number of selected varieties were investigated for starch quality characters, dry matter, starch content and cooking quality. Microscopic study of the raw and cooked tubers was carried out; starch granule size was measured with a stage and eyepiece micrometer by the method of MacMaster [1]. Dry matter content was measured by drying chips at 80°C to a constant weight in an oven. Starch content was determined as the amount of starch recovered from a known quantity of root tuber. Cell condition and cell disorganization were determined by the method of Shewfelt et al [2]. These parameters were compared with the cooking quality of the varieties and M_1V_2 plants, as judged by a taste panel of cassava consumers.

The study on the proximate composition of cassava roots and starch was carried out at two locations. The cooking quality ranking of two sets of varieties harvested at 6 and 13 months were determined in Ghana, and dry chips were taken to the National Nutrition Institute, Rome, Italy where the rest of the research was carried out. The AOAC [3] methods of analysis were used to determine the fat, protein, ash and fibre contents of the samples. The acid hydrolysis method as described by Rickard and Behn [4] was followed to determine starch content. Amylose content was determined by the iodine calorimetric method as described by McCready and Hassid [5].

The changes in viscosity during starch gelatinization and its relationship to cooking quality of the cassava varieties was determined. This was undertaken at the National Nutrition Institute, Rome, Italy. Both the hot paste viscosity and swelling power and solubility of the starch were studied. To determine hot-paste viscosity, a starch suspension (70g l^{-1}, 500 mi) was placed in the cup of the standard Brabender amylograph and heated from 50°C to 95°C, and held for one hour, then cooled to 50°C and held for an hour. The heating rate was 1.5°C min^{-1} and the bowl speed was 75 rev min^{-1} using 700cm g^{-1} measuring cartridge. The flour was run at two concentrations of 70 and 100g l^{-1}.

Swelling power (SWP) and solubility were determined by the method of Schoch [6] using 1 g starch (db) suspended in 50 ml distilled water in 75 ml centrifuge bottles and varieties Ankra, 91934 and 30474 which showed distinct differences in their amylograms were analyzed between 60-95°C at 5°C intervals. All measurements were made in triplicate.

3. RESULTS AND DISCUSSION

3.1. Granular character of starch and cooking quality

In the M_1V_2 population, plants with mealy tubers had large starch granules, while non-mealy types had small granules [7]. There was a strong correlation between mealiness, starch and dry matter content and the size of the starch granules. Similar results have been reported in mealy potato varieties which have relatively large starch granules [8,9]. It seems that the gene(s) controlling mealiness were altered by mutagenic treatment and reflected in the size of the starch granules. This supports the observation [10] that mealiness in potato is under a simple genetic control. In potato, mealiness has been explained on the basis of cell separation; that is a loosening of the pectic intercellular substances leads to the freeing of the cooked cells. When the cells are not easily loosened, the cooked product becomes non-mealy, hard or glassy. The mealiness in cassava may also be explained on the basis of cell disorganization or separation.

3.2. Composition of cassava roots and starch and its relationship to cooking quality

The proximate composition of the roots and starch showed significant difference between varieties harvested at the same age and those harvested at different ages (Tables I and II). Even though many of the correlation coefficients between the components and cooking quality rankings were not statistically significant (Table III), the magnitude of the coefficients were high in many cases. The study showed that the mealiness of the cooked root depends on its starch content. While the elasticity and smoothness of the pounded paste depends largely on the amylose content of the starch. The varieties with high fibre content produced roots with unacceptable cooking quality. The ash and protein contents were the only minor components that were related to cooking quality, which may have implications in fertilizer application in cassava.

TABLE I. PROXIMATE COMPOSITION OF 6-MONTH OLD CASSAVA ROOT (SET B).

VARIETY	STARCH CONTENT gK-1g	AMYLOSE CONTENT OF STARCH (%)	ASH CONTENT gK-1g	FIBRE CONTENT gK-1g
ANKRA	684	20.9	12.2	42.9
AKOSUA TUNTUM	662	22.6	16.5	39.3
60142	680	18.6	16.9	40.6
ISU-W	662	22.0	13.6	45.6
ATRA	665	20.8	21.1	61.2
518-DB	677	21.8	13.5	54.5
3000-W	668	20.9	16.7	51.5
ISU-DB	668	20.3	17.7	55.6
1425-DB	660	20.1	15.8	55.9
LSD (5%)	9.49	1.53	0.8	5.9
LSD (1%)	12.7	2.04	1.1	7.9
CV	0.70	3.63	2.61	6.04

3.3. Cassava starch gelatinization and its rheological properties

There was difference among the varieties in gelatinization or pasting temperature. Moorthy [11] in India observed similar varietal differences. The gelatinization range, estimated as the difference between the temperature of peak viscosity and pasting temperature also varied among the varieties. The peak viscosity also differed among the varieties. This study showed that the varieties differed in the rate and extent of swelling of starch granules. According to Tipples [12], the viscosity at 95°C in relation to the peak viscosity reflects the extent of fragility of the swollen granules, which varied among varieties.

In most cases, correlation between cooking quality scores and points on the amylograph were not statistically significant (Table IV), suggesting that it is difficult to predict cooking quality using the amylograph, confirming previous reports [13]. However, three varieties which differed in their cooking quality showed very distinct differences in their hot-paste viscosities. The variety 91934 had one of the highest peak viscosities and narrowest gelatinization range; its starch granules swelled very rapidly. However, it had the most fragile swollen granules, especially in the flour, and at the end of the 95°C hold, it was no longer a viscous paste. It did not have a good cooking quality. The variety 30474 had the lowest peak viscosity and a relatively wide gelatinization range. This implies that its starch granules swelled very slowly because of strong bonding forces between the granules. It is also not a good cooking variety. The variety Ankra which was the best cooking variety had the highest peak viscosity, and at 95°C, it still produced the most viscous paste.

The differences in the swelling ability of the starch granules and the fragility of the swollen granules were confirmed in the swelling power and solubility studies. The SWP and solubility of the starch at 85°C are shown in Table V. They demonstrate large differences between the varieties. The SWP of an aqueous suspension indicates the strength of the hydrogen bonding between the granules, and this was reflected in the differences observed between the varieties. This became even more evident when the SWP and solubility of varieties 91934, 30474 and Ankra were studied over a range 60-95°C. The results showed that Ankra, the best cooking varierty had granules which did not swell and break down as rapidly as in 91934. The

SWP and solubility studies revealed that for good cooking quality a gradual swelling of the starch granule on heating is desirable. Too rapid swelling with weak bonding forces between granules results in poor cooking quality. Whereas, the proximate composition and the gelatinization properties of the starch may help to explain the difference in cooking quality between cassava varieties, none seems to provide a simple method of objective assessment for selection.

TABLE III. CORRELATION BETWEEN COOKING QUALITY AND PROXIMATE COMPOSITION (SET A).

	MEALINESS	ELASTICITY	SMOOTHNESS
Ash Content of Starch	0.80 *	0.85 *	0.68 ns
Ash Content of Root	0.83 ns	0.55 ns	0.30 ns
Fat Content of Starch	-0.06 ns	-0.36 ns	-0.18 ns
Fat Content of Root	0.03 ns	-0.43 ns	-0.14 ns
Protein Content of Starch	0.64 ns	0.83 *	0.68 ns
Protein Content of Root	0.81 *	0.35 ns	0.71 ns
Starch Content of Root	0.64 ns	0.33 ns	0.36 ns
Amylose Content of Starch	0.22 ns	0.45 ns	0.51 ns
Fibre Content of Root	-0.45 ns	0.17 ns	-0.49 ns
SET B			
Starch Content	0.61 ns	-0.10 ns	0.30 ns
Amylose Content	0.09 ns	0.58 ns	0.33 ns
Ash Content of Root	-0.12 ns	-0.09 ns	0.03 ns

ns Not significant.
* 5% Level of significance.

3.4. The Effect of common salt on hot-paste viscosity of starch

In Ghana, housewives add salt to the pounded starch paste or 'fufu', if a family member is delayed for meal. If this is not done, the paste becomes hardened, loses its elasticity and becomes watery. It is reported that the addition of 2.5% NaCl to wheat starch increased its peak viscosity [14] by enhancing the "granule integrity" of the starch; the granules swelled and remained intact for a long time before fragmentation occurred.

The solutions of 0.43 M, 0.86 M and 1.29 M (2.5, 5.0 and 7.5%) salt (NaCl) were used. Thirty-five grams (db) cassava starch was dissolved in solution, and the standard Brabender procedure was followed. The starch and flour of the three varieties, 'Ankra', 91934 and 30474 were studied. The viscosity changes in the starch and flour in the presence of common salt showed that both the pasting temperature and the temperature of peak viscosity increased (Tables VI), suggesting that salt affected the rate of swelling of the granules. More energy is required to gelatinize the starch granules in the presence of salt. The effect of salt on the hot-paste viscosity changes differed among the varieties. In the varieties 91934 and 30474, high salt concentrations tended to increase the peak viscosity and viscosity at 95°C, but in cv. 'Ankra', there was a reduction in viscosity with increase in salt concentration.

TABLE IV. CORRELATION BETWEEN COOKING QUALITY AND PASTING CYCLE.

	Viscosity changes during gelatinization				
Cooking quality parameters	Peak viscosity	Viscosity at 95°C	Viscosity after 95°C hold	Viscosity at 95°C	Viscosity after 50°C hold
Set A Starch (70 gl^{-1})					
Mealiness	0.16	0.35	0.88	0.74	0.76
Elasticity	-0.39	-0.16	0.44	0.40	0.45
Smoothness	-0.07	0.09	0.63	0.48	0.49
Set A Flour (70 gl^{-1})					
Mealiness	0.45	0.96 **	0.91 *	0.92 **	0.92 **
Elasticity	-0.26	0.62	0.64	0.62	0.64
Smoothness	0.37	0.93 **	0.89 *	0.83 *	0.87 *
Set B Starch (70 gl^{-1})					
Mealiness	-0.02	0.25	0.06	0.15	0.22
Elasticity	0.28	0.40	0.06	0.11	0.19
Smoothness	0.18	0.18	-0.23	-0.12	-0.06
Set B Flour (70 gl^{-1})					
Mealiness	-0.36	-0.34	-0.23	-0.25	-0.22
Elasticity	0.12	0.18	0.35	0.38	0.37
Smoothness	-0.18	-0.29	-0.07	-0.01	-0.07

* 5% level of significance.
** 1% level of significance.

The increase in viscosity when the paste is cooled to 50°C shows the extent of retrogradation of the starch paste. In all varieties, salt reduced retrogradation as compared to the control. Retrogradation leads to increased rigidity of the starch gel as a result of re-association of the starch granules upon cooling, which leads to release of water called syneresis. Therefore, when the consumption of 'fufu' is delayed, retrogradation of the starch takes place, making the paste no longer acceptable. The observed decline in the extent of retrogradation in all varieties with the addition of common salt showed that what the Ghanaian housewife has been practising is to reduce the retrogradation tendency of the pounded paste in order to extend the "table-life" of 'fufu'.

3.5. Effect of harvest time on cooking quality

In Ghana, there is loss in the cooking quality of cassava with the onset of rainy season in March/April. Two local varieties with acceptable cooking quality, 'Ankra', 'Atra' and two introductions 91934 and 30474, from IITA with poor cooking quality, were investigated. Harvesting began at 7 months and continued monthly until 13 months. Harvesting was planned to begin in December in the dry season and end at the peak of the rainy season in June. At each harvest, the cooking quality was determined by a taste panel, and the dry matter and starch contents were determined. The SWP and solubility of the starches were also determined. Starch

samples were sent to the Natural Resources Institute, Chatham Maritime, United Kingdom where the hot-paste viscosities were determined using the standard Brabender. From the tenth month onwards, which coincided with the beginning of the rains, there was a drop in the cooking quality of all the varieties. The cooking quality of the introduced varieties were completely unacceptable at this period. Dry matter content of all the varieties was at its lowest during this period, but there was no distinct drop in the starch content. The swelling power of the starch, however, showed a distinct change in the 9th and 10th months. Since the drop in cooking quality of the varieties coincided with the drop in the dry matter content rather than the starch content, it was concluded that changes in the dry matter content had a more direct effect on cooking quality than changes in starch content. However, the changes in the gelatinization properties of the starch at the onset of the rains may also explain the changes in cooking quality. It has been postulated that the resumption of growth after the dry season may be the main reason for the drop in dry matter content [15]. Since a change in cassava root dry matter content is directly related to the change in cooking quality, root dry matter content may be an important selection criterion for improved cooking quality.

TABLE V. SWELLING POWER AND SOLUBILITY AT 85°C.

Variety	Swelling Power	Solubility (%)
SET A		
ANKRA	33.60	22.46
91934	47.79	31.12
ISU-W	25.96	19.31
30001-W	22.48	21.12
30474	21.71	19.96
60142	27.11	17.69
LSD (5%)	9.93	1.08
LSD (1%)	13.36	1.45
SET B		
ANKRA	24.63	16.33
AD-T	34.96	25.23
ATRA	32.13	20.84
60142	27.21	22.57
ISU-W	38.44	20.49
518-DB	33.31	25.58
30001-W	31.94	27.02
ISU-DB	35.94	19.39
1425-DB	34.51	25.15
LSD (5%)	1.46	0.75
LSD (1%)	1.96	1.00

3.6. Effect of mulching on cooking quality

The local variety 'Ankra' was used to study the effect of mulching on cooking quality, and dry *Panicum maximum* mulch was applied at 3, 6, 9 and 12 tons/ha at the beginning of the dry season, and compared with clean weeding throughout the study and no weeding from the

TABLE VI. VISCOSITY CHANGES OF STARCH DURING GELATINIZATION IN THE PRESENCE OF NaCl.

VARIETY	SALT CONC'N	PASTING TEMP °C	TEMP OF PEAK VISC	PEAK VISC*	VISC AT 95°C	VISC AFTER 1 HR AT 95°C	VISC AT 50oC	VISC AFTER 1 HR AT 50°C
	0	74	82°C	560	460	260	480	420
	0.43M	75.5	89°C	360	340	150	220	180
ANKRA								
	0.86M	74	92°C	300	300	160	200	160
	1.29M	79.3	93.5°C	360	360	200	300	260
	0	74	77°C	500	380	145	280	240
	0.43M	75.5	81.5°C	460	380	30	50	40
91934								
	0.86M	77	84.5°C	500	440	60	90	80
	1.29M	77.8	87.5°C	560	500	100	140	100
	0	71	85°C	340	290	140	280	260
	0.43M	78.5	92°C	340	270	110	160	120
30474								
	0.86M	81.5	95°C	380	380	180	260	200
	1.29M	81.5	95°C	380	380	260	340	280

* Brabender viscosity units (b.U.).

beginning of the dry season. Soil temperature, soil moisture, infiltration rate, bulk density, organic matter content and the amounts of Ca, Mg, K and available P in the soil were recorded at the beginning and end of the experiment. The harvesting was started at the end of the dry season in March when the plants were 10 months old and the final harvest was after the onset of the rains when the plants were 12 months old. The cooking quality, dry matter and starch contents were determined at each harvest.

The no-weeding treatment had the highest dry matter and starch content at 12 months growth and produced some of the most mealy cooked roots and elastic paste. The clean-weeding treatment had the lowest cooking quality. The dry-grass mulch treatment produced better cooking roots than the clean-weeding treatment, though the differences in most cases were not statistically significant (data not shown). The difference between the highest soil temperature (measured at 15:00 hrs) and the lowest (measured at 09:00 hrs) was largest for the bare plots and decreased as the amount of mulch increased. The large diurnal soil temperature fluctuations on the clean-weeded plots may have indirectly affected the root cooking quality through the daily warming and cooling of the root tubers in the soil. Scaramelia-Petri [16] found that in Italy, when the early summer was very warm and dry, the centres of potatoes remained firm after cooking. Infiltration rate increased with increased mulching. Consequently the cumulative soil moisture content, measured fortnightly till the end of the dry season showed that more water was conserved in the soil as the amount of mulch increased. Root dry matter and starch contents were negatively correlated with soil temperature and positively with soil moisture (data not shown). High soil temperatures decreased root dry matter and starch content, and these in turn adversely affected the cooking quality. Similarly, water stress during the dry season leads to a decrease in dry matter and starch content, and has adverse effect on root cooking quality. The practice to leave land unweeded at the beginning of the dry season to prevent deterioration in cooking quality may be instrumental in decreasing moisture stress.

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GENETIC IMPROVEMENT OF SWEET POTATO THROUGH SOMATIC EMBRYOGENESIS AND *IN VITRO* INDUCTION OF MUTATIONS

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Abstract

Mutation breeding is a promising option for the genetic improvement of sweet potato. Callus induction, somatic embryogenesis and plant regeneration was investigated in twenty-two sweet potato varieties of different origin. Plant regeneration was found to depend on the genotype and composition of the induction medium. The regeneration through somatic embryogenesis induced morphological and physiological changes among the regenerated plants. The irradiation with 30 to 50 Gy of meristems before culture on induction medium inhibited somatic embryogenesis. A number of accessions were evaluated in field trials and showed wide differences in yield.

1. INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam.) ranks sixth among the world's most cultivated crop plants, representing an important food source in tropical and temperate regions. Its cultivation for starch production and industrial use has been recently proposed [5]. Sweet potato is a difficult species from a breeding view point - it is highly heterozygous, hexaploid (2n=90) and frequently self and/or cross-incompatible. Flower and fruit set is low, especially in temperate zones. Hence, mutation breeding is an interesting option for the genetic improvement of this crop. Regeneration through somatic embryogenesis (SE) has been suggested as a possible method to regenerate plants from single cells [2, 3] and to avoid or overcome chimerism in mutation breeding programs. It has been also proposed that somatic embryos can be used to produce synthetic seeds of sweet potato [1]. This papers reports the results of a number of experiments aimed to develop methods for mutation induction in sweet potato through irradiation of meristems and induction of somatic embryos from the irradiated meristems. The results on the evaluation of a germplasm collection are also reported.

2. MATERIALS AND METHODS

Twenty-two sweet potato varieties of different origin (Table I) were tested for their potential to regenerate plants through somatic embryogenesis, using callus induction media which contained different levels of 2,4-D (1.4, 2.3, 4.5 and 9.0 μM or 0.3, 0.5., 1.0 and 2.0 mg/l), as described by Sonnino and Mini [6].

3. RESULTS AND DISCUSSION

3.1. Induction of somatic embryogenesis

The formation of embryogenic callus was dependent on the genotype and on the induction medium. Each genotype formed embryos only with a specific concentration of 2,4-D (Table II). Bud meristems of 16 clones formed globular and heart-shaped structures either directly on their surface or indirectly on derived calli after culture between 10 to 40 days. A

further development of the somatic embryos up to plantlets was easily obtained in 14 genotypes by laying the embryogenic calli onto a medium free of 2,4-D and cytokinins (Table III).

The frequency of embryogenic calli was ten times higher from meristems (60%) than intact buds (6%). Small pieces of embryogenic callus and isolated embryos (early and late torpedo, or cotyledonary stage) when subcultured on 2,4-D containing medium produced several secondary embryos.

TABLE I . GENOTYPES OF SWEET POTATO USED FOR SOMATIC EMBRYOGENESIS.

	Genotypes	Source Institute	Country of origin	Description
1	Excel	USDA	USA	USDA: US Department of agriculture
2	C 42	USDA	USA	US Vegetable lab., Charleston, South Carolina.
3	S 2	USDA	USA	
4	S 3	USDA	USA	
5	LT	NRI	Vietnam	N.C.St.Un.: North Carolina State University
6	TDL	NRI	Vietnam	Releigh, North Carolina.
7	196-20 E	N.C.St.Un	-	
8	1135-B	N.C.St.Un	-	AVRDC: Asian Vegetable Research and
9	1508-G	N.C.St.Un	-	Development Center, Tainan, Taiwan.
10	1560-E	N.C.St.Un	-	
11	1525	N.C.St.Un	-	NRI: Nuclear Research Institute,
12	W 190-F	N.C.St.Un	-	Dalat-Vietnam.
13	239-4 C	N.C.St.Un	-	
14	288-6 B	N.C.St.Un	-	
15	CN 1489-89	AVRDC	Taiwan	
16	508 508	USDA	Japan	
17	508 510	USDA	Korea	
18	Q 23728	USDA	Costa Rica	
19	Q 23834	USDA	Costa Rica	
20	Q 23836	USDA	Costa Rica	
21	Q 23837	USDA	Costa Rica	
22	Q 25005	USDA	Peru	

3.2. Assessment of somaclonal variation

To verify occurrence of somaclonal variation, morphological traits on a sample of 81 regenerated plants and 6 control plants of Q 23728 clone were studied. Of the 81 plants, 45 had been micropropagated before. A total of 126 plants plus 8 control plants were tested. Plants were transferred in pot from test tubes, and after a few months transplanted in a field at one plant per square meter, and standard agronomic practices were applied during growing season. For evaluation, descriptors for sweet potato reported by Huamàn [4] were used.

For 9 out of 17 morphological characters studied, there was no difference between the regenerated and control plants. For the other 8 characters (abaxial leaf vein pigmentation; foliage colour in mature and immature leaf, petiole pigmentation, predominant and secondary

skin colour of storage root, predominant flesh colour of storage root and distribution of secondary flesh colour of storage root) some variations were observed. The coefficient of variation for these characters ranged from 33.5% (abaxial leaf vein pigmentation) to 1058.3% (secondary skin colour of storage root). Observations on flowers were not possible because flowering did not occur. Six out of 45 micropropagated plants showed a different secondary flesh colour. This difference may be due to chimerism induced during ontogenesis. If this is the case, somatic embryos did not originate from single cells.

TABLE II. EFFECT OF DIFFERENT CONCENTRATIONS OF 2,4-D ON CALLUS INDUCTION AND SOMATIC EMBRYOGENESIS FROM MERISTEM EXPLANTS OF SWEET POTATO.

Clone	2,4 - D levels (mg/l)			
	0.3	0.5	1	2
239-4 C	-	-	*	* * *
288-6B	-	*	©	* *
1058-G	-	-	©	* *
1560-E	-	-	*	©
508 510	*	©	* *	* *
S2	*	©	* *	* * *
Q 23728	-	-	©	©
Q 23834	-	-	©	*
Q 23836	-	-	*	©

- : whitish, non-watery callus
* : whitish or brownish, non-watery callus
* * : brownish, watery, dispersed callus
* * * : very brown, watery, dispersed callus
© : embryogenic callus

The results suggested that regeneration through somatic embryogenesis induced morphological and physiological changes. This may have important implication in the propagation and breeding of sweet potato.

3.3. Effect of γ-rays irradiation on somatic embryogenesis

Table IV shows the frequencies of somatic embryogenesis (SE), recorded after 30 days of culture from meristems of three clones (Q 23728, Q 23834 and 239-4 C) treated with increasing doses of γ-rays (Co^{60}, 156 rad/min.). It was evident that as the dose increased, the percentage of explants which formed SE tended to decrease. Among the genotypes tested, the 239-4C seemed to be the most radio-sensitive. The effect of γ-rays between 0-20 Gy was not detected; however doses of 30-50 Gy clearly inhibited SE in clone Q 23728 and 23834, and strongly in clone 239-4C. Other criteria such as the dimension and mean number of globular structures per explant were not affected by irradiation (data not shown). Based on these preliminary results, it appears that

TABLE III. EMBRYOGENIC POTENTIAL OF SWEET POTATO GENOTYPES.

Genotypes	2,4 - D level (mg/l)	mean time for embryogenesis (days)	% explant with embryoids	mean number of germinated "seedlings" per explant
C 42	1	28	15. 00	1.47
"	10	10	10. 00	0.98
Excel	2	20	15. 00	1.30
S 2	0.5	40	21. 00	0
S 3	1	-	0. 00	0
"	2	-	0. 00	0
196-20	1	-	0. 00	0
1135-B	2	30	47. 10	0
1508-G	1	30	11. 10	1.55
1560-E	2	15	75. 00	6.80
1525	0.5	-	0. 00	0
W 190 F	1	40	50. 00	2.34
"	2	40	16. 70	1.87
508 508	2	-	0. 00	0
508 510	0.5	40	15. 40	1.5
239-4 C	1	15	25. 00	1.5
"	2	-	0. 00	0
288-6 B	0.5	-	0. 00	0
"	1	30	47. 70	3.57
Q 23728	1	15	37. 90	4.64
"	2	15	60. 00	4.57
Q 23834	1	15	42. 30	-
Q 23836	1	15	35. 30	3.01
Q 23837	1	-	0. 00	0
Q 25005	0.5	-	0. 00	0
TDL	1	23	27. 00	3.22
LT	0.5	30	18. 00	1.08
1489-89	1	15	27. 00	5.00

the radio-sensitivity of meristems with respect to their SE was more or less similar to that obtained in experiments on the effect of γ-rays on *in vitro* micro-cultured plants. In other words, the suitable mutagenic dose for meristem explant could be between 30 to 50 Gy γ-rays.

TABLE IV. EMBRYOID FORMATION (%) IN 3 SWEET POTATO GENOTYPES ACCORDING TO DIFFERENT DOSES OF GAMMA-RAYS.

Clone	Dose (Gy)				
	0	10	20	30	50
239-4C	25	14	10	11	0
Q 23728	56	53	52	32	13
Q 23834	42	43	43	30	9

3.4. Evaluation of germplasm

A number of accessions were tested in a field trial for morpho-physiological characters according to the reported descriptors [4] and for agronomic traits. The average yield of storage root was of 3.15 kg per plant, and ranged between 0.5 to more than 7 kg per plant (Table V). The mean dry matter production was 0.81 kg/plant. It must be emphasized that several genotypes were able to yield more than one kilo of dry matter per plant, and some accessions

TABLE V. AGRONOMIC EVALUATION OF THE GENOTYPE COLLECTION

	1992			1993		
VARIETY	YIELD (kg/plant)	D.M. (%)	D.M. (kg/plant)	YIELD (kg/plant)	D.M. (%)	D.M. (kg/plant)
508506				2.50	34.00	0.85
508507	1.26	29.20	0.37			
508508	1.31			3.75	28.70	1.08
508514	2.28			3.13	27.50	0.86
508519	0.82	28.60	0.23	1.68	22.10	0.37
508532	0.18			0.80	23.60	0.19
Q 23707	0.27					
Q 23835	0.20					
EXCEL	1.15	20.00	0.23	3.76	25.60	0.96
SUMOR	1.14	21.80	0.25			
W 190 B	1.04	34.80	0.36			
W 190 F	0.25	34.00	0.09	1.90	29.00	0.55
239-4c	0.60			7.35	28.10	2.07
239-5b	0.33			6.50	25.50	1.66
288-6b	3.30	21.60	0.71	1.90	23.50	0.45
NC-317D	0.15			2.30	22.40	0.52
1135 B				2.00	21.10	0.42
1560 E	2.70	19.80	0.53	4.32	19.20	0.83
1582 E	0.98	20.60	0.20	1.77	26.60	0.47
2532	0.28	23.00	0.06	5.80	25.32	1.47
2534	2.40	15.40	0.37	5.00	24.00	1.20
2544				1.30	22.30	0.29
CN 1108-13				5.10	20.80	1.06
CN 1345-8				4.15	27.60	1.15
CN 1489-43	0.07	29.20	0.02	4.33	30.80	1.33
CN 1489-89	1.50	26.20	0.39	2.87	31.70	0.91
CN 1656-37	0.50	26.00	0.13	0.90	25.80	0.23
Latina				2.15	35.00	0.75
Leccese				2.32	22.90	0.53
Calabrese				0.51	22.05	0.11
R 71				1.25	24.26	0.30
O 24				6.75	25.60	1.73
L 5				2.21	20.50	0.45
TOTAL				3.15	25.56	0.81

produced almost two kilos. These yields are of interest for the production of food and industrial starch. The morpho-physiologic study showed a wide range in the genetic variability (Table VI), which can be of great interest in breeding.

TABLE VI. BASIC MORPHO-PHYSIOLOGICAL DESCRIPTION OF GENOTYPE COLLECTION

Traits	MINIMUM	MAXIMUM	AVERAGE	S.D.	C.V.
Plant habit	5	9	6.80	1.32	0.20
Soil coverage	3	9	6.23	1.88	0.30
Mature leaf shape	2	7	4.53	1.31	0.29
Leaf lobe type	0	7	3.10	2.01	0.65
Leaf lobe number	0	6	3.23	1.63	0.51
Shape of central leaf lobe	0	7	2.73	1.80	0.66
Mature leaf size	3	5	4.93	0.37	0.07
Abax. leaf vein pigmentation	2	7	4.00	2.20	0.55
Mature leaf colour	2	6	2.17	0.75	0.35
Immature leaf colour	1	9	3.23	2.89	0.89
Petiole length	1	7	4.67	1.40	0.30
Petiole pigmentation	1	9	3.43	2.36	0.69
Storage root shape	1	9	5.47	2.71	0.50
Predominant skin colour	2	9	6.03	2.66	0.44
Intensity of predominant skin colour	1	3	1.93	0.83	0.43
Secondary skin colour	0	9	0.57	2.16	3.81
Predominant flesh colour	1	8	3.30	2.42	0.73
Secondary flesh colour	0	9	2.43	2.42	0.99
Distribution of secondary flesh colour	0	9	4.63	3.96	0.86
Root D.M. (%)	19.20	35.00	25.45	4.02	0.16
Yield (kg per plant)	0.51	8.50	3.30	2.14	0.65
Average root weight (g)	54	800	276.10	187.95	0.68

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CONCLUSIONS AND RECOMMENDATIONS

GENERAL

The participating scientists in this CRP obtained promising results for the improvement of cassava, yam, sweet potato, plantain, sorghum and African rice. For example, several stable mutants of sorghum with long panicle length, improved grain quality and drought tolerance were obtained in Mali. Presently, these mutants are being evaluated in multi-location field trials in co-operation with ICRISAT in Mali. In African rice (*Oryza glaberrima*), several mutants with white caryopsis were developed, five of which are being multiplied for multi-site field trials. White grained varieties have a higher market value than the normal red grain types. In the United Republic of Tanzania, mutants of upland rice (*Oryza sativa*) with increased yield, early flowering and short height were developed, and are in advanced field trials.

In cassava, two mutants were selected with improved cooking quality for the production of fufu, the traditional native dish in Ghana. The mutants have large starch granules in the tubers, high tuber yield, and resistance to African cassava mosaic disease (ACMD). These mutants are being tested in multi-location yield trials. Several local cultivars of Bambara groundnut were collected to conserve genetic diversity and use for breeding of determinate types. In Congo, good progress was reported to induce resistance to leaf-rust disease in basella (*Basella alba* and *B. rubra*), a local leafy vegetable.

The basic research on some of the crops which are important in the African diet was supported by the technical research contract holders in the Italian institutions and the Agriculture and Biotechnology Laboratory, Seibersdorf. These studies made a significant contribution toward developing improved techniques for *in vitro* culture of cassava, banana, potato, sweet potato and yams. *In vitro* techniques in combination with irradiation induced mutations are an efficient tool to obtain variation in crops which are propagated from vegetative parts. An efficient method was developed for regeneration through somatic embryogenesis in sweet potato and adventitious apical and axillary bud proliferation in cowpea (*Vigna unguiculata*). Studies on the organoleptic properties of cassava starch and that of bread and pasta made from mixtures with triticale and wheat flour suggested that it is possible to diversify food products of cassava. Other studies showed that it is possible to transfer DNA by electroporation of plasmids into nodal meristems in planta. The research investigations in the Agriculture and Biotechnology Laboratory, Seibersdorf, revealed that the origin of somatic embryos in cassava is multi-cellular. The studies on chitinase activity in banana clones showed that these enzymes might be involved in resistance to *Fusarium* wilt disease.

RICE

Achievements: Several mutants which combine short height and high yield were obtained from the varieties grown as upland rice in the United Republic of Tanzania.

Recommendations: Experiments to determine the planting density and other agronomic practices of the materials originating from the mutation programme should be conducted using the existing national rice research programme. On-farm trials should be initiated. Some selected farmers should be given seeds of short-statured mutants to grow them according to their normal farm practice along with their own varieties.

To study the fertilizer response of the short-statured mutants, fertilizer experiments based on ^{15}N isotope should be undertaken.

The possibility of testing the mutants in other African countries should be sought in collaboration with other scientists in the region. Efforts should be made to test the mutants in national variety trials and multi-location trials.

The harvest index together with grain quality characteristics of the mutants should be determined. Cross breeding between mutants and mutants × parent should be initiated to obtain recombinants and also to study the inheritance of semi-dwarfism. The possibility to characterize mutants and other local cultivars through molecular markers and probes should be explored in collaboration with laboratories which have facilities to carry out this type of research.

PIGEONPEA

Achievements: Induction of mutations was initiated in pigeonpea to seek high yielding, early maturing mutants with resistance/tolerance to common insect pests and diseases. Some promising high yield mutants were identified in KAT 60/8 (T1P58; T3P28) and in KAST E31/4 (T2P12). Some lines also showed tolerance to *fusarium* wilt. However, little success has been achieved for tolerance to insect pests.

Recommendations: Promising mutants should be tested in different locations to identify those which perform better under different environmental conditions and various input levels. A sufficiently large number of early maturing cultivars should be irradiated and M_2 progenies further evaluated for insect pest tolerance, together with the control populations. Crosses should be made between early maturing Indian type and local pigeonpea varieties. F_1 seeds should be irradiated, and selection should be carried out in the subsequent segregating populations. Exchange of germplasm and elite lines with resistance to insect pests and diseases between ICRISAT and the National Program should be continued. The present seed storage facility for breeders should be upgraded.

COWPEA

Achievements: Regeneration protocols were developed at the University of Naples which can be used in further genetic transformation experiments. The herbicide thidiazuron induced multiple bud cluster formation in the apical and cotyledonary node buds in different cowpea lines. Preliminary genetic transformation experiments (pDNA electroporation into nodal meristems) carried out at the University of Naples showed that transgenic progenies were obtained in cowpea.

Recommendations: Breeding for insect resistance (such as pod borers, pod suckers, seed borers, thrips) is the first priority in cowpeas. However, no cross compatible wild germplasm with such traits has been found to date. In order to get transgenic cowpea lines expressing insect resistant genes (i.e. a-amylase inhibitor), experiments should be continued to improve the *in vivo* electroporation protocol of DNA into nodal meristems and *in vitro Agrobacterium* co-culture of microprojectile bombarded embryos.

BASELLA ALBA AND B. RUBRA

Achievements: Rust caused by *Uromyces bassellae* is the major leaf disease of basella, which damages the commercial and nutritional value of the plant. More than 8000 seeds of the sexually propagated type were irradiated with 400 Gy. The material is in M_2 generation. Reasonable research was carried out to understand the mechanism of the development of the disease. At the Agriculture and Biotechnology Laboratory, a study was conducted to determine the chromosome number of the vegetatively propagated green leaf type. The chromosome number varied from 2n = 36–80. The commonly grown Congo flowering type and a Sri Lankan introduction were found to have 44 and 42 chromosomes, respectively. It was also established that seeds from the red leaf types segregate for both green and red leaves and that under moisture stress situations, the vegetative types sometimes flower and form seeds. However, these seeds did not germinate.

Recommendations: There is a need to introduce germplasm from the surrounding African countries and Asian countries to Congo to widen the genetic variability in these species. A conventional breeding programme involving hybridization should be initiated to generate variation along with mutation induction. It is recommended that research should be continued to create variation, develop mutant resistance to rust disease and obtain changed leaf colour from red to green in the asexually propagated cultivars. There is a need to understand the taxonomy of this species — hence cytotaxonomic studies on different ecotypes are recommended.

SWEET POTATO

Achievements: Sweet potato is recognized as a crop with great potential for solving some of Africa's food problems. The International Potato Center (CIP, Lima, Peru) is already working to improve this crop. Plant regeneration from somatic embryogenesis was developed at ENEA and can be used in combination with radiation induced mutagenesis for the improvement of sweet potato.

Recommendations: It is recommended that African scientists interested in the improvement of this crop should establish contacts with CIP and ENEA. They should develop links with the National Institute of Nutrition, Rome for its industrial use.

POTATO

Achievements: *In vitro* techniques to select potential varieties resistant to late blight in potato were investigated. This method would be of value to select mutants for disease resistance.

Recommendations: Studies should be initiated to elucidate the mechanism and the durability of resistance to late blight provided by glandular trichomes. Studies should be undertaken to obtain recombinants between *Solanum tuberosum* and wild species (such as S. *berthaulti*), that retain the agronomic traits of the cultivated types while being resistant to late blight. Efforts should be made to also achieve the above results in varieties suited for African climatic conditions. To obtain the above objective, both mutation induction and plant regeneration from callus cultures are recommended as useful tools.

CASSAVA

Achievements: Mutants of cassava with improved cooking quality which combine resistance to African cassava mosaic disease were identified. The mutants are particularly suited for making a high quality fufu.

Recommendations: To release the new mutant variety, multi-location field trials should be conducted on large plots. At the same time, on-farm validation trials should be carried out to assess the performance of the mutant under farm conditions. The effect of management practices, such as fertilizer application and inter-cropping on yield and cooking quality of the mutants and other cassava varieties should be studied. The nitrogen studies should involve the use of ^{15}N fertilizer. Inter-cropping studies involving the mutant should be undertaken. This may include cassava/maize/cowpea systems.

In these studies, the reactions of the mutant to various pests and diseases should be monitored. Studies should be conducted to verify the relationship between cooking quality and the specific gravity of the cassava root tubers for use as a selection method. A closer collaboration with the International Institute of Tropical Agriculture (IITA, Ibadan, Nigeria) should be sought. Since tubers of cassava cultivars with special qualities cannot be easily identified on the market by consumers, in the long term attempts should be made to incorporate morphological markers that will facilitate easy tuber identification by the consumer. The need for more studies on cassava starch in relation to its cooking quality and other uses is recognized. There is a need to train more personnel and provide laboratory equipment. The services of experts and greater collaborations with advanced institutions involved in the area of research are recommended. The potential for the utilization of cassava for food products other than the traditional ones should be explored. To achieve this, the expertise of both local and foreign experts and institutions such as the National Nutrition Institute in Rome should be sought.

FOOD TECHNOLOGY

Achievements: Investigations on the physico-chemical and rheological properties of starch from African local crops such as fonio, sorghum and cassava suggested that it was possible to produce pasta and bread with an increased shelf life by blending wheat and triticale flour with cassava.

Recommendations: It is recognized that sustainable agricultural development in Africa will be enhanced by linking improved crop production with appropriate food technologies. It is recommended that governments and international institutions explore means to expedite appropriate crop processing related to crop improvement. Research should include African cereal grains such as fonio, tef, sorghum, pearl millet, barley and triticale. In addition, blending of local cereal flour with starchy products such as cassava, yam, plantains, sweet potato and local grain legumes should be further developed to achieve the required food quality standards, and to provide a well balanced diet from products with increased shelf life.

It is recognized that there are different socio-cultural situations with different food preparations in various regions of Africa; hence the need to develop national capabilities in food technology are also different. It is recommend that scientists should be trained to adapt the available technology to their local situation. The participants, recognizing the potential value of the material produced, recommend research on developing new food products based on local crops.

USE OF BIOTECHNOLOGY IN CROP IMPROVEMENT

There is a considerable interest in the use of techniques of tissue culture, molecular biology and transgenesis to improve crop plants in Africa. The technology of plant tissue culture offers possibilities to produce disease free planting material as well as genetic improvement. However, the following points should be kept in view when such technologies are used:

- Advanced biotechnology techniques should be employed by using appropriate local varieties where possible.

- Advanced biotechnology manipulations (for example, genetic transformation) should be used as complementary techniques to traditional breeding methods. These technologies cannot be recommended as a replacement of the conventional breeding methods.

- Attention must be paid in the release of genetically engineered plants. Such plants cannot be tested directly in countries that do not have safety regulations for the release of such plants. International institutions in some African countries have the necessary requirements to handle this kind of material, and therefore should be involved in the initial field trials.

- Fellowship training and exchanges between African scientists and advanced laboratories are highly recommended.

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