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Contents

International Symposium on Induced Mutations in Plants (ISIMP) - Dr. R. Phillips delivering the keynote speech at the opening session. Also sitting in the front row (from left to right) Mr. Q.Y. Shu (Scientific Secretary), Mr. W. Burkart (DDG/IAEA), Mr. Q. Liang (Director, Joint FAO/IAEA Division), Mr. S. Pandey (Director, Plant Production and Protection, FAO), Mr. P.J.L. Lagoda (Section Head, Plant Breeding and Genetics, Joint FAO/IAEA Division).

To Our Readers

The International Symposium on Induced Mutations in Plants (ISIMP) was successfully held in Vienna, Austria 12-15 August 2008. It attracted more almost 500 participants, demonstrating a broad interest in induced mutations in the plant breeding and research community. The titles of oral presentations are included in this issue for your information. Some papers submitted to the Symposium by authors who were unable to attend the Symposium are included in this issue.

In this issue, you will learn that mutations can be induced in uncommon plants using various means and utilized for various purposes. For example, Kacholam, guar and cocoyam are not widely cultivated crop species and there is very limited genetic variability, **Kanakamanay, Arora *et al*. and Ndzana *et al.*** reported the induction of mutations as a valuable source of genetic variability in these crops. Rice is a staple food crop and has a history of successful application of mutation techniques for its improvement; however, the virus resistant mutant varieties released in the United Republic of Tanzania (**Luzi-Kihupi *et al*.**), the cold tolerant mutant lines developed in Madagascar (**Rakotoarisoa *et al*.**) and the use of proton radiation for mutation induction in

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rice (**González *et al***.) present the continuous progress in this field. The use of mutation techniques for improving tomato productivity in low water supply area (**González *et al***), for increasing crossability and progeny fertility of mungbean crossed with its wild relative species (**Pandiyan *et al***.) and for investigating leaf structure in mungbean (**Chen *et al***.) are convincing examples of the usefulness of mutation techniques.

I hope you enjoy reading these papers.

Qingyao Shu

Contents

Research Article

Induction of Genetic Variability in Kacholam, *Kaempferia galanga* L.

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Abstract

Induction of genetic variability in kacholam, *Kaempferia galanga* L. was undertaken in the local cultivar Vellanikkara, with eight different doses of gamma rays (2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 and 20.0 Gy) and six concentrations of ethyl methane sulphonate EMS (0.25, 0.50, 0.75, 1.0, 1.25 and 1.50%) and MV_1, MV_2 and MV_3 generations were studied. LD_{50} of gamma rays was 20.0 Gy and that of EMS 1.5%.The highest values for yield and yield contributing characters were obtained for 7.5 Gy gamma rays and 0.75% EMS. Gamma rays at 15.0 Gy and EMS at 1% were most effective in inducing variability in rhizome yield and yield attributes. High estimates of heritability (broad sense) coupled with high genetic advance was observed for number of leaves and rhizome number, and direct selection for improvement of these traits will be effective. Mutagenic treatments induced alterations in the association between rhizome yield and components. A high frequency of positive variants at lower doses and of negative variants at higher doses was observed. Mutant characters present in MV_2 were not completely expressed in all MV_3 plants.

Introduction

Kacholam *Kaempferia galanga* L., belonging to the family *Zingiberaceae*, is a highly valued aromatic herb used as a flavouring agent, as a stimulant, expectorant, carminative and diuretic. The oil is extensively used in flavouring confectionery, pharmaceuticals and many other allied industries. These studies were undertaken in kacholam to identify the optimum dose of gamma rays and optimum EMS concentration to induce variability and to isolate the desirable mutants from the population.

Materials and methods

Rhizomes of local Kacholam, *Kaempferia galanga* L., cv. Vellanikkara were used to isolate desirable mutants of economic importance through induced mutagenesis with gamma rays from a Co^{60} chamber and the chemical mutagen ethyl methane sulphonate (EMS). Studies included a standardization of dose/concentration of mutagen for the induction of variability, an analysis of the frequency of variants and an estimation of heritability. The effects of mutagens were assessed based on yield and yield parameters, including number of leaves, plant spread, number of tillers, leaf length, leaf breadth, rhizome number and yield, and crop duration. The performances of treated plants were evaluated over three generations and mean performance was assessed based on yield and yield contributing characters.

Results and discussion

Treated Kacholam exhibited differences in the number of days taken for sprouting, sprouting percentage, duration of sprouting, lethality, floral characteristics and yield and yield attributes with different doses of gamma rays as well as different concentrations of EMS. The percentage of germination revealed that LD_{50} for *Kaempferia galanga* was around 20Gy for gamma rays and 1.5% EMS. There was a gradual inhibition of sprouting as the EMS concentration or doses of gamma rays were increased. The lower germination rate due to mutagenic treatment might be attributed to an inactivation of auxin levels in the plant with increasing exposures as reported by Skoog (1935). According to Sparrow (1961), mutagenic treatment caused chromosomal aberrations could adversely affect cell division. Delayed sprouting was accounted in higher doses. The mutagenic treatment caused both a positive and negative shift in the means of numbers of tillers per plant from that of control. In tuberose, Sambanthamurthi (1983) observed an increased sucker production at a lower EMS concentration and less sucker production at a higher concentration when compared to control. The reduction in number of tillers in Kacholam observed might be due to a retarded growth and development of the plant as a result of higher doses of the mutagen. According to Cherry and Leasman (1967) the reduced growth could be attributed to a delayed onset of first mitosis and an inhibition of DNA synthesis.

Positive shifts in the number as well as weight of rhizomes per plant were observed at lower doses. In *Costus speciosus*, gamma irradiation of rhizomes resulted in a decreased yield (Gupta *et al.,* 1982). The positive shifts to the different yield attributing characters at lower doses were responsible for the increase of yield.

In Kacholam, the crop duration ranged from 6–8 months. Development of types with a shorter growing period which would fit into the cropping system assumed top priority. The mutagenic treatments caused a considerable reduction of the crop duration, which was directly proportional to an increase in dose. Likewise, early maturing mutants were obtained due to mutagenic treatment in cassava by Moh (1976).

The present studies revealed that highest values for yield and yield attributing characters were obtained for the treatment with 7.5 Gy gamma rays. Among EMS treatments 0.75% registered the highest yield. At higher doses

there were negative shifts of all the yield attributing characters whereas with lower doses positive shifts were observed. High estimates of heritability coupled with high genetic advance were noticed for number of leaves and number of rhizomes, which indicated that there is considerable scope for genetic improvement with respect to these traits (Table 1).

Table 1. Heritability (broad sense) and genetic advance as percentage of mean

Characters	Heritability (Percentage)	Genetic advance (Percentage of mean)
Number of leaves	94.24	39.30
Tiller number	95.37	19.49
Leaf length	96.27	13.04
Leaf breadth	95.17	18.56
Plant spread	80.69	14.98
Rhizome number	90.63	37.36
Rhizome yield	64.50	18.68

The performance of the progenies of the selected MV_2 plants revealed that many plants failed to carry either all or some of the mutations to MV_3. This may be due to a chimeric nature of planting materials and, hence, these plants can be considered as variants. Abraham and Desai (1976) pointed out that the low recovery of mutations in the vegetatively propagated plants was due to diplontic selection. The frequency distribution of variants in MV_2 generation indicated a higher frequency of positive variants at lower doses/concentrations and a higher frequency of negative variants at higher doses/concentrations of mutagens in respect of all the important traits studied (Table 2).

Table 2. Frequency distribution of variants in MV_2 generation

Treatments	Rhizome number			Fresh weight of rhizome (g)			Duration (Days)		
Gamma (Gy)	⩽10	10-15	⩾15	⩽40	40-60	⩾60	⩽200	200-225	⩾225
2.5	17.65	50.98	31.37	35.29	43.14	21.57	21.05	75.44	3.51
5.0	21.57	50.98	27.45	43.14	30.19	26.67	40.35	59.65	0
7.5	14.04	45.61	40.35	40.35	42.11	27.54	35.09	64.91	0
10.0	12.28	45.61	42.11	24.56	50.88	24.56	56.14	43.86	0
12.5	17.54	52.64	29.82	29.82	40.36	29.82	53.33	46.67	0
15.0	24.56	47.37	28.07	33.33	35.09	31.58	60.34	39.66	0
17.5	47.06	43.14	9.80	60.78	29.42	9.80	56.14	43.86	0
20.0	62.22	26.67	11.11	68.89	22.22	8.89	65.39	34.61	0
EMS (%)									
0.25	20.51	64.10	15.39	17.95	42.72	33.33	41.03	58.97	0
0.5	0	80.00	20.00	20.00	46.67	33.33	50.00	50.00	0
0.75	0	38.46	61.54	10.26	53.84	35.90	92.31	7,69	0
1.0	0	57.88	42.42	15.15	36.37	48.48	48.48	51.52	0
1.25	0	100.0	0	33.33	40.74	25.93	48.15	51.85	0
1.5	58.33	41.67	0	66.67	33.33	0	66.67	33.33	0

From the MV_2 population several variants could be isolated based on the vegetative and rhizome characters (Table 3). Variegation in leaf might be produced by nuclear and plastid mutation. Sparrow (1961) observed that leaf abnormalities could be due to chromosomal breakage, disrupted auxin synthesis and accumulation of free aminoacids. Among the two mutagens viz: gamma rays and EMS, the latter was found to be the most potent in the induction of chlorophyll mutants in MV_1 and MV_2. As already reported by Ehrenberg *et al*., 1961, the high frequency of EMS mutants might be due to the preferential reaction of ethyl group with DNA possibly with guanine component.

Table 3. Comparison of morphological variants in MV_1 and MV_2 population

Treatments	Morphological variants (%)							
Gamma (Gy)	Cluster of leaves		Crinkled leaves		Long slender rhizome		Short round rhizome	
	MV_1	MV_2	MV_1	MV_2	MV_1	MV_2	MV_1	MV_2
2.5	0	0	3.33	4.17	1.67	1.67	3.33	3.33
5.0	3.33	6.67	1.67	1.67	0	0	1.67	2.50
7.5	1.57	1.67	1.67	1.67	1.67	1.67	1.67	1.67
10.	5.00	5.0	1.67	4.17	3.33	3.33	1.67	1.67
12.5	0	0	0	0	3.33	6.67	3.33	3.33
15.0	5.00	5.83	0	0	0	0	0	0
17.5	1.67	1.67	3.33	3.33	0	0	0	0
20.0	5.00	5.00	1.67	2.5	1.67	3.33	3.33	7.50
EMS (%)								
0.25	3.33	3.33	0	0	1.67	1.67	3.33	4.17
0.50	1.67	1.67	0	0	0	0	3.33	4.17
0.75	1.67	1.67	3.33	3.33	1.67	2.50	1.67	1.67
1.0	0	0	1.67	1.67	3.33	4.17	6.67	6.67
1.25	1.67	3.33	3.33	3.33	0	0	5.0	5.83
1.50	1.67	1.67	1.67	1.67	1.67	1.67	3.33	4.17

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Review

Mutagenesis in Guar [*Cyamopsis tetragonoloba* (L.) Taub.]

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Abstract

Guar or clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.] (2n=14) is a multipurpose legume crop, grown for feed, green fodder, vegetable, green manuring, and grain purposes. Mutagenesis is a powerful tool for creating variation in a crop like guar where exploitable and favourable genetic variability is very meager. Various types of manifestations such as reciprocal translocations, trisomics, reduction in seed germination, seedling survival, pollen fertility, seed yield, number of seeds per pod, and pod length have been reported in the materials treated with mutagens and their progenies. Additionally, some researchers have observed increase in peduncle length, plant height, and number of clusters, number of pods per cluster, number of pods, seed yield, protein content, gum content and early maturity in the mutated material. The doses of 100 to 200 kR have been quoted to be lethal. The application of chemical mutagens like EMS, hydroxyl amine, hydrazine hydrate, kitazin, saturn, sodium nitrate, NMU, and sodium azide have generated chlorophyll mutations, profuse vegetative growth, single stem, regular pod bearing, changed leaf texture or shape and pod size, late flowering, changes in seed colour, determinate and spreading growth habit etc. Heterophylly in guar has also been reported. The effect of hybridization and mutagenesis on the inheritance of various morphological traits in guar has also been studied. Further research and understanding on mutation induction is needed to generate more desirable genetic variability for traits of economic importance to develop better ideotypes in guar.

Introduction

Guar [*Cyamopsis tetragonoloba* (L.) Taub.] is a very important legume and industrial crop in arid areas of India. The guar endosperm contains galactomanan gum, which has several diversified industrial uses. India earns several thousand crores of rupees as foreign exchange from export of guar gum and its derivatives.

Mutation breeding is a powerful tool for enriching variation in a crop like guar where exploitable and useful genetic variability is very meager. Moreover, the creation of genetic variability in this crop through the recombination of genes by hybridization is very difficult and cumbersome owing to small, delicate flower structures resulting in low percentage of crossed seed setting in the manually hybridized buds. Due to these reasons, not much desirable and usable genetic variability has been generated through conventional breeding approaches. Keeping in view the above limitations, concerted efforts have been made to create more and purposeful variability in guar by various scientists through induced mutations and results concerning such an approach reported in the literature are reviewed here.

Description of various types of mutations

Spontaneous Mutations

The first report of the spontaneous occurrence of male sterility and partial male sterility in guar in India was published during 1968 (Mittal *et al.,* 1968). Subsequently, in 1989, the natural occurrence of male sterility was reported in the USA and a mutant having rosette-type raceme (inflorescence) with reduced fertility was reported in South Africa in 1988 (Stafford, 1989, 1988). The mutant trait (rosette raceme) was expressed on every raceme and was inherited in a monogenic recessive fashion. The gene symbol ***ros*** was proposed for this mutant trait. Stafford (Stafford, 1989, 1988) suggested that this phenotype may be useful as a potential genetic marker in the guar breeding programme. But practical utility of male sterility, partial male sterility and rosette raceme mutants has not been demonstrated yet.

Induced mutations

Mutations induced through physical factors

The first successful attempt to induce mutations in guar through physical mutagens was made in India (Vig, 1965, 1969). The plants carrying reciprocal translocation in 20kR treatment showed low fertility. Some off-type plants in the gamma irradiated M_2 progenies of guar identified as trisomics were observed (Singh, 1972). The trisomic plants did not differ from one another in morphology and chiasma frequency but the trivalent frequency differed greatly in each plant. However, the extra chromosome could not be identified. The effects of irradiation on morphology and cytology of two varieties of guar using 10, 20, 40, 60, 80, 100, 150 and 200 kR doses of gamma rays were studied (Lather and Chaudhary, 1972) and found that germination percentage and seedling survival decreased with increased dosage. The dose of 200 kR proved to be lethal as there was no germination at this dose in either variety. Pollen fertility also decreased with the increasing dose resulting in considerable loss in yield. Various types of chromosomal abnormalities such as translocation, anaphase bridges and laggards were found in the progenies obtained from treated seeds. Varietal differences in sensitivity to radiation were also observed. By contrast, Choudhary (Chaudhary *et al.,* 1973) observed increase in yield, protein and gum contents in an M_2 population generated through irradiation with low 2, 5, 10, 15 and 20 kR doses of gamma rays. In M_2 progeny from the 30 kR treatment of variety FS 277 Singh (Singh *et al.,* 1975) noticed 21 self-fertile, three

partially fertile and 12 self-sterile plants. The meiotic studies on reciprocal crosses between fertile and sterile plants revealed that pollen sterility was not due to meiotic irregularities alone. The effects of irradiation ranging from 10-250 kR gamma rays on two varieties of guar for six quantitative characters assessed in the M_2 generation (Chowdhury *et al.,* 1975) showed that the variation for all the characters was generally greater in the irradiated populations. In both the varieties, the number of branches and yield per plant were less in the M_2 generation than the control but the peduncle length was greater. The plant height was also increased by irradiation in one of the varieties. An increase in induced variation in M_2 progenies of guar cv. RGC 197 obtained by gamma irradiation (10, 30, 50, 60, 70 and 80 kR) was noticed in plant height, number of clusters per plant, number of pods per cluster, number of pods per plant and seed yield per plant but induced variation generally reduced the number of seeds per pod and pod length. Based on progeny means, the progeny numbers 108 and 50 recorded higher values for all the traits except 100-seed weight and number of clusters per plant (Amrita and Jain, 2003). The yield potential of M_3 progenies derived from certain high yielding and M_2 progenies of the guar cv. RGC 197 exposed to various doses of gamma rays (10 to 80 kR) showed to be high for pods per plant and seed yield (Yadav *et al.,* 2004).

One of the interesting mutants induced by gamma irradiation was an early flowering and determinate type (Singh *et al.,* 1981). The determinate plants were characterized by non-branching habit, reduced plant height, increased cluster size, synchronous and early maturity and above all the main shoot either terminated into a leaf or into an inflorescence. The populations developed by artificial hybridization of determinate mutants with the normal (indeterminate) plants indicated that two genes controlled determinate habit and that at least two dominant alleles were required for the expression of the character.

The effect of 0-60 kR doses of X rays was studied on seeds of cv. 'Pusa Navbahar' (Rao and Rao, 1982) and low doses were found to be beneficial, while high doses were inhibitory. The inhibitory effects were generally less in the second generation than in the first. A true breeding early flowering mutant having more number of pods was generated from the 10 kR treatment of X-rays.

Guar cv. Pusa Navbahar seedlings were subjected to continuous UV-B radiation for 18 h and post-irradiated with 'white light' and UV-A enhanced fluorescent radiations (Lingakumar and Kalandaivelu, 1998). UV-B treatment alone reduced plant growth, pigment content and photosynthetic activities. However, supplementation of UV-A promoted overall seedling growth and enhanced the synthesis of chlorophyll and carotenoids with a relatively high photosystem I. Post UV-B irradiation under WL failed to photoreactivate the UV-B damage whereas a positive photoregulatory effect of UV-A was noticed in electron transport rates and low temperature fluorescence emission (Lingakumar and Kalandaivelu, 1998). UV-B radiation induced a decline in the amount of photosynthetic pigments and O_2 evolution along with a modification in the absorption spectra of chloroplasts. UV-A+UV-B irradiation partially reversed these changes (Joshi *et al.,* 2007).

Mutations induced through chemical factors

Among the M_1 plants grown from seeds of guar treated with ethyl methane sulphonate (EMS) Gohal (Gohal *et al.,* 1970) observed two chlorophyll deficient plants and five plants with profuse vegetative growth. Unbranched and regular pod bearing mutants were obtained by treating the seeds with hydroxyl amine (Swamy and Hashim, 1979, 1980) and mutants with changed leaf texture or shape, growth habit and pod size were obtained by treating seeds with EMS or hydrazine hydrate. Some of these pod mutants had pleiotropic phenotypes such as extensive branching and late flowering. Some mutants also showed changes in seed colour from normal violet to light grey or light brown. Determinate and spreading variants were observed in the M_1 and M_2 generations obtained from the seed of cv. 'Pusa Navbahar' soaked in 200, 400 and 600 ppm kitazin and 1000, 2000 and 3000 ppm Saturn for 12 and 24 h (Rao *et al.* 1982).

Mutations induced through physical and chemical factors

The cytological effects of 50, 100 and 150 kR doses of gamma rays or 0.5, 1.0, 1.5 and 2.5 per cent sodium nitrate were studied in a buffer solution (Yadava and Chowdhury, 1974). They found that 100 and 150 kR doses of gamma rays were lethal, while other treatments caused cytological abnormalities such as chromosome stickyness, laggards, anaphase bridges, fragments, univalents and translocations. They observed that radiation caused the highest number of abnormalities. High yielding mutants were induced by treating the soaked seeds of cv. 'PLG 143' and 'Suvidha' with 80 and 100 kR gamma rays and aqueous solutions of 0.1-0.3 per cent EMS and 0.01-0.03 per cent solution of N-methyl-N-nitrosourea (NMU) either alone or in various combinations. The M_2 mutants thus obtained had long pods, increased number of pods and early maturity. These mutants showed increased yields and gum contents in the endosperm or whole seed (Singh, Aggarwal and Suman, 1986). Mutagenic effects of gamma rays at 20, 40 and 60 kR; magnetic fields at 3000, 4000 and 5000 G; sodium azide at 1×10^{-3}, 2×10^{-3} and 3×10^{-3} M, and a combination of treatments were studied in guar varieties Pusa Navbahar and FS 277. The higher levels of each treatment were more efficient at inducing chlorophyll mutations in each variety. However sodim azide was the most efficient mutagen (Basha and Rao, 1988). Heterophylly in guar was observed when the seeds were exposed to gamma rays and sodium azide treatment (Badami and Bhalla, 1992). Gamma rays were less effective although, at 45 kR, leaf margins were changed from serrate to entire and combined treatments of 35 and 45 kR with 100 and 200 ppm sodium azide, respectively, produced two and four leaflets, instead of three. Treatment with 35 kR plus 200 ppm sodium azide reduced the number of secondary and tertiary veins from 15 and 69 to 4 and 5, respectively.

Effect of hybridization and irradiation

Genetic analysis of seed mass following hybridization and irradiation in guar revealed that the additive component of genetic variance was more important in irradiated generations, i. e. F_1M_1 and F_2M_2, whereas non-additive gene action was more important in case of non irradiated populations, i.e. F_1 and F_2, for the inheritance of 100-seed weight (Arora *et al.,* 1997).

Further Challenges in mutation breeding in guar

The foregoing review clearly demonstrates that lower doses of various mutagens, either alone or in combination, induce much more useful variability than higher doses. However, work on mutation breeding of guar is limited and only a few mutants carrying one or two useful attributes have been obtained so far. Therefore, there is a need to initiate extensive research work using large number of lower doses of various mutagens alone or in combination to induce really desirable variability *in vitro* as well as *in vivo* in order to exploit the same in breeding for developing early maturing, resistant to major diseases, high seed and gum yielding improved cultivars in guar which is a highly drought tolerant and industrially important crop for semi arid and arid regions.

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Short Communication

Preliminary Study on Radiation Sensitivity of *In Vitro* Cultures of *Xanthosoma* (Macabo) in Cameroon

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Abstract

In vitro grown cocoyam genotypes were exposed to ^{60}Co γ-irradiation at varying doses (4 to 20 Gy) to determine a suitable lethal dose (LD) for eventual use as orientation for selection of effective mutagenic treatments that can induce useful genetic changes. The three cocoyam cultivars studied (a white flesh tuber, a red flesh tuber and a hybrid clone derived from the White and Red accessions) differed in their reaction to the irradiation, with the Red accession being more sensitive than the White and the hybrid being the most sensitive. Phenotypic changes following irradiation included growth reduction and transformation of plantlet leaf shape, indicating the possibility of distinct changes in the plants' genetic makeup. This study indicates that variability within cocoyam species could be increased through induced mutations at a dose rate of ~ 9 Gy.

Introduction

Cocoyam (*Xanthosoma sagittifolium* (L) Schott), known in Cameroon as '*Macabo'*, is a 'neglected' traditional plant species, widely cultivated in the Tropics and sub-Tropics for its edible leaves and tubers (Oknopise *et al.*, 1999). However, there has been a progressive disappearance of the crop during the past three decades. In Cameroon, this has been a consequence both of a considerable decline of cocoyam yield and a severe reduction of its quality. These defects have been attributed to the *Pythium myriotylum* induced Root Rot Disease (RRD; Nzietchueng, 1985: Pacumbaba *et al*. 1992).

Research to address this has so far consisted of screening the available germplasm and performing gene recombination of edible cultivars using classical breeding procedures. However, limited success has been obtained because cocoyam, as most vegetatively-propagated crops, has a narrow genetic base and the available germplasm provides limited variability. One of the main objectives of the cocoyam breeding programme in Cameroon is to increase the scope of its improvement by enhancing the genetic variability of the crop and induce resistance to RRD through induced mutations.

Progress in breeding requires adequate sources of genetic variation. Mutation breeding is considered as a reliable strategy with considerable potential for generating sufficient genetic variability in most plant species. Its application on some "neglected" crop species such as the cocoyam may result in substantial progress in the effort of selecting clones with desirable characteristics.

It has previously been shown that physical mutagens, such as radiation have been efficient in inducing variability in many crop species and can also be applied to Cocoyam to increase its genetic variability. In the past, induced mutations in *Xanthosoma* using γ irradiated micro sets have resulted in the recovery of several morphologically robust mutants. Cocoyam plants with different levels of tolerance towards RRD, have been obtained through irradiation with γ rays of *in vitro* grown apices (Saborio *et al*., 2004). *In vitro* mutation breeding can therefore be presented as a technology that may significantly contribute to genetic improvement of *Xanthosoma*.

This study gathers information about the sensitivity of *in vitro* derived shoots exposed to γ irradiation treatments of different cocoyam genotypes with the aim of determining the most appropriate dose to be used as an effective mutagenic treatment to induce useful genetic changes.

Materials and methods

Preparation of plant materials

Corms and cormels of three genotypes of edible cocoyam were used for this study. The genotypes included the White flesh tuber cocoyam (accession RO1054), the Red flesh tuber cocoyam (accession RO2063), and the hybrid derived clone 92007-305 from the cross between White and Red accessions of cocoyam. These corms and cormels were cut into sets, cured for about an hour and planted in non-sterilized topsoil to sprout. Sprouted sets of each genotype were collected and washed under running tap water for about 30 min to remove external contaminants. The sprouts were trimmed to obtain shoot tip explants of 5-7 mm length by carefully eliminating the outermost leaves. Explants were surface disinfected for 15 min under a laminar flow hood with 15% Clorox containing a few drops of Tween 20. The disinfected explants were rinsed thoroughly with sterile distilled water containing 0.1% ascorbic acid to prevent exudation of phenolic acid. All explants were transferred aseptically to the initiation medium. Explants were excised from *in vitro* growing plantlets (elongated micro-shoots) and the shoots along with their pseudostems were isolated and transferred aseptically into a culture medium.

Culture medium

The culture medium was based on B5 medium (Gamborg *et al*., 1968) with micro- and macro-nutrients and vitamins supplemented with 30 g/L sucrose as the carbon source. The pH of the medium was adjusted to 5.8. Aliquots of 10 ml were dispensed into culture tubes (25 mm × 150 mm) with covers, and sterilized at 121°C for 15 min. The cultured explants were incubated in a growth room at 26 ± 2°C under 16 h light and 8 h darkness. After 4 - 6 weeks, the explants were transferred into a multiplication medium based on MS basal salts and vitamins supplemented with 3 mg/L BAP. Proliferating

shoots were sub-cultured unto fresh medium every 4 to 5 weeks and maintained in the growth room.

The growing shoots were isolated and trimmed to about 5 mm size with a *pseudostem* of about 0.5–2 mm diameter. The explants were then transferred to a slow growth medium supplemented with 0.5% sucrose and solidified with 0.2% Gelrite. The basal medium was Murashige and Skoog (MS 1962) and contained: 0.1 mg/L thiamine HCl, 100 mg/L myo-inositol, 27.8 mg/L iron ($FeSO_4.7H_20$), 37.2 mg/L EDTA.$2H_20$, 1.0 mg/L glycine, 0.5 mg/L nicotinic acid and 0.5 mg/L pyridoxine HCl. Five explants per cocoyam genotype were cultured aseptically on the slow medium contained in 90 mm diameter Petri dishes and the latter sealed with parafilm for protection before the irradiation.

Irradiation

Irradiation was performed at the Biotechnology and Nuclear Agriculture Research Institute, in Legon (Ghana). An irradiation experiment was carried out with ^{60}Co γ irradiation at increasing doses from 0 to 20 Gy with 4 Gy intervals; the dose rate used was 1.88 Gy/min.

Of the 900 explants prepared, 815 were successfully irradiated (91% success rate). The latter were comprised of 400 explants of the White flesh tuber cocoyam (accession RO1054), 205 explants of the Red flesh tuber cocoyam (accession RO2063) and 210 explants of the hybrid derived clone 92007 - 305. Irradiated explants were individually transferred onto a fresh MS medium with 30 g sucrose and incubated for 35 days in a growth room at 27±2°C under continuous cool white fluorescent light. Data were collected on the fresh weight, mean height (petiole length) of plantlets and the production of leaves and roots.

For the radio-sensitivity test, lethal dose (LD) determination was done by assessing the mean fresh weight and the petiole length of plantlets. Two LDs were assessed to determine the level of sensitivity that matched with phenotypic changes observed after irradiation. *In vitro* growth was assessed as the increase in the weight of treated plantlets as compared to untreated ones, the number of leaves produced, as well as the number of roots per plant and the petiole length of tallest leaf.

Results and discussion

Determination of suitable lethal dose (LD)

The result obtained from the comparison of the two lethal doses (LDs) showed remarkable differences on fresh weight and petiole length (Figure 1). The fresh weight in unirradiated plants ranged from 9.2 mg for the White, 8.1 mg for the Red and 7.1 mg for the hybrid derived accessions. For the dose that caused 30% fresh weight reduction (LD_{30}), estimates made using regression analysis gave 4.6 Gy for the White accession, 3.6 for the Red accession and 2.5 Gy for the hybrid derived clone (Figure 1a). This trend was similar for the petiole length across the three cultivars (Figure 1b).

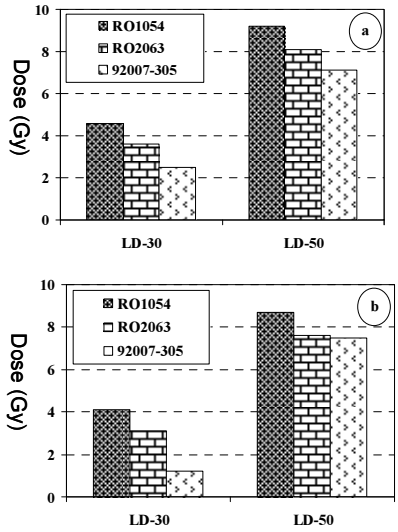

Figure 1. Estimated treatment doses using regression analysis of data on (a) mean fresh weight and (b) petiole length at both LD-30 and LD-50.

The petiole length, on its part, showed the same trend, with LD_{50} ranging from 8.7 Gy for the White, 7.6 Gy for the Red and 7.5 Gy for the hybrid clone. Treatment doses at the LD_{30} resulted in no marked morphological changes while the doses at the LD_{50} resulted in marked phenotypic changes among the plants when compared with non-irradiated plantlets. All three genotypes showed the same pattern indicating that the LD_{50} dose might be the better than the LD_{30} dose for generation of mutants.

Response to Gamma radiation

There was a progressive decrease in fresh weight as irradiation doses increased irrespective of the cocoyam genotype (Figure 2a). The decrease mean fresh weight was more abrupt at lower doses of irradiation (0-8 Gy). The reduction in mean weight seemed to tapper off at higher irradiation doses (>10 Gy), indicating some differential response of cocoyam to gamma ray irradiation, with the Red accession being more sensitive to irradiation than the white. The hybrid clone derived from the cross between the Red and the White accessions was most sensitive.

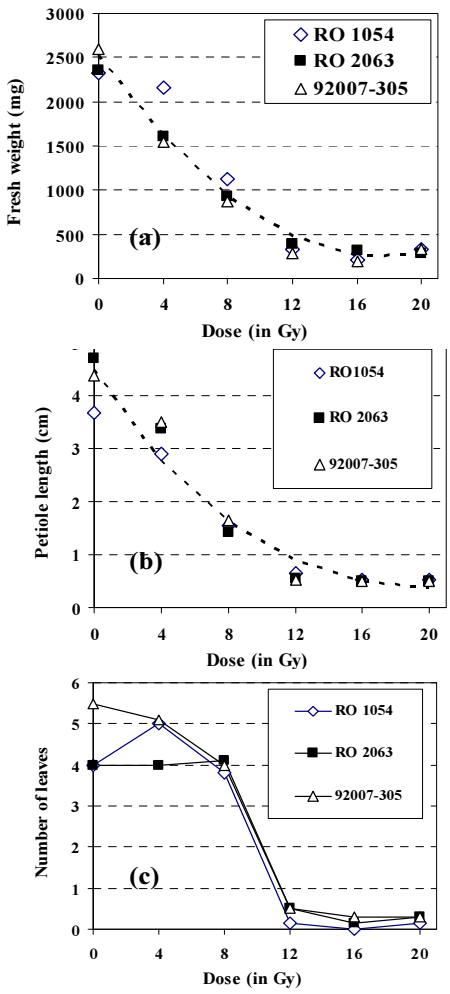

Figure 2. Effect of radiation doses on the (a) Fresh weight of plantlets, (b) Petiole length of plantlets, and (c) Number of leaves of *Xanthosoma in vitro* cultures 35 days after irradiation.

The trend observed for the mean fresh weight was similar to that of the plant height or petiole length (Figure 2b) and that of the number of leaves produced during post irradiation (Figure 2c). The height of derived plantlets considerably reduced as doses increased from 8 - 20 Gy. At doses above 12 Gy, the production of leaves was almost abolished, irrespective of the cultivar, leading thereby to the death of irradiated plantlets. These observations were made across the three cultivars indicating that the physical mutagens significantly reduced the growth of derived plantlets and consequently induced some morphological and phenotypic changes in the cocoyam species.

Phenotypic observations

Considerable phenotypic changes were observed on plantlets derived from irradiated shoots (data not shown). Most changes were observed on the leaves of the plantlets. Plantlets were of smaller size with reduced leaf area. The leaves generated were shrunken and spear-shaped with some of them showing mosaic-like characteristics and/or with no distinct lamina and petiole. Root formation was inhibited, as none of the treated plantlets of any of the cultivars produced roots post irradiation.

Conclusion

In this study, *in vitro* derived shoots of different cocoyam genotypes were exposed to γ radiation treatments in order to determine a suitable lethal dose to be used as an effective mutagenic treatment that can induce useful genetic changes. The results indicated that the LD_{50} was a more appropriate treatment to be applied on cocoyam shoot tips. Differences observed among the three cocoyam cultivars (White, Red and the Hybrid) in their response to irradiation treatments, revealed that the Red cocoyam cultivar was more sensitive to irradiation than the White, with the derived hybrid exhibiting the most pronounced sensitivity. Irradiation doses between 8 and 10 Gy were more prone to induce stable changes that could be used to increase variability within the cocoyam species. Induced mutations can therefore be considered as a reliable alternative in achieving genetic improvement of some neglected crops such as Cocoyam.

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Mutant Variety

Mwangaza – A New Early Maturing, RYMV Resistant Rice Mutant Released in the United Republic of Tanzania

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Abstract

A new rice mutant was developed at the Sokoine University of Agriculture through gamma ray irradiation. The original popular variety ' Supa' was irradiated with 170, 201, and 240 Gray (Gy) gamma rays at the International Atomic Energy agency (IAEA) Seibersdorf Laboratories in May 1994. The evaluation of resulting mutants identified five mutants that were early maturing and resistant to rice yellow mottle virus as compared to the parents. The selected mutants were tested in replicated multi-location trials and farmers' fields. In 2005, one mutant line was recommended for release and named 'Mwangaza'.

Key words: rice, mutant variety, early maturing, gamma rays, RYMV resistant

Introduction

Rice (*Oryza sativa* L) is a staple food in many countries of Africa and constitutes a major part of the diet in many others (Oteng and Sant' Anna, 1999). In sub-Saharan Africa it is estimated that the cultivated area is 6.8 million hectares (Jones, 1999).

United Republic of Tanzania, the second largest rice producer after Malagasy (IRRI, 1994) produces about 800,000 tons of rice from an estimated area of 548,100 hectares (Ministry of Agriculture and Co-operative, 1998). The average for Africa is estimated at 2.1 tons/ha. In the United Republic of Tanzania, about 74% of rice is grown by small-scale farmers under rainfed lowland conditions. Upland rice comprises about 20% and irrigated rice constitutes about 6% of the total area under rice (Kanyeka *et al*., 1994).

Major constraints that limit rice production in the United Republic of Tanzania include poor weed management, pests and diseases, lack of improved varieties with acceptable grain quality, inadequate fertilizer and soil amendments, inadequate soil and water management and unavailability of inputs. Many farmers still grow specifically adapted local varieties many of which are photoperiod-sensitive, late maturing, late and weak-statured. Mutagenesis was employed in the rice breeding programme at the Sokoine University of Agriculture (SUA) in order to reduce the plant height and maturation period of popular indigenous cultivars while maintaining the good qualities of the parents.

Materials and methods

Dry seeds of the popular 'supa' cultivar were irradiated with 170, 210 and 240 Gray (Gy) gamma rays from Cobalt 60 at the International Atomic Energy Agency (IAEA) Seibersdorf Laboratory, near Vienna, Austria in May 1994. The irradiated seeds and control were sown in July, 1994 at the Sokoine of Agriculture Crop Museum field.

M_1 primary panicles were harvested, panicle fertility determined and M_2 seeds planted as M_2 panicle to progeny rows. About 70 panicles were selected per dose (treatment). The M_2 plants were selected using Single Seed Descent method whereby, one seed was randomly selected from M_2 plants to raise the M_3 and M_4 generations. From the M_4 generation, five lines from the 170 Gray (Gy) treatment which were found to be early maturing and resistant to rice yellow mottle virus (RYMV) were selected. In 2001 and 2002, the five selected lines plus the original cultivar (Parent) were grown in replicated trials at two sites: SUA and Dakawa Agro-scientific research Centre in Morogoro, United Republic of Tanzania. Subsequently, the selected lines were evaluated in multi-location trials and in farmers' fields (Luzi-Kihupi & Zakayo, 2001). The data collected included plant height, days to 50% flowering, panicle length, number of panicles per square meter, panicle weight, 1000 grain weight, percent filled grains per panicle and grain yield. All the data were collected in accordance with Gomez (1972) and subjected to analysis of variance using MSTAT C (Michigan State University, 1990) computer programme. Apart from agronomic data, grain quality analysis was done to determine the grain appearance, cooking and eating quality according to the procedure outlined by Jennings *et al.* (1978).

Results

Agronomic characteristics of the mutants and their parent are presented in table 1. From the pooled data of two sites and two seasons, the characters grain yield, days to 50% flowering, plant height and panicle length and percent filled grains per panicle showed significant differences among the genotypes while number of panicles per square meter, panicle weight and 1000 grain weight did not show any significant differences among the genotypes. Days to 50% flowering of the mutants were reduced by about 22-24 days compared to the parent. All

the mutants except SSD 35 out yielded the parent with SSD 3 yielding the highest across the two locations.

Agronomic performance of the mutants and parents across five sites in the farmers' fields is shown in table 2. Significant differences were observed for the characters plant height, days to 50% flowering and grain yield. Mutant SSD1 was the highest yielding line. Table 3 shows the grain quality characteristics of the mutants and parent. None of the mutant was as aromatic as the parent.

In 2005, with the help of participating farmers in Southern Highlands and Eastern Zone of the United Republic of Tanzania, one mutant line, SSD 35 which is early maturing and resistant to rice yellow mottle virus was recommended to the National Variety Release Committee for release. The new variety is now registered under the new name 'Mwangaza'. The characteristics of the new variety and its parent are as shown in the table 4. The new variety which is early-maturing, non- photoperiod sensitive is suitable both under rainfed upland and lowland conditions of the United Republic of Tanzania especially in areas affected by the rice yellow mottle virus.

Table 1. Agronomic characteristics of mutants and their parents (SUA and Dakawa, United Republic of Tanzania, 2001 & 2002)

Genotype	Treatment	Grain yield kg/ha	Days to 50% flow	Plant Height (cm)	No. of Panicle/ sq. m	Panicle length (cm)	Panicle wt (g)	1000 grain wt (g.)	% filled grain/ panicle	RYMV Score
SSD1	170Gy	4116b	71bc	118.3a	160.7	21.9c	4.3	32.9	82.2ab	1
SSD3	170Gy	5296a	72bc	127.0ab	167.2	23.9ab	4.6	32.5	81.2ab	3
SSD5	170Gy	4655b	72bc	120.0b	163.7	23.9ab	4.4	31.9	79.7b	3
SSD7	170Gy	3799b	72bc	121.1ab	168.3	23.7a	4.7	31.9	83.4ab	1
SSD35	170Gy	2956c	70c	120.0b	146.5	24.9bc	4.7	34.7	84.5a	1
Supa	Control	2935d	94a	122.1a	184.3	25.5	4.1	32.1	71.2c	7
Mean		45,56	75	120.6	165.1	23.5	4.4	32.7	80.4	
Sx (+/-)		150	0.53	12.77	9.97	0.54	0.17	0.4	1.23	
CV(%)		8.46	1.73	8.63	14.8	5.62	3.1	3.1	3.76	

Table 2. Performance of selected mutants on farmers' fields (Mean of five fields, Ifakara, United Republic of Tanzania, 2002)

Variety/Line	No. of Tillers/sq.m.	Plant Height(cm)	Days to 50% Flowering	Yield kg/ha
Salama M-19	153.2	145.6b	86.6c	3969b
Salama M-57	153.8	144.8b	85.6c	3769b
SSD 1	150.4	106.6e	60.6de	4689c
SSD 5	159.2	110.0d	62.0d	2867c
SSD 35	155.0	114.2c	58.6e	3049c
Supa	155.4	155.2a	94.6b	3951b
Mean	155.83	123.5	78.91	3527
Sx (+/-)	3.72	0.968	0.779	0.643
CV (%)	5.34	1.76	2.21	20.13

Table 3. Grain quality characteristics of the rice mutants and their parent

Mutant / Parent	Hull colour	Kernel length (mm)	Length/ Breadth (mm)	Shape	Opacity (% chalkiness)	Amylose content%	Gel Consistency	Gelatinization Temperature	Aroma
SSD 1	Gold	7.8	2.91	Medium	None	29.51	Medium	Int.	None
SSD 3	Gold	6.8	2.45	Medium	< 10%	24.29	Soft	High	Slight
SSD 5	Gold	6.7	2.73	Medium	< 10%	22.08	Soft	High	Slight
SSD 7	Gold	7.3	2.89	Medium	< 10%	24.68	Soft	High	Moderate
SSD 35	Brown	8.0	3.02	Slender	< 10%	22.08	Soft	Int.	None
Supa	Gold	7.9	2.87	Slender	None	25.79	Soft	Low	Aromatic

Int. = Intermediate

Table 4. Distinguishing characteristics of Mwangaza variety and its parent

Variety name	Mwangaza	Supa (Parent)
Former Designation	Supa SSD 35	Supa
Leaf		
Leaf blade colour	Green	Dark green

Variety name	Mwangaza	Supa (Parent)
Leaf sheath Colour	Green	Green
Collar colour	Pale green	Pale green
Leaf blade pubescence	Glabrous	Glabrous
Basal leaf colour	Green	Green
Leaf angle	Horizontal	Horizontal
Flag leaf angle	Intermediate	Intermediate
Culm length (cm)	92	137
Plant Height (cm)	114-120	130-155
PANICLES		
Panicle length	23.5	27.3
Panicle type	open	Intermediate
Panicle exertion	Well- exerted	Well-exerted
Shattering	low	Mod-Low
Threshability	Intermediate	Intermediate
Days to 50% Flowering	60-65	90-100
Photoperiod sensitivity	Not sensitive	Sensitive
Grains		
Grain length (mm)	8.0 (Extra long)	7.9 (Extra long)
Grain width (mm)	2.5	2.8
Grain shape	Slender	Slender
Seed coat colour (bran)	Brown	Straw
Awn presence	Awned on some spikelets	Absent
1000 grain weight	34.7	32.1
Translucency	<10% chalkiness	Translucent
DISEASE RESISTANCE		
RYMV	Highly resistant	Susceptible
Blast	Resistant	Susceptible
Spikelet fertility (%)	80-90	90
GRAIN QUALITY		
Amylose content (%)	Intermediate	Intermediate
Gelatinization temperature	Intermediate	Low
Gel consistency	Soft	Soft
Scent (aroma)	Non-scented	Scented
Grain Yield (tons/ha)	2.5-3	2.5-3

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Research Article

Inducing Cold Tolerance in Malagasy Rice Varieties IR 58614, Malady and Rojofotsy through *In Vitro* Mutagenesis

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Abstract

The use of induced mutagenesis to develop cold tolerant mutants from Malagasy rice varieties was investigated with the aim of developing rice mutants that could be planted during the cold seasons in the country. The strategy involved the induction of calli from mature rice embryos and exposing the calli to different doses of gamma rays. The efficacy of different media compositions were evaluated both for callus induction and for plantlet regeneration. Selections for cold tolerance were carried out by attempting to induce the irradiated calli to regenerate at 12°C. The putative mutants were evaluated for agronomic performance under controlled environments and field conditions. Data are presented on the optimal media compositions for both callus induction and plant regeneration for both indica and japonica rice varieties. In all, 3 cold tolerant induced mutants with high yield and seed set were identified. The implications of the findings and suggestions for the integration of the mutants into Malagasy rice agriculture in order to achieve 2 crops per year are discussed.

Key words: Rice, *indica, japonica*, induced mutation, cold tolerance, callus induction, plantlets regeneration.

Introduction

Rice is the staple food of over half of the world's population, accounting for 60 to 70 percent of the energy intake of more than 2 billion people in Asia alone (FAO, 2003). Its productivity is of increasing importance to food security in an increasing number of low-income food-deficit countries such as Madagascar. At 2t/ha (IRRI, 1995), rice productivity in Madagascar is still very low relative to the potential yield of 2.5 t/ha and yields averaged 1.7-1.8 t/ha between 1960-1989; 1.8-2.1 t/ha between 1989 and 1995 and 2.1-1.9 t/ha between 1995 and 2000 http://www.irri.org/media/facts/pdfs/madagascar. Most rice cultivation in Madagascar, sustained by irrigation, is in the highlands at altitudes of 800 to 1000 m above sea level and with mean annual temperatures ranging from 15 to 19°C (with minimum of 5°C having been recorded). Annual rainfall varies from 1200 mm to 1500 mm. There are 2 main rice cropping seasons in the highlands of Madagascar, the *vakiambiaty* season from October to May (although some varieties could be planted at the end of January); and the *vary aloha* season from April / May to January / February. At these high altitudes, cold, especially during the winter months of June to September, is probably the most limiting factor for rice cultivation in Madagascar. This is because rice germination requires a temperature above 21°C (Vergara, 1970). It is for this reason that the farmers plant rice in April or May when the temperature is favourable for germination, a compromise that effectively negates the possibility of two croppings per year as the cold period persists from May to September (Vergara, 1970; Dechannet *et al.*, 1996; Rakotonjanahary, 1993). Additionally, the cultivation of local unimproved varieties, not adapted to the unfavourable climatic conditions, leads to high spikelet sterility. It is therefore necessary to produce a cold tolerant short cycle rice variety in order to be able to cultivate the same parcel of land twice in a year. The conventional method of crop improvement, involving hybridisations, is still used for rice improvement in Madagascar. The drawback for this method is the long time, 8-10 years, required for producing an improved variety. There is therefore a need to complement the conventional crop improvement strategies with novel techniques with potential for shortening the time required for the development of new varieties. Induced mutations facilitated by modern biotechnologies, such as *in vitro* techniques, hold promise for mitigating this constraint. There are ample examples on the use of mutagenesis to improve many different types of crop and ornamental plant varieties with more than 2,250 officially released mutant crop varieties from 175 plant species being cultivated by farmers in 59 countries of Africa, Asia, Australia, Europe, South America and North America (Maluszynski et al, 2000; FAO/IAEA Mutant Varieties Database http://www-mvd.iaea.org/MVD/Default.htm; The improvements are in plant architecture (such as dwarfness); a myriad of quality traits; and resistance to both biotic and abiotic stresses (IAEA, 1977; Maluszynski *et al*., 2000; Ahloowalia *et al*., 2004; MBNL, 2005). Out of these officially released crop mutant varieties, 440 are rice mutants, thereby making rice the crop plant with the greatest number of mutant varieties officially released to farmers and hence with the greatest potential therefore for improvement using this technology. The exposure of plant propagules to ionising irradiation, such as gamma rays, is a proven method for the induction of mutations in plants (IAEA, 1977; Van Harten, 1998).

The objective of this work was to develop cold tolerant rice lines using induced mutations facilitated with *in vitro*

techniques. This involved the determination of favourable media for callus induction and for plantlet regeneration; evaluation of the effects of irradiation on growth and development *in vitro* and *in vivo* of the irradiated explants; and the determination of the effect of irradiation on phenology and on spikelet sterility as a function of temperature during the beginning of the growth cycle, compared with the parent or the untreated control.

Materials and methods

In vitro mutagenesis

Plant material and callus induction

Three local Malagasy rice varieties Rojofotsy (1285), IR58614 and Malady (2509) provided by 'Foibe Fikarohana ho Fampandrosoana ny eny Ambanivohitra' (FOFIFA) were used for the studies. Their most important agronomic characteristics, for which they are widely cultivated by farmers, are high yield, tolerance to submersion, and dwarfness, respectively. Rojofotsy and IR 58614 belong to *indica* subspecies while Malady is of the *japonica* subspecies. The explants used for the studies were rice mature embryos that were induced to callus in culture media. To obtain the embryos, rice seeds were dehusked, sterilized with 70% ethanol for 5 minutes, disinfected by soaking for 20 minutes in 5.75% sodium hypochlorite mixed with 3 drops of Tween 20; and then rinsed 3-4 times with distilled water. Eight seeds per variety were plated in Petri dishes containing 8 different media, the Murashige and Skoog (MS, Murashige and Skoog, 1962), N6, Linsmaier and Skoog (LS, Linsmaier and Skoog, 1965) basal media supplemented with various components denoted A, B, C, D, F, G, H and I, (Table 1). These were replicated 6 times. The Petri dishes containing these plated seeds were incubated at 25±2 °C in darkness until the induction of callus.

Table 1. Composition of callus induction media evaluated for 3 Malagasy rice varieties

Media	Ingredients
A	The MS (Murashige and Skoog, 1962) basal medium (MS-5519 made by SIGMA) supplemented with 2 mg/L of 2,4 - Dichlorophenoxyacetic acid (2,4-D), 0.5 mg/L of kinetin, 30 g/L of sucrose and 5.5 g/L of agarose I-A. At pH of 5.8
B	The same as A medium but with 8 g/L of agarose I-A
C	The MS (Murashige and Skoog,1962) basal medium (MS-5519 made by SIGMA) added with 100 mg/L of Myo Inositol, 0.4 mg/L of Thiamine-HCl, 2 mg/L of 2,4-D, 0.2 mg/L of Benzyl Amino Purine (BAP), 50 mg/L of Proline, 60 g/L of sucrose, and 5 g/L of agarose I-A. At pH of 8
D	The same as C medium but with 8 g/L of agarose I-A. At pH of 8
F	The LS (Linsmaier and Skoog, 1965) basal medium supplemented with 2mg/L de 2,4-D; 0.1mg/L of Thiamine; 100 mg/L of Inositol; 30g/L sucrose; and 8g/L agarose I-A. At pH of 5.8
G	The MS (Murashige and Skoog, 1962) basal medium (MS-5519 made by SIGMA) supplemented with 2 mg/L of 2, 4-D, 1 g/L of Casein hydrolysat, 30 g/L of sucrose, 8 g/L of agarose I-A
H	The LS (Linsmaier and Skoog, 1965) basal medium supplemented with 1 mg/L of 2,4-D, 100 mg/L of Tryptophan, 0.3 mg/L of kinetin, 30 g/L of sucrose, 8 g/L of agarose I-A
I	The MS (Murashige and Skoog, 1962) basal medium (MS-5519 made by SIGMA) added with 2 mg/L BAP, 1 mg/L kinetin, 1 mg/L Naphthalene Acetic Acid (NAA), 30 g/L sucrose, and 8 g/L agarose I-A. At pH of 5.8

Induction of mutation

Large calli aged eight weeks were divided into 5 to 8 mm small pieces in order to achieve profuse callusing. The latter pieces were transferred to the same fresh callus induction medium and then irradiated by gamma rays using a ^{60}Co source at the International Atomic Energy Laboratories, Seibersdorf, Austria. The following doses were applied for each medium: 0, 10, 15, and 20 Gy, and the dose rate was 286.10^{-3} Gy /min for A, B, C, D medium and $28 .10^{-3}$ Gy/min for F, G, H, and I medium.

Post-irradiation treatment of calli

Calli derived from pieces of calli that had been subjected to different doses of irradiation per variety and per medium were sub-cultured again onto fresh callus induction medium and divided in 3 groups:

- The first group was incubated at 16°C for 1 month at the end of which calli that were still viable (with the 'cauliflower' morphology, i.e. roughness aspect, rounded, globular, waterish, shiny with white-

yellowish coloration called embryogenic structure) were sub-cultured onto the same fresh induction medium and incubated again at 12°C for 1 month. The non-embryogenic ones (necrotic - brown to black colouration, smooth surface and the ones that are neither round nor globular are opaque and white) were discarded.

- The second group was treated exactly as the first except for their incubation at 28°C.
- The third group was transferred immediately onto regeneration medium.

Regeneration of plantlets

The irradiated calli were dehydrated for 24 hours in Petri dishes containing sterile filter paper, transferred onto different freshly prepared media. Three different media compositions J (for *japonica* variety), K and L (for *indica* variety), were used (Table 2). The Petri dishes containing these plated calli were incubated at 25 ± 2 °C under a photoperiod of 16 hours light of 3600 Klux alternated with 8 hours of darkness for the regeneration of plantlets.

Table 2. Composition of regeneration media used in culturing irradiated calli from 3 Malagasy rice varieties

Media	Composition
J	The MS (Murashige and Skoog, 1962) basal medium (MS-5519 made by Sigma Aldrich) added with 1 mg/L of kinetin, 1 mg/L of NAA, 30 g/L of sucrose, 1.7 g/L of gelrite, at pH of 5.8.
K	The MS (Murashige and Skoog, 1962) basal medium (MS-5519 made by Sigma Aldrich supplemented with 2 mg/L of BAP, 1 mg/L of kinetin, 1 mg/L of NAA, 30 g/L of sucrose, and 8 g/L of agarose I-A. At pH of 5.8.
L	The N6 (CHU C. C. and al., 1978) basal medium with MS (Murashige and Skoog, 1962) vitamins, supplemented with 2 mg/L of BAP, 1 mg/L of kinetin, 30 g/L of sucrose, and 8 g/L of agarose I-A. At pH5.8.

Rooting and acclimatization

Regenerated young plantlets were plated onto hormone-free MS basal medium (Murashige and Skoog, 1962) (MS-5519 made by SIGMA) with 30 g/L of sucrose and 8 g/L of agarose I-A for rooting. Then, they were transferred to Yoshida's liquid medium (Yoshida *et al*., 1965) for acclimatization. Vigorous plantlets were planted in pots containing soil for rice cultivation in the greenhouse until the first generation of seeds (M_1) was harvested.

Evaluation of the progenies

Seed germination tests

M1 seeds were incubated at 52°C for 3 days without water, then immersed in tap water and incubated at 28°C until germination at the Biotechnology and Plant Breeding Unit at the Plant Physiology Laboratory, University of Antananarivo, Madagascar.

Two sets of 10 M_3 seeds per pot, per variety and per irradiation dose and replicated twice were planted at the International Atomic Energy Agency Laboratories in Seibersdorf, Austria. One set of treatments was planted in the incubator at 15°C for 2 months while the second set was left on bench tops in the greenhouse at 28°C, under a luminosity of 3500 klux and a humidity of 70%. The 3 parents, ' Rojofotsy (1285), IR58614 and Malady (2509), and the local cold tolerant variety, Latsibavy (widely grown at Faratsiho, the coldest region of Madagascar) were used as controls and reference.

Field Evaluation

The experiments were carried out at the experimental field of the Faculty of Sciences, University of Antananarivo, Madagascar. Single young seedlings aged 21 days were planted, with a spacing of 25 cm × 25 cm and forming a square to facilitate weeding-out on the 23rd of June 2001. A mixture of 60 kg/ha of NPK (16-22-11), 45 kg/ha of urea and 45 kg/ha of a local biological fertilizer called ' Taroka' was applied.

The percentage of surviving seedlings was recorded for each variety and each dose. Among the surviving lines, those which showed good size and shape of panicle, high rate of fertile seeds and low rate of sterile seeds per plant were selected. The seeds of the selected lines were used for the 2nd selection in the field the following year.

Multiplication and yield evaluation

The variants that germinated and survived under the cold treatments were considered putatively cold tolerant mutant lines and tested further under field conditions leading as well to multiplication of the seeds. For the field trials, an area of 100 m^2 was divided into 3 plots, corresponding to 3 replications. Each putative cold tolerant line was planted on 2.5 m row containing 40 plants in each of the 3 plots. There was 25 cm spacing between the plants and 25 cm spacing between the rows. The plots were separated by 50 cm. Data on plant height, number of tillers,

number of leaves per plant during vegetative phase, were collected from 10 randomly selected plants from each row. At maturity, data were also collected from the same 10 plants for percentage sterility of the spikelet, auricular distance, number of fertile seeds, number of sterile seeds per plant, 1000 seeds weight and disease symptom severity scores for *Pyricularia oryzae, Pseudomonas fuscovaginae, Trischipa sp*. Yield was calculated according to Vilain (1988), that is, Yield (t/ha) = Total number of plants /ha × average number of spike per plant × average seeds number per spike × 1 grain of paddy average weight (g) .

Statistical analyses

Data were subjected to analysis of variance (ANOVA) and Correspondence Factorials Analysis (AFC) using version n°4 of the computer programme, STATITCF. The means were compared using the NEWMANN-KEULS test and inferences of statistical significance made at a threshold 5%; and the PEARSON X^2 test (Benzécri, 1973).

Results and discussions

In vitro mutagenesis

Callus induction

Callus induction in eight different types of medium

Table 3 summarises the production of calli by the 3 rice varieties on the 8 callus induction media evaluated. All of the varieties produced calli when cultured on each of the media, with a range of 13-100% of the plated seeds callusing. The exception was for medium A that did not support callus induction for the Rojofotsy variety. The F and G media (100%) followed by G and H (85%) were the best for callus induction for variety IR 58614 *indica* sub species while for variety Malady *japonica* sub species, calli were induced on all media tested at up to a rate of 90% of the plated seeds. For the Rojofotsy variety *(indica* sub species), the media F, B and G showed percentages of induced calli ranging from 70 to 90% implying therefore that media compositions F and G were the most appropriate for the induction of callus formation in the Malagasy *indica* rice varieties Rojofotsy and IR 58614 used in this study. In general however, the media composition F, (LS - Linsmaier and Skoog, 1965 basal medium supplemented with 2 mg/L of 2,4-D, 1 mg/L of thiamine-HCl, 100 mg/L of inositol, 30 g/L of sucrose and 8g/L of agarose I-A) yielded the highest callus induction for all varieties in this experiment, achieving up to 90-100% of callus induction from the plated seeds. These differing results suggest an interaction between genotype and media composition for the induction of calli from Malagasy rice seeds, an inference that accords with earlier suggestions (Andrianjaka *et al*., 2000; Rakotoarisoa, 2001).

Table 3. Percentage of induced calli and embryogenic calli of the 3 varieties on the 8 callus induction media

Variety	% of induced calli on 8 media							
	A	B	C	D	F	G	H	I
IR 58614 *indica* ssp	58	56	50	80	100	100	85	67
Malady *japonica* ssp	83	75	90	88	90	90	90	60
Rojofotsy: *indica* ssp	0	76	28	55	90	70	60	38
	% of induced embryogenic calli on 8 media							
IR 58614: *indica* ssp	60	56	60	45	9	2	4	0
Malady *japonica* ssp	9	4	12	8	70	38	60	64
Rojofotsy: *indica* ssp	0	5	0	40	62	30	14	29

Induction of Embryogenic calli

Embryogenic calli contain globular cells, are nodular, shiny and watery in appearance. These calli are able to produce plantlets if environmental conditions are favourable. Table 3 shows that most of the media supported the induction of embryogenic calli for the varieties studied except media composition I for IR58614; and A and C for Rojofotsy varieties.

For IR58614 variety, media compositions A, B, C and D supported the most copious production of embryogenic calli with a range of 45 to 60%. It is probable that this was because of the similarity in the composition of the 3 media with each containing MS basal medium supplemented with 2 mg/L of 2.4-D. The D and F media were the most favourable for embryogenic callus production for the Rojofotsy variety with a range of 40-60%. Thus, the induction of embryogenic calli in the Malagasy rice *indica* sub species could be deduced to be dependent on genotypic response. With the embryogenic calli induced from the variety Malady *japonica* sub species at the range of 60 to 70%, media compositions F, I and H were considered to have elicited its production most. Also the F medium was the overall best for the induction of em-

bryogenic calli for all the varieties except IR 58614. The conclusion therefore is that callus induction is influenced by interplay between the composition of the induction medium and the genotypic response of the test variety while the reversal to embryogenesis by the callus is controlled solely by the composition of the media. Zapata-Arias (1998) have reached the same conclusions based on his work with *indica* rice varieties.

Effects of irradiation on calli

Table 4 gives the rate of production of embryogenic calli for the different temperature regimes, 28°C and 16°C. At 28°C, all the irradiated calli from the two varieties IR 58614 and Malady presented a rate of embryogenic calli formation superior or equal to 50% with some reaching up to 70%. This demonstrates that a temperature of 28°C would be about the optimal for callus development within a period of 1 month. Regarding the impact of irradiation on callus development, for IR58614 and Rojofotsy varieties *indica* sub species, the higher the radiation dose, the higher the rate of induced calli. By contrast, this trend was reversed for the *japonica* subspecies Malady, where there was an inverse relationship between the dose of irradiation and the rate of callus induction. At 16°C, the rate of induced embryogenic calli for IR 58614 was slightly less, 40 to 60%, than when incubated at 28°C for the same duration. For Malady and Rojofotsy, there was no difference compared to those incubated at 28°C.

Table 4. The production of embryogenic calli at the different temperature regimes

Variety	Dose of irradiation	28°C (Month 1)	28°C (Month 2)	16°C (Month 3)	12°C (Month 4)
IR 58614	0 Gy	55	35	40	22
japonica ssp	10 Gy	58	37	48	25
	15 Gy	72	41	60	35
	20 Gy	70	42	59	36
Malady	0 Gy	65	33	70	30
japonica ssp	10 Gy	58	28	52	22
	15 Gy	49	27	49	20
	20 Gy	50	26	30	11
Rojofotsy	0 Gy	29	13	29	2
japonica ssp	10 Gy	35	16	50	5
	15 Gy	43	28	51	8
	20 Gy	62	30	69	11

After leaving the cultures to incubate for 2 months, the rates of production of embryogenic calli for all 3 varieties at both temperatures decreased relative to the values observed following incubation at 16°C for1 month. These trends imply that prolonged treatment at 28°C, beyond the optimum of 1 month, had even more drastic effects on the totipotency of the cells than the effects caused by short duration cold treatments (incubations at 16°C).

Regeneration of plantlets

The regeneration of plantlets from the different irradiation dosage and temperature treatments are summarised in Table 5. It was observed that for IR 58614, after incubation at low temperature for 2 months, plantlets were regenerated from only the embryogenic callus arising from calli irradiated at 20 Gy. For Malady, plantlets were recovered from calli that had been irradiated at all the doses used in the study and with no plantlets arising from the control. Interestingly, the rate of plantlets regeneration decreased with increase in irradiation dose. It is noteworthy that the indica Rojofotsy variety did not yield any plantlets from either the control or the irradiated calli. It seems plausible therefore to infer that irradiation did not enhance totipotency for this variety as the cold treatment seemed to have led to a complete loss of the cellular totipotency of the calli, which therefore could not regenerate any plantlets.

The overall rate of regeneration of plantlets for all varieties was relatively higher among the samples incubated at 28°C than from those incubated at 12°C (Table 5). The data suggest that irradiation of calli had a stimulatory effect on plant regeneration after both prolonged incubation as well as incubation under low temperature regimes. Also, both the duration and temperature of incubation seemed to have had debilitating effects on the totipotency of the cells as the calli that were transferred immediately to regeneration media yielded an average regeneration rate of 60% compared to 25% for those incubated at 28°C for 2 months and 0% for those incubated at 16°C then at 12°C for 1 month each treatment (for Rojofotsy). Similar

trends as before seemed to hold true with irradiation appearing to stimulate plantlet regeneration which is modulated however by variations in genotypic response and probably an interaction between the genotype and the media composition (Table 5). An interesting observation deserving further exploration is the seeming contrasting responses to irradiation in terms of plantlet regeneration elicited in the *japonica* and *indica* sub-species. The data suggest an inverse relationship between irradiation dose and plantlet regeneration in the former compared with the direct relationship observed for the latter. Anon (1972) had recommended the immediate culturing of irradiated explants in order to minimise damage, a position that has been corroborated by the data in this study in which calli transferred immediately to the regeneration medium consistently produced more plantlets than those kept for longer durations in the same medium

Table 5. The percentage of plantlet regeneration from different calli derived from irradiation dosage and temperature treatment

Variety	Dose of irradiation	Treatment at 28°C during 2 months		Treatment at 16°C (1month) then at 12°C for (1 month)		Transferred directly onto the regeneration medium	
Medium		K	L	K	L	K	L
IR 58614	0 Gy	0	0	0	0	37	34
indica ssp	10 Gy	8	6	0	0	52	32
	15 Gy	14	9	0	0	54	15
	20 Gy	25	14	25	0	51	12
Medium		J		J		J	
Malady	0 Gy	0		0		35	
japonica ssp	10 Gy	25		20		32	
	15 Gy	15		14		25	
	20 Gy	7		2		10	
Medium		K	L	K	L	K	L
Rojofotsy	0 Gy	0	0	0	0	0	1
indica ssp	10 Gy	4	13	0	0	24	33
	15 Gy	16	15	0	0	24	25
	20 Gy	20	25	0	0	28	60

Agronomic evaluations in the field

Seed germination

Table 6 summarises the percentage of germination of seeds harvested from plantlets from the 3 varieties subjected to various treatments. Irradiation at low doses seemed to have stimulated seed germination in the Malady variety as the highest percentage of 80, higher than 70% for the control, was recorded for seeds originating from 10 Gy irradiation. The trend was different for IR 58614 where irradiation seemed to decrease the rate of germination, 69% compared with 83% for the control.

Table 6. Percentage germination of seeds from rice varieties

Variety	% of germination
Malady 0 Gy	70
Malady 10 Gy	80
Malady 15 Gy	72
IR 58614 -0 Gy	83
IR 58614-20 Gy	69

Comparative growth and development of the putative mutants

Survival rate under cold regimes

The putative mutants, their parents (control) and the cold tolerant check variety Latsibavy were grown in the field at an average minimum temperature of 10°C and a very low precipitation, 2.1 mm. The control materials all died within 6 weeks with the rapid yellowing and withering of the leaves culminating eventually in the death of the plants. On the other hand, the putative cold tolerant mutant lines, Md1-10 Gy n°2, Md1-15Gy n°3 and the line M1-IR-20Gy n°1, grew vigorously with green leaves and following the standard sigmoid curve (data not shown). Table 7 shows the percentage survival of the seedlings sown in the field. The putative mutant Md1-10Gy had a survival rate of 82% while all the controls (non-irradiated parents) had died. In general, the *japonica* sub-species tolerated the low temperatures in the field better than the *indica* sub-species, a finding that corroborates the earlier work of Vergara (1970).

Table 7. Percentage survival of the seedlings sown in the field

Variety	% of the survival seedlings sown in the field
Malady 0 Gy	0
Malady 10 Gy	82
Malady 15 Gy	45.4
IR 58614-0 Gy	0
IR 58614-20Gy	29

Agronomic characters

Table 8 shows a summary of the data on selected agronomic traits for the putative mutants, the control parents and the check variety. The progeny from the calli incubated at 28°C were taller, had more fertile seeds per panicle, longer auricular distance than those from calli incubated at 15°C. The 1000 seed weights of progeny from both temperature regimes were comparable. While irradiation led to the reduction in plant heights of the putative mutants, the seed set and auricular distance of the putative mutants were higher than in their controls. The yields of the putative mutant lines M4-Md -10 Gy, M4-Md-15 Gy and M4-IR-20Gy were 4 t/ha, 4.5 t/ha and 5.4 t/ha, respectively. These figures were higher than the yields of their respective parents (control) that were 3 t/ha, and 4 t/ha, respectively.

The objective of this study, to develop putative cold tolerant mutant rice lines by *in vitro* mutagenesis, was successful as putative mutants with high germination percentages, that produced reasonable yields, and were more fertile under cold regimes were developed. Their cultivarion can reduce the auto sterility of the spikelet and can ensure double cropping of the same land in a year. The duration of their vegetative cycle was 174 days for the putative mutant lines M4-Md-10 Gy and M4-Md-15 Gy developed from Malady; and 180 days for the putative mutant line M4-IR-20 Gy developed from IR 58614. With harvests in December and January for Malady and IR 58614 respectively, a second crop on the same land could be established by the end of January for the ‘ vakiambiaty’ planting season.

Table 8. Data on selected agronomic traits for the putative mutants, the control parents and the check variety

Variety	Malady (CK)	Malady 10 Gy		Malady 15 Gy		IR58614 (CK)	IR58614 20 Gy	
	28°C	28°C	16°-12°C	28°C	16°C-12°C	28°C	28°C	16°C-12°C
Height (cm)	78	74	59	64	60	77	75	62
Fertile seeds number / panicle	47.1	64.2	52.3	42.2	41.3	47.1	58	58
Auricular distance (cm)	9	12.2	7	10	7.5	14.4	17.7	15
1000 seeds weight (g)	24.2	26	26	22	22	22	24	24
% Sterility of spikelet /panicle	14.2	10.4	9.7	14	10	22.4	10.8	10.7
Yield (t/ha)	3	-	4	-	4.5	4	-	5.4

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Short Communication

Cuban Rice Mutants Obtained from Protons Radiations

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Abstract

Rice mutation breeding can be considered especially successful in obtaining new cultivars and broadening the genetic base of this crop. In order to obtain new Cuban varieties of rice with either improved agronomic characteristics or biotic and abiotic stress tolerance, proton radiation was used to generate novel mutants of the Amistad-82 rice variety. Amplified fragment length polymorphism (AFLP) techniques were used to evaluate the genomic diversity among the mutants and the Amistad-82 variety. Several rice mutants were obtained and the use of AFLP markers confirmed the adequacy of proton radiation for inducing genetic variability in rice

Key words: rice, mutants, proton radiation, AFLP markers

Introduction

Rice (*Oryza sativa* L.) is one of the most important crops in the world. Rice is planted on about one tenth of the earth's arable land and is the single largest source of food energy to half of humanity (Eckardt, 2000; Kurata and Yamazaki, 2006).One of the major limitations of Latin American rice breeding programs is their narrow genetic source. The use of a narrow source of germplasm has become a common trend in breeding programs.

Rice mutation breeding can be considered especially successful in obtaining new cultivars with good agronomic characteristics, plus either resistance to biotic stress and/or tolerance to abiotic stress, and in broadening the genetic base of this crop (Alvarez *et al.,* 2000), however, no rice mutants obtained by proton radiation appear to have been reported.

This work was undertaken to evaluate the agronomic characteristics of selected mutants obtained by proton radiation and to determine the genetic diversity among these mutants using amplified fragment length polymorphism (AFLP) techniques.

Materials and methods

Dry seeds from the Amistad-82 rice variety were irradiated with 20 Gy of protons in DUVNA**.** The irradiated seeds were cultured on MS medium with 2 $mg.L^{-1}$ 2-4D, 2 $mg.L^{-1}$ BAP, 100 $mg.L^{-1}$ inositol, 30g refined sugar and 2 g Gelrite. The cultures were incubated at 25 ± 2 ºC in the dark for callus induction. The calli were transferred to MS medium supplemented with 2 $mg.L^{-1}$ kinetine, 2 $mg.L^{-1}$ AIA, 100 $mg.L^{-1}$ inositol, 30g refined sugar and 2 g Gelrite.

A bulk harvest was obtained of the M_1 population and promising lines were selected in M_2 to M_5 generations.

Preliminary yield trial (M_5 generation)

Five rice mutants plus the Amistad-82 rice variety (donor) were sown in the field at 'Los Palacios' Rice Research Station in a random block design with three repetitions, using plots of 8 m^2. Cultural practices followed the Technical Pattern for Rice Cultivation. Final height, cycle, agricultural yield ($t.ha^{-1}$ at 14% humidity), industrial yield (% of whole grains in a sample of 1 kg) and lodging resistance were dtermineddetermined.

Statistically significant differences were detected through Variance Analysis, using Duncan's Multiple Range Test with $p \leq 0.05$ (Cochran and Cox, 1971).

Molecular evaluations

DNA extraction -

LLeaves from 45 day-old plants of the five mutants and Amistad-82 variety were immediately frozen in liquid nitrogen (N_2L) and stored at -70°C for DNA extraction. Three g of leaves were powdered under liquid N_2 and DNA was isolated by the procedure of Dellaporta *et al.* (1983). The quality of the extracted DNA was evaluated by electrophoresis on 0.8% agarose.

AFLP analysis -

The AFLP method of Vos *et al.,* (1995) was performed using the AFLP Analysis System I Kit (GIBCO BRL, Life Technologies). Genomic DNA was digested with an *Eco*RI/*Mse*I enzyme combination. The preamplification step was carried out with AFLP primers having one selective nucleotide (*Eco*RI+A, *Mse*I+C). Selective amplification was performed with three selective nucleotides (*Eco*RI+ANN, *Mse*I+CNN). Four primer combinations (E-ACG/M-CAG, E-AGC/M-CAG, E-AGC/M-CAC, E-ACG/M-CTC) were tested. PCR samples were denaturated by adding an equal volume of formamide buffer (98% formamide (v/v), 10 mM EDTA, pH 8.0, 0.05% bromo-phenol blue (w/v), and 0.05% xylene cyanol (w/v), heating for 5 min at 93°C and then chilling on ice. The samples were loaded on a 6.5% polyacrilamide gel under standard sequencing conditions. AFLP fingerprints were visualized using the silver nitrate staining method according to the manufacturer's instruction (Promega). Each primer combination was scored by eye for the number of polymorphic fragments detected and overall sharpness and intensity of polymorphic fragments. The scored fragments ranged in size from 200 to 700 base pairs (bp). The size of the fragments was determined by comparing sequencing ladders of control template DNA to AFLP patterns.

Only polymorphic and clearly repeatable bands between 200 and 700 bp were used for analysis. Gels were scored visually for the presence (1) or absence (0) of bands. Genetic distance (GD) was calculated according to the Dice coefficient. Associations among lines were determined from cluster analysis based on *GD* estimates. The unweighted pair group method with arithmetic averages (UPGMA) was used for hierarchical clustering using appropriate procedures of the computer package NTSYS-PC version 1.8 (Rohlf, 1993).

Results and dDiscussion

Performance in preliminary yield trial

All the selected mutants showed greater lodging resistance than the Amistad-82 variety. The genotypes 8551, 8552, 8553 and 8554 had greater agricultural yield than the Amistad-82 variety, particularly genotypes 8552 and 8554. The 8554 genotype had greater percentage of whole grains than other genotypes (Table 1).

The rice variety Amistad-82 had been eliminated from National Rice Production because of its lodging susceptibility. Mutations induced by proton irradiation made possible high productive potential with lodging resistance**.**

These appear to be the first beneficial mutants developed by proton radiation according to the IAEA database.

Table 1. Agronomical behavior of mutant and donor variety in field conditions

Genotype	Final Height (cm)	Cycle (days)	Yield		Lodging resistance
			Agricultural (t/ha^{-1})	Industrial (% whole grains)	
Amistad-82	80,15	125	6,5	55 bc	S
8551	83,44	130	7,2	57 b	R
8552	82,52	134	8,0	55 bc	R
8553	83,18	135	6,6	54 c	R
8554	78,25	135	7,8	60 a	R
8555	79,18	135	5,8	55 bc	R
ES x	2,92 ns		0,78	1,95	

S: susceptible; R: resistant

Molecular evaluation

AFLP analysis effectively detects large numbers of polymorphic genetic loci in a single PCR reaction. The number of scored bands and polymorphic bands obtained for the different primer combinations used is showed in Table 1. The four primer combinations tested produced good polymorphism results showing a total of 200 bands with fragments sizes ranged from approximately 50 to 850 bp. One hundred and three bands, ranging from 200 to 700 bp, had a good sharpness and intensity able to discriminate the polymorphic bands. Of these, 33 bands were clearly polymorphic between two or more genotypes for 32.03% of amplified polymorphic fragments per primer combination. On average, 8 polymorphic bands were scored per primer pair, with a range of 4-14 (26.6 to 35% of polymorphism) (Table 2).

Table 2. Total scored bands and polymorphic bands obtained for the different primer combinations used

Primer combination	Bands scored 200-700 bp	Polymorphic bands	Polymorphism (%)
E-ACG / M-CAG	45	14	31.1
E-AGC / M-CAG	20	7	35
E-AGC / M-CAC	15	4	26.6
E-ACG / M-CTC	23	8	34.7
Total	103	33	32.03

Analyses of the genetic distance between lines based on the Dice index allowed us to define 2 groups. The dendrogram (Fig. 1) depicts the clustering of rice mutants into two groups of individuals as well as a clear distinction between mutants and the variety Amistad-82. Cluster A is composed by three mutants (8551, 8553 and 8554), whilst 8552 and 8554 mutants are present in cluster B, showing a very small genetic distance between them. The variety Amistad-82 (A-82) appears ungrouped and more genetically distant from all mutants.

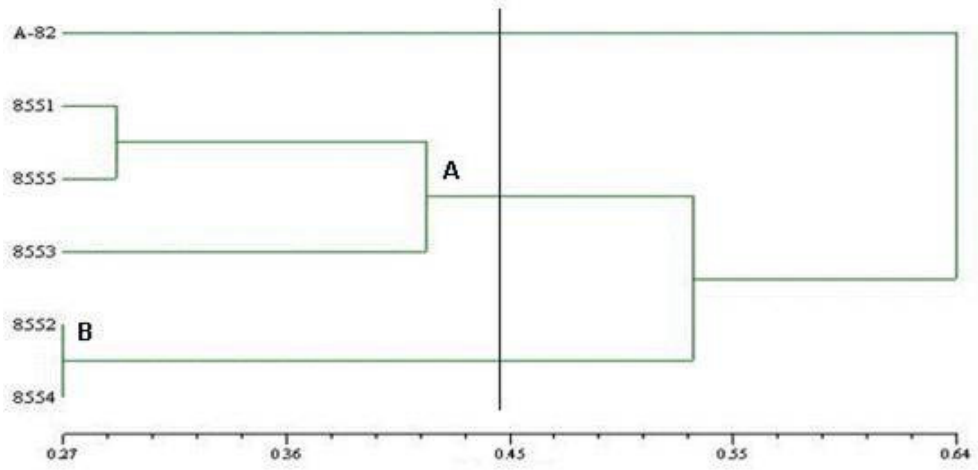

Figure 1. Dendrogram of the six rice genotypes revealed by UPGMA cluster analysis of AFLP-based genetic distance (Dice's coefficient) calculated on the basis of 33 polymorphic AFLP bands.

The level of variation detected using AFLP techniques depends on the combination of restriction enzymes selected for the first stage, on the number of combinations of primers used, and on the genetic distance between the genotypes analyzed. The AFLP technique permits the detection of the variation of many loci simultaneously. AFLP markers are widely used for the evaluation of genetic variation between genotypes with a differentiated degree of relatedness, especially between genotypes with a small genetic distance, such as between a variety and the mutants derived from it. In this case, the detection of a polymorphism requires the use of a sensitive marker system, due to the fact that an unusually low frequency of mutations occurs when agents and methods of mutation are used which do not lower plant viability and induce desirable changes in the genetic material (Witkowicz *et al.,* 2003).

In the present work was possible to identify some mutants with good agronomic behavior and to confirm the genetic diversity present in rice mutants using the method of amplified fragment length polymorphism (AFLP) as well as to demonstrate the adequacy of proton radiations for inducing genetic variability in rice.

Acknowledgements

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Research Article

Selection and Characterization of Tomato Mutants Tolerant to Low Water Supply

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Abstract

Genetic variability was induced in tomato (INCA-9-1) by means of ^{60}Co gamma rays and four high-yielding mutant lines were selected under low water supply conditions. Agronomic, biochemical and molecular evaluations were carried out for the selected mutants. The mutant lines had up to 100% yield advantage over their parent variety under low water supply. These mutants also had quality characteristics similar to, or better than, the parent and other commercial varieties. RAPD analysis showed that the genetic differences between mutants and INCA-9-1 might be larger than between mutant lines.

Key words: tomato, drought tolerance, induced mutations

Introduction

The climatic alterations associated with global changes occurring in recent years seem to affect considerably the rainfall pattern in many countries of the planet; consequently, there are more frequent severe and prolonged droughts (Science, Innovation and Development, 2001), which have become one of the most damaging factors affecting productivity of many crops, such as tomato, that are essential for feeding people in the world.

Tomato (*Lycopersicon esculentum* Mill) is the most important horticultural crop in many countries and its production surpasses 100 million tons per year in a 110 million-ha-seeding area. In Cuba, this crop occupies 42% of the vegetable-producing areas (FAOSTAT, 2006); however, most seedings are conducted in dry months when rainfall can not satisfy crop water demands. Irrigation is becoming a more expensive activity at world level, since it requires costly energy and technological resources. In addition to the enormous and growing difficulties to establish irrigation in crops, water restrictions sometimes reach dramatic levels in more arid regions. Just 15% of the cultivated land of the planet depends on irrigation, where 50% of the agricultural production is obtained (ACTAF, 2002).

Mutation induction has contributed significantly to plant breeding at world level (Maluszynski *et al*., 1995; Nichterlein, 2000). Keeping in mind the low level of genetic diversity in tomato (Saavedra and Spoor, 2002), mutation induction constitutes a valuable strategy to create genetic variability, which in turn reduces the time required to breed new varieties compared with traditional methods (Cornide, 2001). A breeding program was developed in order to enhance tomato genetic variability by using ^{60}Co gamma radiations and to select new high-yielding mutant lines under low water supply conditions.

Materials and methods

Based on radiation sensitivity studies, two doses (300 and 500 Gy) were chosen to irradiate 3 000 seeds per dose of the INCA-9-1 tomato variety in an MPX-25 irradiator with a power of 11.3 Gy.minute^{-1}. Thirty days after seed germination, plants taller than 10-cm-were transplanted to field conditions. At harvest time, four fruits per plant were collected in bulk.

The selection of high-yielding potential mutant lines was made under low water supply conditions (Dell'Amico, 1992). Selection of individual plants was done every generation from M_2, to M_5 under field conditions, taking into account the following visual criteria: healthy plants, determinate growth habit, greater number of fruits per plant and/or bigger fruit compared to the pareantal variety. Yield per plant, fruit number per plant, average fruit weight, equatorial and polar fruit diameters were recorded in individually selected plants. The best genotypes were chosen by means of multivariate and genetical distances (Sigarroa and Cornide, 2002), according to the highest-yielding genotypes.

Morphological and agronomic evaluation

One hundred seedlings per genotype of the selected mutant lines at the M_5 generation along with the parental variety (INCA-9-1) were transplanted in a randomized block design with four replications. Only three irrigations were applied. At harvest time, yield per plant, fruit number per plant and average fruit weight were evaluated in a sample of 10 plants per line. A sample of 10 mature fruits per genotype were taken for analyses of total soluble solids (Brix), acidity, dry matter and water content

Biochemical and molecular evaluation

Seeds from the selected genotypes (20 per genotype) were sown along with the parental variety (INCA-9-1) in 50x50-cm^2 boxes containing Eutric Compacted Red Ferralitic soil, which was sterilized and kept under glasshouse conditions at a temperature of 23+/-2^{0}C, relative humidity between 80-85% and natural photoperiod at CENSA. Thirty days after seed germination, from five to seven young leaves per genotype were sampled randomly, in order to extract proteins according to the procedure described and standardized by Solorzano (2002). Protein concentration was determined by the Bradford method (1982). The specific enzymatic activities (µmoles.mn^{-1}.mg^{-1}) of polyphenoloxidase (PPO), peroxidase (PO), chitinase, ß 1-3 Glucanase and phenylalanine ammonium liase (PAL) were calculated as described by

Solorzano (2002). For determining random amplified polymorphism (RAPD), DNA was extracted following the protocol of Dellaporta *et al*. (1983). Amplification took place in a Progene Techme thermocycler programmed for 45 cycles of one minute at 94°C, one minute at 36°C and two minutes at 72°C, and a cycle of 10 minutes at 72°C.

PCR products were visualized following 1,5% agarose gel electrophoresis in a TBE 0.5X buffer solution and stained with ethidium bromide before being observed in a transiluminator, classifying the most intensive bands as present [1] or absent [0] in each genotype, so creating a matrix of binary values. To determine the degree of similarity in the analyzed genetic material, the similarity coefficient of Nei and Li (1979) was used, to later build a dendrogram by means of a nonweighed arithmetic average link (UPGMA).

In case of significant differences detected through Variance Analysis, Duncan's multiple range test (Duncan, 1955) was applied, with $p \leq 0.05$.

Results and discussion

Selection of mutant lines

Genotypes were grouped according to yield. In the M_2, M_4 and M_5 generations, two groups were formed whereas in M_3, there were three groups. Table 1 gathers yield variation ranges per generation. Groups of 11 genotypes in the M_2 generation with an average yield of 2540,45 g per plant, 10 genotypes in M_3 with yield of 3313,50 g per plant, eight genotypes in M_4 with an average yield of 2939,75 g per plant and four genotypes in M_5 with an average yield of 2626,23 g per plant, were identified as high-yielding. The donor variety INCA-9-1 was markedly inferior to these genotypes, reaching yield values of 1385,00; 1446,80; 1464,00 and 1137,33 g per plant, respectively.

Table 1. Mean yields per plant (g) and standard deviations (g) of each group

Generations	Groups	Genotypes (L)	Mean ± Deviations
M_2	I	7 L300 + 4 L500	2540,45 ± 213,19
	II	8 L300 + 10 L500 + varieties	1712,43 ± 363,96
M_3	I	8 L300 + 2 L500	3313,50 ± 275,69
	II	1 L300 + 7 L500	2481,25 ± 221,76
	III	Varieties	1432,27 ± 183,99
M_4	I	8 L300 + 4 L500	2825,00 ± 196,76
	II	varieties	1518,00 ± 200,52
M_5	I	8 L300 + 3 L500	2358,12 ± 233,28
	II	varieties	1230,23 ± 80,76

Varieties: INCA-9-1, Amalia and Campbell-28
L 300 : lines selected in the field from INCA 9-1 variety irradiated with 300 Gy
L 500 : lines selected in the field from INCA 9-1 irradiated with 500 Gy

Yield performance of selected lines

Highly significant differences ($P \leq 0,01$) were observed among the mutant lines, their parent, and other commercial varieties for yield per plant, fruit number per plant and average fruit weight (Table 2). R4-300 and R19-500 had higher yields per plant compared with their parent INCA-9-1 and commercial varieties (Amalia and Campbell-28). So far as the fruit number per plant is concerned, the behavior was similar to the one recorded in yield per plant, except the insignificant differences among average values reached by R4-300 and INCA-9-1. Regarding average fruit weight, all selected genotypes significantly surpassed INCA-9-1. The co-variation of selected mutant lines, with respect to yield per plant, was relatively smaller than INCA-9-1, indicating the mutant lines had better yield stability (Table 2).

Fruit quality performance

Regarding fruit quality characteristics, shown by evaluating total soluble solid content (brix), acidity, brix/acidity ratio as well as dry fruit weight content, there were variations among the selected mutants, which formed two groups (Table 3).

Table 2. Average yield, average fruit number per plant and average fruit weight in the material analyzed

Genotypes	Yield/		Fruit number/plant	Fruit average weight (g)
	gram per plant	CV (%)		
INCA-9-1	1348,07[e]	11,46	32,70[d]	41,61[f]
R19-500	2639,95[a]	5,13	39,30[c]	67,17[d]
R4-300	2716,57[a]	5,05	33,10[d]	82,15[b]
R20-300	2160,47[b]	1,34	53,10[a]	40,70[f]
R16-300	2051,62[c]	1,74	46,10[b]	44,63[e]
AMALIA	1554,45[d]	4.67	17,60[e]	88,45[a]
C-28	1269,96[e]	4.76	16,70[e]	76,26[c]
± E.S.	32,90***		0,60***	0,96***
VC (%)	5,30		5,64	13.56

Means with common letters do not differ significantly, according to Duncan's multiple range test at 5%
***Significant at 1% ($p < 0,001$)

Table 3. Index values of internal fruit quality per genotype

Group/Subgroup	Genotypes	Brix (B) (%)	Acidity (A) (%)	Dry weight (%)	Water content (%)
A	R4-300	7,12	0,56	7,78	92,22
B/1	INCA-9-1	6,52	0,50	7,12	92,88
	R19-500	6,12	0,50	7,22	92,78
	R20-300	6,52	0,53	7,27	92,73
Means per subgroup 1		6,38	0,51	7,20	92,80
B/2	R16-300	6,12	0,46	6,67	93,33
	Campbell-28	5,92	0,46	6,08	93,92
	Amalia	6,12	0,56	6,48	93,52
Means per subgroup 2		6,05	0,49	6,41	93,59

Varieties: INCA-9-1, Amalia and Campbell-28
R4 300, R20-300 and R16-300 : Genotypes selected from INCA 9-1 variety irradiated with 300 Gy
R19-500: Genotype selected from INCA 9-1variety irradiated with 500 Gy

Quality attributes of the R4-300 mutant surpassed those of all other genotypes and donor variety (INCA-9-1) as well as other commercial varieties (Campbell-28 and Amalia). In general, both selected genotypes and varieties obtained brix, acidity and brix and acidity ratio values within the range for commercial cultivars (Pratta *et al*., 1996).

Based on the established parameters for internal fruit quality, all genotypes could be appraised for their industrial use and R4-300 genotype as a double purpose variety, when its average fruit weight is considered.

Biochemical and genetic evaluation

Specific activity of several enzymatic systems

Figure 1 shows a dendrogram based on enzymatic activities (polyphenoloxidase, ß1-3 Glucanase, chitinase, peroxidase and phenylalanine ammonium liase) in different genotypes. Two main groups were observed, the first constituted by R16-300, R20-300, R4-300 and R19-500 genotypes and the second by the donor variety INCA-9-1. Thus, it can be concluded that all selected genotypes differ from INCA-9-1 in their enzymic activities, which implies that at the biochemical level, selected genotypes are new phenotypic variants. Within the first group, the greatest relatedness was shown by R16-300 and R20-300 genotypes, followed by R4-300 and then R19-500.

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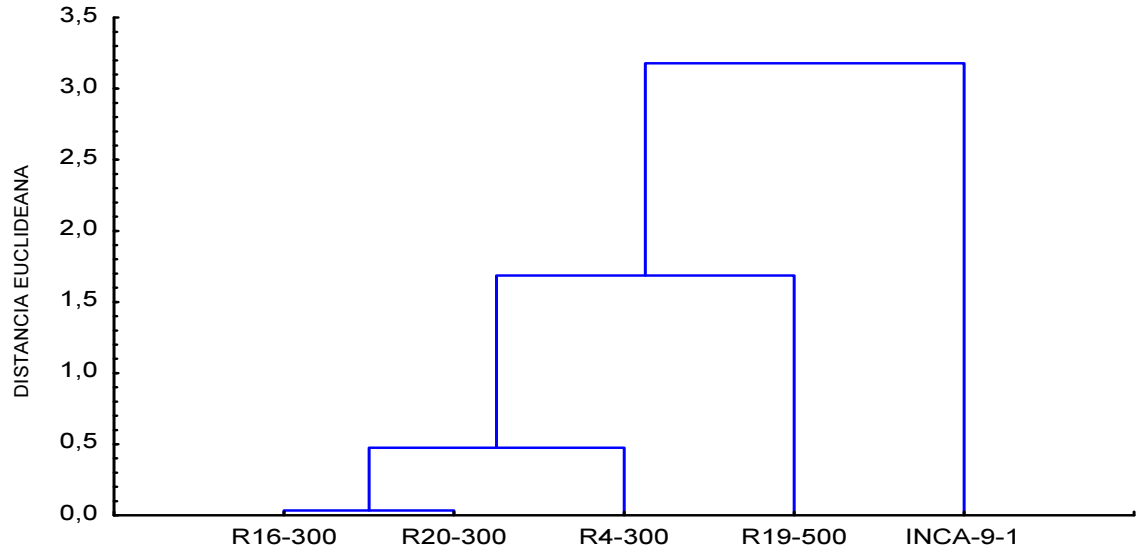

Figure 1. Dendrogram derived from similarity indexes in the mutants and donor variety INCA-9-1.

Genetic analysis

The 14 OPA and OPF primers used produced 90 bands upon amplification (Table 4), 58 of which were polymorphic. OPF 06, OPF 13, OPF 14 and OPF 15 primers were most informative, with 100% polymorphic bands.

Table 4. Amplification products obtained using RAPD technique in DNA through the polymerase chain reaction (PCR)

Primer	Sequence	Total of bands	Polymorphic bands	Polymorphism percentage
OPA 02	TCCCGAGCTG	6	4	67
OPA 03	AGTCAGCCAC	5	3	60
OPA 12	TCGGCCATAG	4	1	25
OPA 13	CAGCACCCAC	5	0	0
OPF 01	ACGGATCCTG	9	4	44
OPF 03	CCTGATCACC	5	1	20
OPF 04	GGTGATCAGG	4	2	50
OPF 05	CCGAATTCCC	5	2	40
OPF 06	CGGAATTCGG	9	9	100
OPF 07	CCGATATCCC	3	0	0
OPF 10	GGAAGCTTGG	8	5	63
OPF 13	GGCTGCAGAA	14	14	100
OPF 14	TGCTGCAGGT	4	4	100
OPF 15	CCAGTACTCC	9	9	100

Two main groups were observed in the dendrogram derived from similarity indexes calculated within each pair of genotypes employed (Figure 2). The first group was formed by INCA-9-1 donor variety and the R19-500 genotype, whereas the second one was formed by the R16-300, R20-300 and R4-300 genotypes. The similarity degree recorded between R19-500 and INCA-9-1 donor variety might be due to the fact that primers used in this study do not amplify to other regions with more remarkable differences between these two genotypes, which are located in regions of the genome not yet explored by the primers. In contrast, it was observed that R16-300, R20-300 and R4-300 genotypes differed markedly from the parental variety, thus, dominant variations were induced in these genotypes.

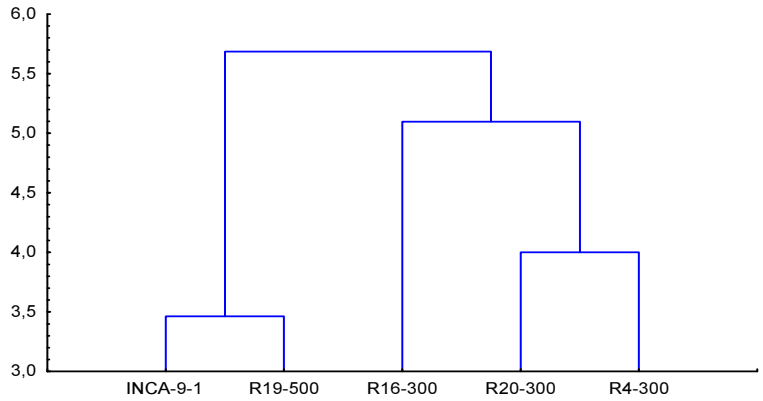

Figure 2. Dendrogram derived from genetic distances in the material studied DNA polymorphic markers of Random Amplification (RAPD).

Thus, genetic relatedness recorded by means of RAPD technique between the donor variety and the R16-300, R20-300 and R4-300 genotypes resembled the biochemical relatedness (Figure 52). Similarly, the R19-500 genotype was closer in both genetic and biochemical relatedness to the donor variety than other mutant genotypes.

Peteira *et al.* (2001) and Florido *et al.* (2002) detected little natural genetic variability in tomato. However, using induced mutation it has been possible to increase the genetic variability and obtain new tomato genotypes. Based on the large differences between the selected mutants and the donor variety in their agronomic, morphological and biochemical characteristics, together with the RAPD analysis, it can be concluded that R16-300, R20-300 and R4-300 genotypes are beneficial mutants and that mutation induction can be used effectively to obtain drought tolerant tomato varieties.

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Research Article

Broadening the Genetic Base and Introgression of MYMV Resistance and Yield Improvement through Unexplored Genes from Wild Relatives in Mungbean

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Abstract

Introgression of unexplored genes from the wild relatives could be rewarding for broadening the genetic base of important traits such as yield, yield attributes and resistance to biotic and abiotic stresses in pulses. Aimed at developing superior segregants for yield coupled with yellow mosaic virus resistance (MYMV), interspecific direct crosses were attempted in *Vigna radiata* var. VRM (Gg) 1 with two accessions of *Vigna umbellata (*yellow and red). Even though crossability barriers were predominant, it was possible to recover interspecific hybrids in direct crosses. F_1 plants of V. *radiata* x *V. umbellata* were found to be intermediate in phenotype with light green colour leaves. The reproductive parts tend to resemble V. *umbellate*, with double peduncle in one leaf axis. No pod set was observed when F_1s were selfed nor in their corresponding backcrosses with the parents. The F_1 plants produced more than 4000 flowers per plant, but spontaneous sterility was observed both in female and male parts of the flowers. Detailed cytological studies were carried out for male and female sterility. Male sterility was due to meiotic irregularities viz., unequal separation of tetrads and female sterility was due to degeneration of megaspore during megasporogenesis. Hence, irradiation techniques were applied to recover fertile plants in F_1 hybrids. The parental seeds were irradiated with 100, 200, 300, 400 and 500 Gy doses. The pod set percentage was increased due to irradiation. In normal crosses, pod set ranged from 2.00% (VBN (Gg) 2 × *Vigna umbellata* red) to 4.40% (VRM (Gg) 1 × *V. umbellata* red). In crosses resulted from irradiated parents, pod set ranged from 2.70% (CO 6 × *V. umbellata* yellow) to 4.90% (VRM (Gg) 1 × *Vigna umbellata* yellow) among crosses involving parents treated with 100Gy. Fertile F_1 hybrid plants were obtained from a cross between *Vigna radiata var. VRM (Gg) 1* and *V. umbellata red (*both parents treated with 200 Gy). The fertile F_1 phenotype was generally towards the female parent, but traits like orientation of top leaves, tendrilness and number of seeds per pod shifted towards the male parent.

Introduction

Vigna radiata (L.) Wilczek, commonly known as greengram or mungbean is the most widely distributed species among the six Asiatic wild *Vigna* accessions. The cultivated species *V. radiata* has desirable characters like short cycle duration, high yield, amenability for crop rotation and undesirable characters like susceptibility to bruchids and yellow mosaic virus, the latter provoking 100% yield loss on severely affected plants. There is therefore a need to improve the greengram by hybridisation with wild species (Boling *et al.,* 1961). Among the wild *Vigna* species studied, *V. umbellata* (rice bean) has high test weight and resistance to bruchids and yellow mosaic virus. However, the recovered F_1 is both male and female sterile, and to overcome this problem and recover fertile F_1s mutation studies were undertaken. The material generated through mutagenesis can also contribute as a reservoir of novel genes for an improvement of yield and yield components. Thus, this study was taken up to attempt coupling mutation and interspecific hybridization of *V. radiata* with species in secondary pools to generate variability for better yield and resistant to yellow mosaic virus, and to compare such variability created among the segregants generated.

Materials and methods

Two *Vigna* species, *V. radiata* (mungbean) and *V. umbellata* (rice bean) were used. Normal, *i.e.* non irradiated, and irradiated crosses of six accessions of *V. radiata* (VBN1, VBN(Gg)2, KM2, K1, VRM(Gg)1 and CO 6) as female parents and two accessions of *V. umbellata* (red [Vur] and yellow [Vuy]), both as male parents, were programmed. Parental seeds and F_0 seeds (seeds set after crossing) were irradiated with 100, 200, 300, 400 and 500 Gy doses. The crossing block consisted of three rows of female parents and two rows of male parents (raised two weeks before the female parents to synchronize flowering), spaced at 50 × 30 cm, during rabi 2006-2007. The trial was conducted at Tamil Nadu Agricultural University, National Pulses Research Centre, Vamban Pudukkottai, Tamil Nadu. India.

Pollen fertility was analysed in the parents and their hybrids by acetocarmine staining technique

$$\text{Pollen fertility} = \frac{\text{No. of viable pollen}}{\text{Total no. of pollen observed}} \times 100$$

Cytological studies of parents and their hybrids were performed. Flower buds (1-2 mm) were fixed in modified Carnoy fluid (Ethyl alcohol : Chloroform : Glacial acetic acid; 6 : 3 : 2, v/v) for 24 h, at 10-15^{o}C, washed and preserved in 70% ethanol. For preparing slides, the anthers were squashed in 2% acetocarmine and the slides were slightly warmed and observed under a transmission microscope. The chromosome association at meiosis was studied for the hybrids. Cells at diakinesis, metaphase and anaphase were examined to obtain the frequencies of univalents, bivalents and quadrivalents. Twenty five PMCs were observed for estimating the frequencies of chromosomal abnormalities.

Photomicrograhs were taken of the various abnormalities observed in the hybrids.

Results

The results with normal (non-irradiated) crosses are presented in Table 1.

Table 1. Performance of normal interspecific crosses of *Vigna radiata* x *Vigna umbellate**

Parents and Crosses	PF (No.)	PS (No.)	PS (%)	CSO (No.)	CSG (No.)	G (%)	SAM (No.)	HB (%)	HL (%)
VBN1 × Vuy	550	12	2.40	55	35	63.64	22	62.86	37.14
VBN1 × Vur	660	14	2.12	149	110	73.83	80	72.73	27.27
VBN(Gg)2 × Vuy	700	18	2.50	40	28	70.00	15	53.57	46.43
VBN(Gg)2 × Vur	650	13	2.00	101	85	84.16	68	80.00	20.00
KM2 × Vuy	750	19	2.50	29	12	41.38	6	50.00	50.00
KM2 × Vur	820	35	4.26	27	12	44.44	6	50.00	50.00
K1 × Vuy	575	16	2.70	119	73	61.34	60	82.19	17.81
K1 × Vur	653	18	2.70	132	85	64.39	65	76.47	23.53
CO 6 × Vuy	625	20	3.20	125	68	54.40	45	66.18	33.82
CO 6 × Vur	628	24	3.80	139	79	56.83	61	77.22	22.78
VRM(Gg)1 × Vuy	750	28	3.70	127	102	80.31	93	91.18	8.82
VRM(Gg)1 × Vur	850	38	4.40	134	106	79.10	98	92.45	7.55

*: *V. umbellata* yellow = Vuy, *V. umbellata* red = Vur; PF = pollinated flowers; PS = pod set; CSO = Crossed seeds obtained; CSG = Crossed seeds germinated; G = Germination; SAM = Seedlings attaining maturity; HB = Hybrid breakdown; HL = Hybrid lethality.

The maximum number of flowers emasculated and pollinated was 850 for the cross VRM(Gg)1 × Vur followed by 820 flowers in the cross KM2 × Vur. The number of pods set ranged from 12, in VBN1 × Vuy, to 38 in VRM(Gg)1 × Vur. The percentage of pod set ranged from 2.0 (VBN (Gg) 2×Vur) to 4.40 (VRM(Gg)1 × Vur). The highest number of seeds, 149, was yielded by the cross VBN 1 x Vur and the lowest, of 27 seeds, was produced by the cross KM2 × Vur. The pollen fertility percentage recorded in F_1s was zero. The highest hybrid germination was 84.16%, observed in the cross VBN(Gg)2 × Vur and the lowest of 41.38% was recorded in the cross KM2 × Vuy. The highest hybrid breakdown of 92.45% was recorded in the cross VRM(Gg)1 × Vur, and the highest hybrid lethality, of 50.00%, was observed in crosses having *V. radiata* KM2 as female, whichever the *V. umbellata* genotype used as male parent. The lowest hybrid lethality (of 7.5%) was recorded for the cross VRM(Gg)1 × Vur.

Table 2 gives the results observed in irradiated crosses.

The maximum number of flowers emasculated and pollinated was 900 in the cross VBN(G)2 (400Gy) × Vuy (400 Gy), followed by 895 flowers in the cross K1(500 GY) × Vuy (500Gy). The number of pods set ranged from 18 in five crosses, namely VBN (Gg)2 200Gy × Vuy (200 Gy), VBN (Gg)2 300Gy × Vuy (300 Gy), KM2 (400GY) × Vuy (400GY), CO6 (200 Gy) × Vuy (200 Gy) and VRM (Gg)1 (500Gy) × Vur (500 Gy), to 38 in K1 (200 GY) × Vuy (200 Gy). Percentage of pod set ranged from 2.6 in the cross CO6 (200GY) × Vuy (200Gy) to 4.90 (VBN 1 (300GY) × Vuy 300 Gy.

The highest number of seeds obtained was 80, for the cross VRM(Gg)1 (500Gy) × Vur (500Gy) and the lowest number of seeds obtained was 6, for the cross VBN 1 × Vur 500 Gy. The range of pollen fertility recorded in the F_1s was from 43% (VBN(Gg)2 300 GY × Vur 300 GY) to 75% (VRM(Gg)1 100 Gy × Vuy 100 Gy). The highest germination recorded was 90.63% in cross VRM(Gg)1 200Gy × Vuy 200Gy, and the lowest, at 28.57%, occurred in cross CO6 (100Gy) × Vuy 100 Gy. A hybrid breakdown of 93.3% was observed in the cross VRM(Gg)1 100Gy × Vuy 100 Gy. Hybrid lethality ranged from 60.00% for VBN(Gg)2 200 Gy × Vur 200 Gy to 6.70%, recorded in cross VRM(Gg)1 100 Gy × Vuy 100 Gy.

To asses the reasons for the high pollen sterility in the F_1, the cytogenetic analysis through meiotic studies in PMCs was carried out. The results are presented in Table 3. The two parental species, *V. radiata* and *V. umbellata* had 2n = 22 chromosomes and meiosis was normal with regular formation of 11 bivalents. In F_1 of their cross, all types of abnormalities were observed. Out of 25 PMCs studied at Anaphase I, only one cell revealed 11 bivalents. The occurrence of abnormal associations, namely univalents and quadrivalents, was frequently observed. The number of univalents varied from 0 to 14, while the number of

quadrivalents ranged from 0 to 5. The average chromosome association per cell was IV (1.28) + II (4.96) + I (6.96). Premature separation of chromosomes and formation of anaphase bridges was commonly observed in many PMCs.

Table 2. Performance of irradiated interspecific crosses of *Vigna radiata* × *Vigna umbellate* (see Table 1 for abbreviations)

Parents and Crosses	PF (No.)	PS (No.)	PS (%)	CSO (No.)	CSG (No.)	G (%)	SAM (No.)	HB (%)	HL (%)
VBN1 100 Gy × Vuy 100 Gy	700	28	4.0	0.0	0.00	0.00	0.0	0.0	0.0
VBN1 200 Gy × Vuy 200 Gy	750	30	4.0	0.0	0.00	0.00	0.0	0.0	0.0
VBN1 300 Gy × Vuy 300 Gy	710	35	4.9	0.0	0.00	0.00	0.0	0.0	0.0
VBN1 400 Gy × Vuy 400 Gy	750	35	4.7	0.0	0.00	0.00	0.0	0.0	0.0
VBN1 500 Gy × Vuy 500 Gy	725	32	4.4	0.0	0.00	0.00	0.0	0.0	0.0
VBN1 100 Gy × Vur 100 Gy	700	21	3.0	48.0	25.0	52.08	18.0	72.0	28.0
VBN1 200 Gy × Vur 200 Gy	722	21	2.9	45.0	21.0	46.67	16.0	76.2	23.8
VBN1 300 Gy × Vur 300 Gy	750	23	3.1	33.0	18.0	54.55	15.0	83.3	16.7
VBN1 400 Gy × Vur 400 Gy	720	31	4.3	8.0	3.00	37.50	2.0	66.7	33.3
VBN1 500 Gy × Vur 500 Gy	720	21	2.9	6.0	2.00	33.33	1.0	50.0	50.0
VBN(Gg)2 100 Gy × Vuy 100Gy	700	23	3.3	0.0	0.00	0.00	0.0	0.0	0.0
VBN(Gg)2 200 Gy × Vuy 200 Gy	650	18	2.8	0.0	0.00	0.00	0.0	0.0	0.0
VBN(Gg)2 300 Gy × Vuy 300 Gy	670	18	2.7	0.0	0.00	0.00	0.0	0.0	0.0
VBN(Gg)2 400 Gy × Vuy 400 Gy	900	35	3.9	0.0	0.00	0.00	0.0	0.0	0.0
VBN(Gg)2 500 Gy × Vuy 500 Gy	710	21	3.0	0.0	0.00	0.00	0.0	0.0	0.0
VBN(Gg)2 100 Gy × Vur 100Gy	755	23	3.0	28.0	11.0	39.29	6.0	54.5	45.5
VBN(Gg)2 200 Gy × Vur 200 Gy	675	25	3.7	35.0	15.0	42.86	6.0	40.0	60.0
VBN(Gg)2 300 Gy × Vur 300 Gy	520	28	3.8	38.0	12.0	31.58	6.0	50.0	50.0
VBN(Gg)2 400 Gy × Vur 400 Gy	568	20	3.5	0.0	0.00	0.00	0.0	0.0	0.0
VBN(Gg)2 500 Gy × Vur 500 Gy	685	28	4.0	0.0	0.00	0.00	0.0	0.0	0.0
KM2 100 Gy × Vuy 100Gy	870	35	4.0	0.0	0.00	0.00	0.0	0.0	0.0
KM2 2 200 Gy × Vuy 200 Gy	650	28	4.3	0.0	0.00	0.00	0.0	0.0	0.0
KM2 300 Gy × Vuy 300 Gy	589	19	3.2	0.0	0.00	0.00	0.0	0.0	0.0
KM2 400 Gy × Vuy 400 Gy	562	18	3.2	0.0	0.00	0.00	0.0	0.0	0.0
KM2 500 Gy × Vuy 500 Gy	556	19	3.4	0.0	0.00	0.00	0.0	0.0	0.0
KM2 100 Gy × Vur 100Gy	675	20	3.0	28.0	15.0	53.57	8.0	53.3	46.7
KM2 200 Gy × Vur 200 Gy	655	21	3.2	35.0	18.0	51.43	8.0	44.4	55.6
KM2 300 Gy × Vur 300 Gy	655	23	3.5	42.0	19.0	45.24	10.0	52.6	47.4
KM2 400 Gy × Vur 400 Gy	682	23	3.4	0.0	0.00	0.00	0.00	0.0	0.0
KM2 500 Gy × Vur 500 Gy	652	22	3.4	0.0	0.00	0.00	0.0	0.0	0.0
K1 100 Gy × Vuy 100Gy	655	22	3.4	0.0	0.00	0.00	0.0	0.0	0.0
K1 200 Gy × Vuy 200 Gy	855	38	4.4	0.0	0.00	0.00	0.0	0.0	0.0
K1 300 Gy × Vuy 300 Gy	845	39	4.6	0.0	0.00	0.00	0.0	0.0	0.0
K1 400 Gy × Vuy 400 Gy	745	28	3.8	0.0	0.00	0.00	0.0	0.0	0.0
K1 500 Gy × Vuy 500 Gy	895	35	3.9	0.0	0.00	0.00	0.0	0.0	0.0
K1 100 Gy × Vur 100Gy	875	35	4.0	25.0	10.0	40.00	8.0	80.0	20.0
K1 200 Gy × Vur 200 Gy	785	28	3.6	25.0	10.0	40.00	7.0	70.0	30.0
K1 300 Gy × Vur 300 Gy	885	35	4.0	28.0	10.0	35.71	7.0	70.0	30.0
K1 400 Gy × Vur 400 Gy	785	28	3.6	0.0	0.00	0.00	0.0	0.0	0.0
K1 500 Gy × Vur 500 Gy	655	21	3.2	0..0	0.00	0.00	0.0	0.0	0.0
CO 6 100 Gy × Vuy 100Gy	700	19	2.7	0.0	0.00	0.00	0.0	0.0	0.0
CO 6 200 Gy × Vuy 200 Gy	700	18	2.6	0.0	0.00	0.00	0.0	0.0	0.0
CO 6 300 Gy × Vuy 300 Gy	755	21	2.8	0.0	0.00	0.00	0.0	0.0	0.0
CO 6 400 Gy × Vuy 400 Gy	786	25	3.2	0.0	0.00	0.00	0.0	0.0	0.0
CO 6 500 Gy × Vuy 500 Gy	785	25	3.2	0.0	0.00	0.00	0.0	0.0	0.0

Parents and Crosses	PF (No.)	PS (No.)	PS (%)	CSO (No.)	CSG (No.)	G (%)	SAM (No.)	HB (%)	HL (%)
CO 6 100 Gy × Vur 100Gy	785	28	3.6	35.0	10.0	28.57	5.0	50.0	50.0
CO 6 200 Gy × Vur 200 Gy	650	25	3.8	38.0	12.0	31.58	6.0	40.0	60.0
CO 6 300 Gy × Vur 300 Gy	800	32	4.0	42.0	15.0	35.71	6.0	40.0	60.0
CO 6 400 Gy × Vur 400 Gy	725	22	3.0	0.0	0.00	0.00	0.0	0.0	0.0
CO 6 500 Gy × Vur 500 Gy	715	20	2.8	0.0	0.00	0.00	0.0	0.0	0.0

Discussion

In the present investigation, interspecific hybridization was attempted between greengram and rice bean with the aim of transferring useful traits from the wild relatives into greengram. The extent of crossability, fertility of hybrids and possibility of obtaining superior hybrids through recombination of genomes were studied. The wild relatives of greengram, such as *V. umbellata,* possess desirable genes for many yield components, coupled with resistance to bruchids and MYMV. Transfer of these genes into the cultivated species could result in development of high yielding resistant types. The use of wild *Vigna* accessions in greengram breeding has been problematic because of problems encountered in obtaining successful F_1 hybrids due to crossability barriers. In spite of these difficulties, wide hybridization between *V. radiata* and its wild relatives was successfully accomplished by many workers (Ganeshram, 1993; Pandae, *et al.,* 1990; Renganayaki, 1985; Subramanian and Muthiah, 2000; Uma Maheswari, 2002). Crossability is a prerequisite for gene transfer in wide hybridization. Understanding crossability relationships among species has been helpful in choosing methods to produce F_1 hybrids, but also in tracing phylogenic relationships among species.

Table 3. Meiotic behavior of chromatin in V. radiate x V. umbellate cross

Description	I (Univalent)	II (Bivalent)	IV (Quadrivalent)
PMC 1	-	11	-
PMC 2	10	2	2
PMC 3	-	1	5
PMC 4	4	7	1
PMC 5	10	2	2
PMC 6	2	-	5
PMC 7	4	9	-
PMC 8	10	2	2
PMC 9	2	10	-
PMC 10	4	9	-
PMC 11	8	3	2
PMC 12	10	6	0
PMC 13	12	5	-
PMC 14	12	5	-
PMC 15	-	5	3
PMC 16	6	4	2
PMC 17	12	5	-
PMC 18	8	3	2
PMC 19	12	5	-
PMC 20	14	-	2
PMC 21	4	9	-
PMC 22	10	2	2

PMC 23	2	10	-
PMC 24	14	2	1
PMC 25	4	7	1
Total	174	124	32
Average chromosome association	I (6.96)	II (4.96)	IV (1.28)

In the present study, successful pod set was observed in all 12 interspecific crosses with *Vigna* radiata as female. This result is in agreement with previous reports (Ahuja and Singh, 1977; Egawa, 1990; Gopinathan *et al.,* 1986; Mendioro and Ramirez, 1994; Parida and Singh, 1985; Uma Maheswari, 2002).

The percentage of lethality among interspecific hybrids varied from 6.70% to 60.00%. Similar observations on hybrid lethality and inviability were noticed in interspecific crosses involving different wild *Vigna* accessions in the past (Adinarayanamurty *et al.,* 1993; Al-Yasiri and Coryne, 1966; Chen *et al.,* 1989; Ganeshram, 1993; Uma Maheswari, 2002). Stebbins (1958) had attributed the hybrid weakness, inviability, lethality and sterility as mechanisms of nature for maintaining the integrity of related species.

In general, the pollen fertility among the normal crosses was zero compared to irradiated crosses, which indicated that the mutational approach using irradiation is likely to generate better fertile hybrids and segregants. Similar results were reported by various authors for differential pollen fertility among interspecific crosses of wild *Vigna* accessions (Anandabaskaran and Rangaswamy, 1996; Mendioro and Ramirez, 1994; Monika *et al.,* 2001; Pandae *et al.,* 1990; Ravi *et al.,* 1987; Sidhu and Satija 2003; Subramanian and Muthiah, 2000; Uma Maheswari, 2002). Among crosses, pollen fertility was highest in the cross *V. radiata* × *V. radiata* var. *sublobata*, supporting the view of Pandae *et al.,* 1990 and Mendioro and Ramirez, 1994 that *V. radiata* var. *sublobata* is a probable progenitor for *V. radiata*.

In normal crosses, the range of pollen sterility observed in all the F_1 hybrids was high and no viable F_2 segregants could be generated. Considering the importance of this cross for the resistance related traits, it was essential to device methods enhancing fertility in F_1 that could aid in developing breeding materials with resistance, and cytological analysis was carried out for this hybrid. In irradiated crosses, seed set was observed only for the lower doses (100,200,300 Gy) of all crosses with *V. umbellata* yellow.

Some of the hybrids that could be recovered from these promising interspecific crosses might serve as better breeding base for the improvement of yield and yield components. Such interspecific hybrids were also reported before (Ganeshram, 1993; Subramanian and Muthiah, 2000; Uma Maheswari, 2002). In this situation, selection for traits in early generations will not be fixable; hence, selections in later generations or by adopting modified breeding procedures such as inter-mating the segregants followed by recurrent selection may shift the gene action towards additive effects. Since sterility factors will be gradually reduced over generations in interspecific crosses and more recombined populations will be available for selection, selection in the later generations will be more effective.

Chromosomal analysis of *V.radiata* x *V.umbellata* F_1 hybrids and their parents revealed that chromosomal pairing was normal in the parents, with 11 bivalents, whereas F_1 hybrids showed loose pairing between chromosomes leading to precocious separation at Anaphase I. This had already been observed by some of the earlier workers (Bhatanagar *et al.,* 1974; Kaur and Satija, 1998; Machado *et al.*, 1982; Uma Maheswari, 2002). Formation of univalent, dicentric bridges and laggards also indicated lack of homology between the parental species. The average association of IV (1.28) + II (4.96) + I (6.96) indicated abnormal chromosomal association due to structural chromosomal differences among parental genomes. For restoring fertility in this hybrid, adoption of chromosome doubling through colchiploidy and recovery of fertile amphidiploids would be a viable solution for recovering useful segregants as suggested by Machado *et al.*, (1982) and Sidhu and Satija (2003).

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Research Article

Rough Texture of Mungbean (*Vigna radiata* L.) First True Leaves Induced by Gamma Irradiation

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Abstract

Mature seeds of 6 mungbean varieties were treated with gamma irradiation at 100, 300, 500 and 700 Gy. Both adaxial and abaxial surfaces of the first true leaves of all plants treated by 500 and 700 Gy irradiation showed severe rough texture. For the 100 Gy treatments, the plants possessed either the severe rough texture or normal first true leaves, and the proportion of the plants with textured first true leaves varied among varieties. However, only very light rough texture of first true leaves of some of the plants was observed when the dose was 300 Gy. While lethality differed between varieties and with irradiation dosage, all of the varieties showed the same type and extent of appearance of textured first true leaves at each of the doses analysed. The rough texture of first true leaves was caused by the rugged surface of those leaves, which most likely resulted from the irregular size and or uneven density of mesophyll cells in the palisade layer.

Key words: Mungbean first true leaves, rough texture, gamma irradiation, radio-sensitivity, mesophyll cell

Introduction

Ionizing irradiation may cause injury to cells, and the effect of ionizing irradiation on organisms is complicated by the indirect action of active oxygen species generated by water radiolysis, which may also cause damage to different compounds in cells (Nagata *et al.,* 1999). Whilst the level of injury caused is generally dose dependent, different species or different varieties of the same species may differ in their levels of sensitivity to irradiation, in regard to parameters such as survival rate, sterility, growth and mutation frequency. Mutants found in *Arabidopsis* indicated that growth could be arrested as a result of blocking of the cell cycle induced by gamma irradiation, and such effect could be suppressed in the irradiation-resistant mutant sog (suppressor of gamma) (Preuss and Britt, 2003). DNA damage caused by irradiation may induce the phosphorylation of a suite of proteins involved in DNA repair, cell cycle control, and apoptosis (Garcia *et al.,* 2003). Besides antioxidants that may reduce the detrimental effect caused by active oxygen species generated from irradiation, chemicals rendering chromatin compaction may inhibit irradiation induced DNA breaks (Goel *et al.,* 2003; Newton *et al.,* 2004). Nevertheless, the mechanism of radio-sensitivity is still largely unknown.

Ionizing irradiation has been shown to affect many aspects of metabolism and morphology of organisms. Besides DNA damage, which may result in gene mutation, gamma irradiation may also influence the production of chemicals such as ethylene (Young, 1965), sucrose (Hayashi and Aoki, 1985), soluble carbohydrates and some protein species (Ferullo *et al.,* 1994). Enzyme activities such as phenylalanine ammonia-lyase (Pendharkar and Nair, 1975) and peroxidase (Sah *et al.,* 1996) and level of plant growth regulators such as auxin (Sax and Schairer, 1963) and gibberellins (Machaiah *et al.,* 1976) are also affected by gamma irradiation.

Typical morphological changes in plants in response to X ray irradiation have been described in early reports, and include abnormal leaf morphology, increased lateral branching, altered flower morphology, reduced number of flowers and fruits, and abnormal fruit development (Johnson, 1936). Similar changes were also observed in plants after gamma irradiation (Cordero, 1982). Recently, Nagata *et al.* (1999) observed that additional trichome formation on the adaxial surface of mature leaves of *Arabidopsis* plants was induced within seven days after gamma irradiation, and active oxygen species may also be involved in the induction of new trichomes. In the investigation of gamma irradiation effects on mungbean, we observed a series of morphological changes such as stunted plants, reduction of the length of primary roots and abnormal leaves, among which the rough texture of first true leaves induced by gamma irradiation has not been reported previously. We report here the morphological characterization of rough texture of mungbean first true leaves and the relation between texture changes and doses of gamma irradiation.

Materials and methods

Plant materials

Mature seeds of 6 mungbean varieties were used. They included the varieties Emerald, Celera, Delta, Green Diamond, White Gold and Yellow Sun.

Gamma irradiation and planting

The seeds of 6 mungbean varieties were treated to a dose of 100, 300 500 and 700 Gy of gamma irradiation from cobalt-60. For each dose, the seed samples of 6 varieties were mounted onto cards and placed on stands for processing in the Australian Nuclear Science and Technology Organisation (ANSTO) GATRI facility. Fricke (ammonium ferrous sulfate) dosimeters were sited throughout the samples in the predetermined minimum and maximum dose zones. The samples were then irradiated for times calculated to give the exact doses. The dose rate was 6.4 Gy. min^{-1}. ANSTO's dosimeters have measurement compliance with the Australian and UK Standards

for Absorbed Dose. The estimated overall uncertainty in each dose is ±3.0%. Two months after treatment, the seeds were grown in plastic tubes in a glasshouse, at one seed per tube. Three repeats were set for each variety / dose combination by randomizing tubes in different trays, and each repeat included 30 tubes. The growing condition was 25˚C with 16 hours day and 8 hours night. The number of surviving plants with textured first true leaves of all treatments was scored at the 10th day after germination, and their first true leaves were collected at the same time for microscopic observation.

Sample preparation and Scanning Electron Microscope analysis

Leaf material was preserved for 2 hours in a solution of 10% formalin with a neutral pH (10% formaldehyde, disodium hydrogen orthophosphate, potassium dihydrogen orthophosphate). Samples were then rinsed in distilled water and dehydrated in an ethanol concentration gradient series from 25% to 100%. Specimens were then dried using a Polaron 3100 Critical Point Dryer using liquid carbon dioxide. After the samples were dried they were coated with 10nm of gold using a Baltec Sputter Coater. Samples were then imaged on the Leo 440 Stereoscan Scanning Electron Microscope (SEM). Settings for the microscope were: Probe-100 pA, EHT-10kv, Working Distance-10mm.

Histological observation

Leaf materials were cut into approximately 2×3 mm pieces and fixed in 5% formalin for 6 hours. After being washed in tap water for 10 minutes, the leaf slices were placed on tissue to remove water that remained on them and then embedded in O.C.T. compounds (Elkhart, IN, USA) onto cryotome holders and frozen in liquid nitrogen (Ishii *et al.,* 1990). Cryosectioning was performed using Reichert-Jung Cryocut 1800. Sectioned tissues were then transferred onto slides and preserved in 50% ethanol then 20% ethanol and hydrated in distilled water, each for 10 minutes, followed by Fast Green staining. Finally, the sectioned tissues were observed under a light microscope.

Results

Rough textured first true leaves induced by gamma irradiation

After planting, mungbean seeds germinated and the folded first true leaves of the plantlets gradually opened. Once the first true leaves opened, both the adaxial and abaxial surfaces of the 2 first true leaves of each plantlet germinated from the seeds treated by gamma irradiation at 700 Gy showed significantly different texture (rough) from that of the nonirradiated control (smooth) (Fig. 1). This rough texture existed for all the life span of the first true leaves, while such texture change did not appear on other leaves of the same plant. Conversely, the colour, size and shape of the rough textured first true leaves showed no obvious and regular changes as compared with the nonirradiated control. This phenomenon happened on all the 6 mungbean varieties analyzed.

Figure 1. Appearance of first true leaves with rough texture. The plant on the left shows rough texture of first true leaves induced by 700 Gy gamma irradiation, and the plant on the right side of it is the nonirradiated control of mungbean variety White Gold

Factors influencing plant growth and rough texture of first true leaves

After gamma irradiation, some of the seeds were not able to germinate, and some of the seedlings died soon after germination. While different varieties differed in lethal rates at specific doses, on the whole, the lethal rate of the varieties was relatively low at 100 Gy, and increased at 300 Gy and 500 Gy, and especially at 700 Gy. (Table 1). Variance analysis indicated that the lethal rate was affected significantly by variety, dose and the interaction

between variety and doses (Table 2). The incidence of rough texture of mungbean first true leaves appeared to be significantly dose dependent. The rough texture was observed on the first true leaves of all the plants of all the mungbean varieties germinated from the seeds treated by irradiation at 700 and 500 Gy. The rough texture was much reduced and could not be easily distinguished for the first true leaves of some of the plants germinated from the seeds treated at 300 Gy. However, at 100 Gy, the plants were either as heavily textured as first true leaves of plants treated at 700 Gy, or appeared untextured like the nonirradiated control. Whilst this happened to all varieties, the percentage of plants with rough textured first true leaves differed among different varieties (Table 1). Variance analysis indicated that at 100 Gy, the varietal effect on the percentage of plants showing textured first true leaves was significant (Table 3).

Table 1. Average percentage of textured plants induced at 100 Gy and the lethal rate at different doses among varieties

Variety	Textured plants at 100 Gy (%)	Lethal Rate (%)			
		100 Gy	300 Gy	500 Gy	700 Gy
Celera	8.05	3.3	16.7	14.3	14.3
Emerald	5.70	2.3	11.0	19.0	35.7
Delta	15.86	2.3	20.0	20.0	20.0
Green Diamond	13.82	3.3	22.3	22.3	20.0
White Gold	38.11	6.7	45.7	60.0	50.0
Yellow Sun	27.87	12.3	43.3	51.0	73.3

Table 2. Variance analysis of the effects of variety and dose on lethal rates

Source of variance	Sum of Squares	df	Mean Square	F	$F_{0.01}$
Variety	12385.19	5	2477.04	58.16**	3.43
Dose	9788.89	3	3262.96	76.61**	4.22
Inter variety and dose	4359.26	15	290.62	6.82**	2.44
Internal (Error)	2044.44	48	42.60		
Total	71600.00				

Table 3. Variance analysis of the effect of variety on the percentage of plants with textured first true leaves at 100 Gy

Source of variance	Sum of Squares	df	Mean Square	F	$F_{0.01}$
Variety	2319.29	5	463.86	17.80**	5.06
Internal (Error)	312.69	12	26.06		
Total	8617.47	18			

Morphological observation of textured first true leaves

The surface of textured first true leaves was observed by means of SEM. The cuticle and the epidermis of both the adaxial and abaxial leaf surface did not show differences between rough textured first true leaves and the control. Although an increase in the number of straight and glandular trichomes was observed on the adaxial surface of textured first true leaves, the number of trichomes was very limited, and the trichomes - especially straight trichomes - are mainly distributed on the edge of the first true leaves. The cause of the rough textured first true leaves was the structural change beneath the epidermis resulting in the rugged leaf surface (Fig. 2 B). The structure beneath the epidermis is the palisade and spongy layer. Mungbean first true leaves have a thick palisade layer but a thin spongy layer. In the first true leaves of control plants, the mesophyll cells are regularly organized in the palisade layer, and the epidermis layers are relatively flat (Fig. 2 C). Typical structure of a first true leaf with rugged surface is shown in Fig. 2 D. Whilst a distinct palisade layer is observed, the density as well as the longitudinal size of mesophyll cells in the palisade layer is uneven in different parts of the leaf (Fig.2 D). Such structure in the rough textured first true leaves does not show significant variation among varieties, which indicates that the change of internal structure of first true leaves may be the cause of rugged surface induced by gamma irradiation in those leaves.

Discussion

The current study indicated that distinctly different incidence of rough textured first true leaves occurred after the gamma irradiation treatment at 100, 500 and 700 Gy. Treatment of 100 Gy caused severe rough texture on some of the plants and 500 and 700 Gy resulted in severe rough texture on the first true leaves of all of the plants, while the 300 Gy treatment only caused very light rough

texture on some of the plants. It is possible that 100 to 300 Gy gamma irradiation and or related biochemical reaction may have stimulated a repair mechanism suppressing the texture symptom, which reached the maximum effect at approximately 300 Gy and diminished at 500 and higher doses because of severe injuries. Lethal rate is important in indicating the sensitivity of a variety to irradiation. In the current study, the lethal rate at definite doses varied among varieties (Table 1), and both the varietal and dose effect on lethal rate were extremely significant (Table 2), which indicated that the varieties used varied widely. The different incidences of rough textured first true leaves appeared dose dependent; for example, all varieties presented some light texture first true leaves at 300 Gy and severe texture first true leaves at 500 Gy. Despite differences in lethal rate, all of the varieties presented the same type of appearance, whether severe or light, of textured first true leaves at each dose. Those observations suggested that the different incidences of rough textured first true leaves might be a common response of mungbean to a range of doses of gamma irradiation. On the other hand, at 100 Gy, the varietal effect on the percentage of plants with textured first true leaves was significant (Table 3). The biochemical and genetic mechanism of resistance or susceptibility of texture first true leaves at this dose is of interest but its cause remains unknown.

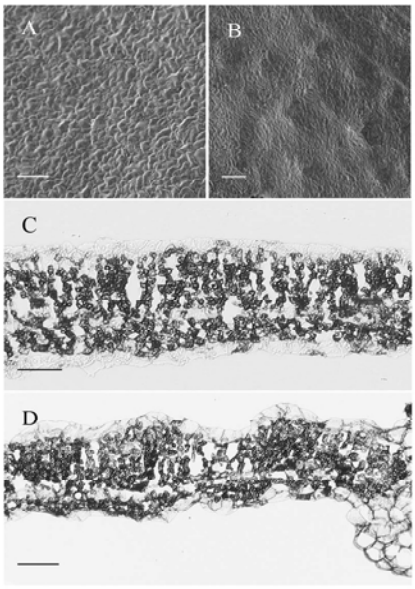

Figure 2. Morphological observation of first true leaves of White Gold with rough texture. A. Control. Flat adaxial surface. B. 700 Gy treatment. Rugged adaxial surface. C. Control. Regularly lined mesophyll cells in palisade layer and flat epidermis layers. D. 700 Gy treatment. Uneven density and size of mesophyll cells in palisade layer and rugged epidermis layers. A, and B, bar=200 μm; C and D, bar=180 μm

Obliquely divided cells and enlarged cells were observed in chronically irradiated regions of the vascular system in lupin (Cordero, 1982). Whilst irregularly shaped mesophyll cells also exist in the rough textured first true leaves, the uneven density and longitudinal size of mesophyll cells observed in the current study are most likely related to the dimpling or raising of the epidermis layer. It is also possible that uncoordinated divisions of some of the cells in the epidermis layer may result in the forming of irregular areas leading to rugged surface of the epidermis layer. However, among such morphological changes, which is the cause and which is the consequence still needs to be determined.

A mutant with disrupted leaf development was identified in *Arabidopsis* (Reiter *et al.,* 1994), but only in the late stage of development, where the mutant leaves have enlarged intercellular spaces, and the palisade layer can no longer be distinguished. In addition, it contains low levels of chlorophylls, which differs from the rough textured first true leaves we observed. For the rough textured first true leaves induced by gamma irradiation, whether the changes that happened to mesophyll cells were caused by irradiation directly or by active oxygen produced, the reasons why the rough textured first true leaves occurred only in some plants at the 100 Gy treatment, and why only slight texture change happened at 300 Gy treatments, still need to be examined. Nevertheless, that the rough texture happened evenly in all parts of the first true leaves of a plant and existed throughout the life span of the leaf suggests that rough texture may be a systemic response of the plant induced by gamma irradiation. The irradiation system described in the current study may be helpful for the study of the development of plant leaves.

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Y.J. Wu	China	A Novel Dominant Semidwarf Mutant and its Plant Height Revertants Induced with Ion Irradiation in Rice (*Oryza sativa* L.)
P.M. Gresshoff	Australia	Mutational and Functional Genomic Analysis of Systemic and Local Regulation of Legume Noduclation
B. Meyers	USA	The Use of Mutants for Dissecting and Understanding Plant Small RNAs and their Functions
K.H. Engel	Germany	Metabolite Profiling of Induced Mutants of Rice and Soybean
M. Hansson	Sweden	A Microarray Approach to Identify Genes Known only by their Mutant Phenotype
M. Talon	Spain	Genomics Meets Induced Mutations in Citrus: Identification of Deleted Genes through Comparative Genomic Hybridization

Speaker	Designating Member States/Organization	Title of Paper
P.J. Facchini	Canada	Mutagenesis as a Functional Genomics Platform for Pharmaceutical Alkaloid Biosynthetic Gene Discovery in Opium Poppy
L. Munck	Denmark	From Discovery of High Lysine Barley Endosperm Mutants in the 1960-70ties to New Holistic Spectral Models of the Phenome and of Pleiotropy in 2008
G. Rowland	Canada	The Effect of Plants with Novel Traits (PNT) Regulation on Mutation Breeding in Canada
Q.Y. Shu	IAEA	Turning Plant Mutation Breeding into a New Era: Molecular Mutation Breeding
T. Ishige	Japan	Plant Mutagenesis in Genomics Era: Opportunities and the Way Forward (Summary of the Symposium)

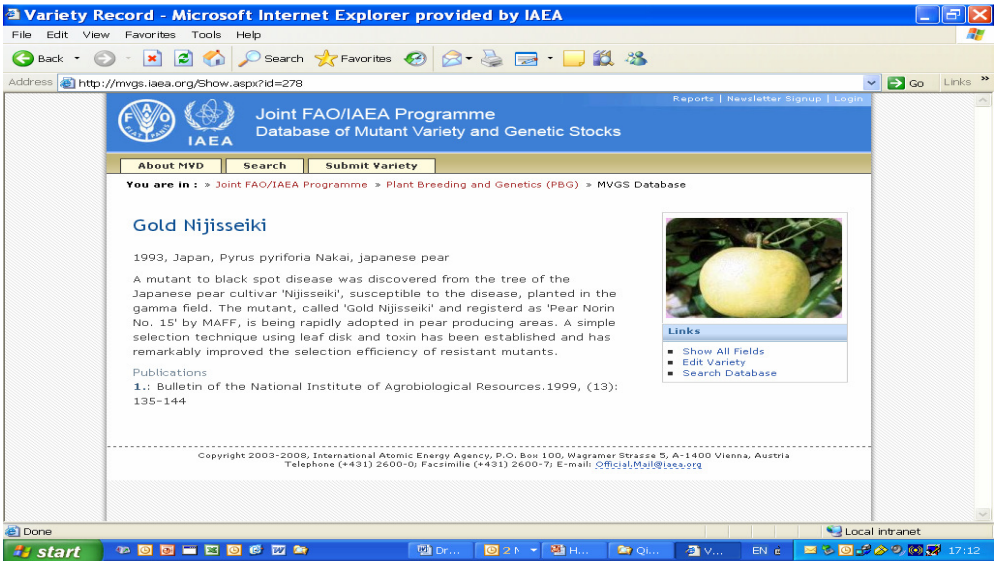
New FAO/IAEA Database of Mutant Varieties and Genetic Stocks

Welcome to our new FAO/IAEA Database of Mutant Varieties and Genetic Stocks! At the moment, we just completed construction of the part for Mutant Variety Database, which is still in the process of information updating. We will add the other part for Mutant Genetic Stocks in due time. The new database has improved over the FAO/IAEA Mutant Variety Database in many ways. We are working to make the new database as the global information source of mutant varieties and mutant genetic stocks, as well as activities and events related to plant mutation breeding and research.

The key feature of the database is that you can register your mutant varieties from your desktop. For this purpose, you need first register an account; then you will be authorized to submit or edit a mutant variety.

We would greatly appreciate your support by registering your mutant variety in our database. Once the variety is registered, it will have its own 'homepage' (see below). Therefore, you can use it as an important platform to showcase your new varieties (The introduction of this variety may be shown in local language).

Please visit the website http://mvgs.iaea.org and send us your valuable suggestions and comments regarding the structure and content of this database. Please also send us other information, related to plant mutation breeding and mutant varieties, genetic stocks; we may post them on the website.



YOU MAY STILL SEND US INFORMATION ON YOUR MUTANT VARIETY AND WE WILL UPLOAD THEM INTO THE SYSTEM, IF IT IS DIFFICULT FOR YOU TO DO SO.

PLANT MUTATION REPORTS AUTHOR'S GUIDELINES

Scope

Plant Mutation Reports (PMRs) publishes (mini) reviews, short communications and complete research papers in all areas of plant mutation research which focuses on mutagenesis, mutation induction, mutant characterization, and mutant applications. It also publishes description papers on mutant germplasm and mutant varieties. Papers on social-economic impact analysis of induced mutations and mutant varieties are also accepted.

Style

The manuscript should be concisely written with the following sections:

Title page

- Title: the title should be as short as possible, but should contain adequate information regarding the contents.
- Authors: Initials of given name followed by full family name.
- Affiliation(s)/Address(es):
- Email address: the corresponding author's email address should be given.

Abstract and Keywords

A brief and informative summary of the paper not exceeding 150 words. Optional for short communications. Each paper should have 3-5 keywords.

Main text

- Review articles may be organized according to their specific requirements.
- Research articles should include: Introduction, Materials and Methods, Results (and) Discussion (this could be combined for Short communications).
- New mutant germplasm should include a short description of initial material used and the mutagen and doses applied; selection process; mutated characteristics and its genetic and agronomic analysis. Description of mutant variety should, in addition, include its performance in yield trials for varietal release and the releasing committee, when applicable.

Acknowledgements

- Acknowledgements of grants, support etc, should follow the text and precede the references.

References

The literature references should be cited either as John (1990) for single author paper, John and Johnson (2000) for papers with two authors, or John et al. (2000) for papers with more than two authors throughout the text, and alphabetically listed in the Reference following the style shown below:

- Periodicals: Shamsuzzaman K.M. and Shaikh M.A.Q. (1991) Early maturing and high seed yielding chickpea mutant. Mut Breed Newslett 37: 4-5.
- Books (edited by someone other than author of article): Maluszynski M. (1990) Gene manipulation in plant improvement. In: Gustafsson J.P. (ed), Induced Mutations in Plant Improvement. Plenum press, New York. Pp239-250.
- Books (identical author and editor) van Harten A.M. (1998) Mutation Breeding, Theory and Practice. Cambridge University Press, Cambridge, U.K. pp. 237-240.

Figures and Tables

- All tables and figures, i.e., photographs, graphs and diagrams should be referred to as either "Table" or "Fig." and be numbered consecutively (1, 2, etc.) in the text.
- In tables, footnotes are preferred over long explanatory material in the heading or table body. Such explanatory footnotes, identified by superscript letters, should be placed immediately below the table.
- Do not use boxes; use horizontal lines only. Figures and tables should be placed on separate pages.

Units and symbols

The standard SI units and symbols should be used throughout (www.scenta.co.uk/tcaep/science/siunit/index.htm).

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Index

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IAEA International Atomic Energy Agency

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