CONCURRENT SESSION 10

Induced Mutations in Seed Crop Breeding (2)

A *UGPase1*–blocked Male Sterility Mutant and Its Possible Use in Hybrid Seed Production of Rice

H J Koh* & M O Woo

Abstract

A rice genic male-sterile mutant was induced by chemical mutagenesis using N-methyl-N-nitrosourea, which had a pleiotropic effect on chalky endosperm. The mutant gene, *ms-h*, was isolated through a map-based cloning approach. The suppression of UGPase activity, caused by a splicing error at the 3' splice junction of the 14th intron of the *UDP-glucose pyrophosphorylase 1* (*UGPase1*; EC 2.7.7.9) gene, was the cause of male sterility. This was confirmed by both RNAi and complementation-transgenic experiments. The endosperm of the mutant had more roundish and smaller starch granules, a higher frequency of long glucose chain amylose, higher ratio of Fr. III to Fr. II chains, and shorter branching of amylopectin than the wild type parent. A hybrid seed production system was proposed using the pleiotropic effect of *UGPase1* gene on male-sterility and chalky endosperm. Relatively low density of chalky seeds may facilitate the early detection of male-sterile seeds in segregating populations prior to sowing by density-gradient method.

Introduction

Male-sterile (MS) mutants have been reported in many species of higher plants as the result of both spontaneous and induced mutations [1]. Male sterility is conditioned by either cytoplasm-specific (CMS) or genetic male sterility (GMS) genes. In rice, male sterility is classified into four major groups: cytoplasmic male sterility (CMS), photoperiod-sensitive genic male sterility (PGMS), thermo-sensitive genic male sterility (TGMS) and other genic male sterilities [2]. CMS and PGMS/TGMS have been used for hybrid seed production. However, genic MS lines have hardly been used due to the difficulty in purifying MS plants in segregating mixtures of MS and heterozygous plants. Several efforts have been made to develop a genic male-sterility system involving a closely linked phenotypic marker so that the male-sterile plants could be readily distinguished from normal plants in segregating populations. Initially, it was envisioned to produce F_1 seeds using the linked marker to indicate which plants were male sterile. However, this approach has not been generally successful [1, 3, 4, 5]. We induced a new genic MS mutant, Hwacheong ms-h, by chemical mutagenesis on a Korean japonica cultivar, Hwacheong, using N-methyl-N-nitrosourea [6]. Here, we review the previous studies on the phenotypic characteristics of the mutant, isolation of the MS gene, and its feasibility in hybrid seed production system.

Phenotypic characterization and inheritance of the mutant

MS plants, Hwacheong *ms-h*, showed a shorter plant height, incomplete panicle exertion, and smaller panicle number compared to the parental plants as reported so far on the growth reduction in MS plants [6]. Meiosis was aborted in MS plants generating abnormal microspores. This implies that the MS gene affects whole stages of growth and development. MS phenotype was stable regardless of air temperature and day length. When the MS plants were pollinated with the parent, the F_2 seeds were segregated into normally transparent and chalky grains, and the segregation ration ratio fit to 3:1. Interestingly, all of the chalky grains grew into MS plants while transparent grains grew into normally fertile plants (**Fig. 1**). After examination of cosegregation over generations, it was concluded that MS phenotype was controlled by a single recessive gene, which had a pleiotropic effect on chalky endosperm [7]. The gene was designated as *ms-h* and was mapped on chromosome 9.

Analysis of chemical composition of grains revealed that the MS grains, having homozygous-recessive MS genotype, showed lower amylose content in starch, higher lipid content, higher potassium content, and much lower gel consistency than corresponding normal grains (**Table 1**). Starch granules in endosperm of the MS grains were more roundish polyhedral and smaller than normal grains, and accordingly, some viscosity parameters also changed in the MS grains. Elution patterns on a Sephadex G-75 column of starch debranched by isoamylase revealed that the MS grains presented a higher Fr.III/Fr.II ratio and a shorter branching of amylopectin compared to the normal grains. This indicates that starch metabolic pathway in MS grains was modified by the MS gene [8].

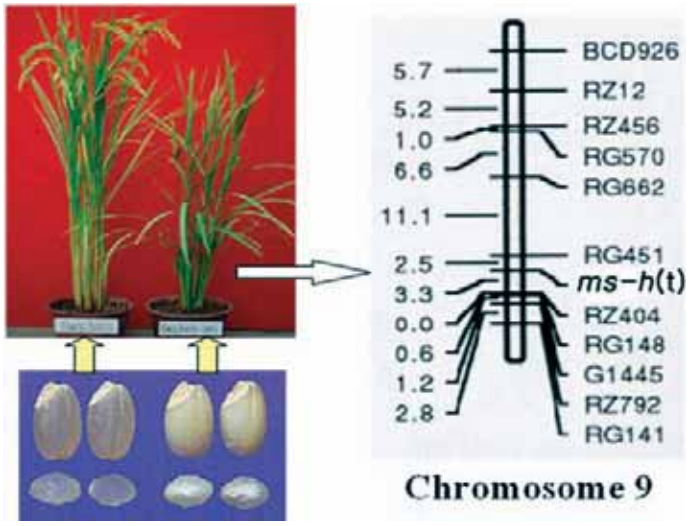

Figure 1 Appearance of plants and grains of Hwacheongbyeo **(A)** and MS mutant **(B)**. The normally clear grains grow into normal-fertile plants and chalky grains into male-sterile plants as arrows indicate. The ms-h(t) gene was mapped on chromosome 9 **(C)**.

Splicing error of UGPase1 gene caused by single base substitution resulted in male sterility

Target locus of '*ms-h*' region was narrowed down through saturated mapping using STS and CAPS markers and finally was delimited to 60kb region containing 11 candidate genes [9]. After sequence comparison of the candidate genes between parent and the mutant, a single nucleotide substitution of Guanine to Adenine at the 3' splice junction of the

Dept of Plant Science, Seoul National University, Seoul 151-921, Korea

* Corresponding author. E-mail: heejkoh@snu.ac.kr

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14th intron of the *UGPase1* gene was found to be critical (**Fig. 2A/B**). RT-PCR analysis using *UGPase1*-specific primers revealed that the mutant produced two types of transcripts which were 74-bp longer and 1-bp shorter compared to the wild type transcript (**Fig. 2C**). The deduced amino acid sequences of the mutant transcripts displaying two abnormal sizes suggests that both the 1-bp deletion and the 74-bp insertion cause frame shifts that generate two, independent stop codons in the process of translation, resulting in truncated 299-aa and 298-aa proteins instead of the 469-aa protein encoded by the wt *UGPase1* transcript. To confirm that the *UGPase1* gene is causally related to male fertility, we generated UGPase1-RNA*i* transgenic plants through transforming the parental plants with UGPase1-specific RNA*i* vector construct by *Agrobacterium*-mediated method. The RNA*i* transgenic plants were found to be male sterile. On the other hand, using the same transgenic technology, we complemented the MS phenotype by introducing an over-expression construct containing the wt *UGPase1* sequence into homozygous *ms-h* mutants. As expected, successfully transformed plants produced fertile panicles [9].

Table 1. Some physicochemical properties of chalky and normal grains

	1000 grs. Wt. at 10% MC (gr)	Absolute grain density (gr/cm3)	Hardness at 10% MC (gr/grain)	Amylose content (%)	ADV at 1.2% KOH (1~7)	Protein content (%)	Gel consistency (mm)
Normal grains	18.9	1.486	6.27	20.3	5.5	9.80	100
Chalky grains	17.7	1.430	3.07	17.9	5.5	9.52	26
Difference	**	**	**	**	ns	ns	**

MC: moisture content

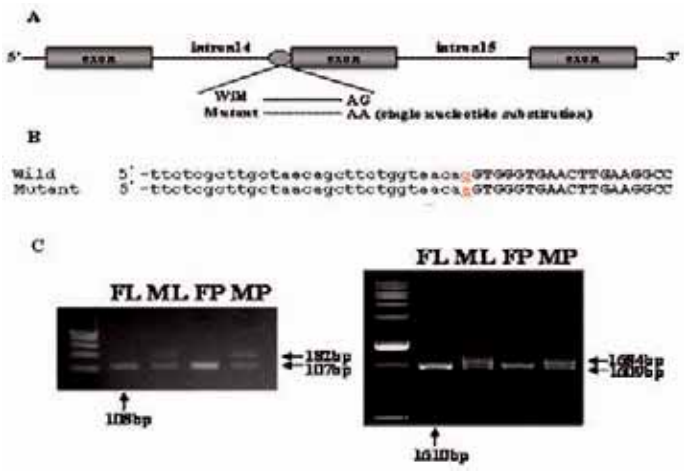

Figure 2 Schematic diagram of the UGPase1 gene presenting single base substitution in the mutant (A, B) and unstable splicing in the mutant RNA(C). A. The mutation within the UGPase1 gene in the ms-h mutant. B. Comparison of sequences between the wild type and the mutant indicating the one-base substitution at the 3-splice junction of the 14th intron. C. RT-PCR analysis with *UGPase1*-specific primers. As a result of each RT-PCR using the UGP1-PRT primer set (left) and the UGP1-FRT primer set (right), wt Hwacheong displayed only the expected size fragment in both reactions, while the ms-h mutant contained two fragments; one similar to wt and a second, longer fragment. The same banding pattern was observed in both leaf and panicle. The arrows pointing upward indicate the fragment size of the wt Hwacheong and the arrows pointing left represent the fragment size of the ms-h mutant. FL: fertile leaf, ML: ms leaf, FP: fertile panicle, MP: ms panicle.

To confirm that chalky endosperm results from a pleiotropic effect of the *ms-h* gene, we evaluated both male sterile and male fertile transgenic progeny to determine whether opaque seeds were always associated with *ms-h*. In the case of UGPase1-RNA*i* transformants, male-sterile transgenic T_0 plants were crossed to wt Hwacheong as the male parent. Some of the F_1 progenies produced fertile panicles which segregated opaque F_2 seeds. The T_1 seeds harvested from 11 T_0 transgenic lines produced in complementation test were also examined for chalkiness after hulling. Chalky grains were segregated in two of the 11 plants. The opaque T_1 seeds harvested from c10 and c13 transformants were planted with other normal T_1 seeds to verify the pleiotropism with male-sterility. After maturing, we confirmed co-segregation of the *ms-h* gene and seed opaqueness based on phenotypic examination and molecular analysis of *UGPase1*-RNA expression patterns. These results clarify that the *ms-h* gene has a pleiotropic effect on chalky endosperm [9].

Feasibility of the mutant in producing hybrid seeds

Due to its pleiotropic effect on MS and chalky endosperm despite its nature of genic male sterility, the Hwacheong ms-h can be applicable to the hybrid seed production system. As seen in **Table 1**, since grain density of chalky grains which were MS grains was lower than normal grains, it might be possible to select MS grains by density gradient method. We tried to pick chalky grains out of seed mixture, in which normal and chalky grains are segregating, using NaCl solution. At the specific gravity level of 1.14~1.16 gr/ml, 85~90% of chalky seeds and 10~15% of normal seeds were floated [6]. If the MS seeds were chosen by this gravity method, 10~15% of normal seeds might be mixed in the seed bulk. However, the plant height of the seedlings of the mutant was significantly shorter, and was readily distinguishable in mixed seedling populations (**Table 2**). This may enable to rogue out most of the remnants of normal seedlings in seedling nursery that still remain after pre-screening by specific gravity method. Nevertheless, its feasibility for hybrid seed production still remains to be studied more in relation to the effectiveness and cost.

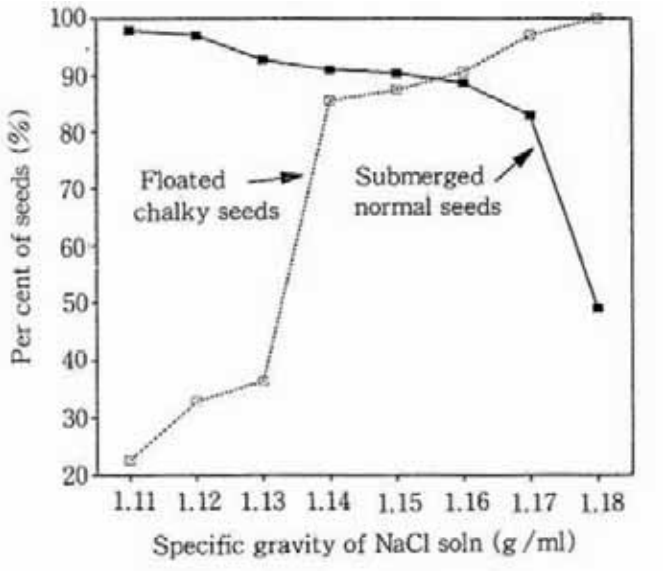

Figure 3 Percent of submerged and floated seeds in NaCl solution of different specific gravity.

Table 2. Plant height of the MS mutant line, Hwacheong ms-h.

	Days after seeding		Days after transplanting		
	15	30	15	30	45
Hwacheong	14.2±2.1	25.4±2.1	27.7±2.9	46.8±2.9	76.7±2.5
Hwacheong ms-h	9.2±1.9	18.9±2.7	22.6±2.8	37.5±3.2	64.0±3.5
Difference	**	**	**	**	**

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Barley (*Hordeun vulgare*) and Kiwicha (*Amaranthus caudatus*) Improvement by Mutation Induction in Peru

L Gómez-Pando*, A Eguiluz, J Jimenez, J Falconí, & E Heros Aguilar

Abstract

In order to increase food availability and household incomes of families in the Andean region of Peru, a mutation induction method was applied to improve barley (*Hordeun vulgare*) and kiwicha (*Amaranthus caudatus)* cultivars. Barley cultivar *Buenavista* was treated with 200 and 300Gy inducing different kinds of mutations. Twenty promising mutant lines were selected and have been evaluated at the national trials. From them Mbv-Earlier, from 300Gy dose was selected and released in 2006 as a new cultivar denominated *Centenario*. This cultivar has a high yield potential (5,552 kg/ha), resistance to stripe rust (*P. striiformis f sp hordei*) and better food quality than the parental cultivar. Kiwicha traditional cultivar *Seleccion Ancash* treated with 400Gy , identified a higher yield mutant denominated *Centenario* Cultivar. At farmer location in the coast the yield has a variation of 3,500 to 5,500 kg/ha and in the highland from 2,500 to 3,700 kg/ha. The better yield potential, tolerance to *Sclerotinia sp*, color and size of its grains have contributed in the preference of *Centenario* over other commercial cultivars.

Introduction

In Peru it is very important to produce more food, especially in the rural areas, where the population lives in extreme poverty. The increase of food must be made in amount and quality using improved cultivars, among other factors. Due to bad weather and soil, there are few crops that are able to grow in these marginal conditions and among them are the cereals and native grains. There is evidence that cereal and native grains in a mixture can produce food of a high nutritive value because of the amino acids and the other nutrients that are complementary. The improved cultivars should have the capability to overcome most of the limiting factors of the marginal conditions of the highlands of Peru, such as shallow and low fertility soils, drought, and frost and hail storms. The crop technology used in the cereal and native grains production are very low. The mutation induction methodology can be applied to improve many plant species. Two thousand, five hundred and forty three mutant cultivars of 175 plant species have been developed trough induction mutation; some of them are grown at large scales [3, 7]. Valuable progress has been reported on the improvement of several characters of plants by mutation induction such as yield, life cycle, disease resistance, quality characters, grain quality, abiotic stress resistance, improved plant type and others [5; 6, 8, 12, 13, 14, 15,16, 18].

Barley

In Peru, barley is the fourth most important food crop in terms of area of land dedicated to its production. Barley is one of the few crops suitable for the extensive and marginal highlands where frost and drought are very frequent.

Materials and Methods

The dried seed of barley cultivar Buenavista was treated with Gamma-rays at 200 and 300Gy doses. The treated seeds along with the control were sown to rise the M_1 generation. Spikes were harvested individually and raised as M_2 progeny in spike/row. In the M_2 generation, chlorophyll mutants were scored and classified following the classification presented in reference [9, 10]. Individual spikes were selected in normal plants within all the rows showing mutations of any kind in the M_2 and M_3 generations to handle initial germplasm. This approach was made looking for micro mutants-forms without clear difference from the parental cultivar. The promising mutant lines and their progeny have been tested in different locations and years following the conventional breeding procedures in the generations M_4 to M_7.

Results and Discussion

On the basis of M_2 seedlings, the number of plants with chlorophyll mutations and the spectrum observed are presented in **Table 1**. The frequency of chlorophyll mutation varied from 0.05 to 0.91 percent for 200Gy and from 0.05 to 0.72 per cent for 300Gy . The frequency values followed an irregular trend. This result it was a preliminary index of effectiveness of mutability of the cultivar Buenavista, similar observation was reported [4, 11, 17]. The following different kinds of chlorophyll mutations were found: albino, xhanta, xantha-alba, viridis- albino, albo-viridis, virescens, chlorina, lutescens, albescence, tigrina, striate and maculate. Among the 12 chlorophyll mutants, albino was found to be the maximum followed by virescens in the two doses.

Table 1. Frequency and spectrum of chlorophyll mutants in M_2 generations of Buenavista cultivar irradiated with Gamma-rays.

Chlorophyll mutations	Gamma-rays (Grays)	
	200	300
Albino	0,91	0,72
Xantha	0,05	-
Xantha- alba	0.06	0,08
Viridis-albino	0.08	0,05
Albo-viridis	0.02	0,03
Virescens	0.69	0,57
Chlorina	0,35	0,44
Lutescens	0.07	0,20
Albescens	0,09	0,21
Tigrina	0,20	0,05
Striata	0,05	0,15
Maculata	0,30	0,23

In the M_2 population, 16 types of different mutations have been observed such as life cycle, plant height, habit of growth, shape of leaves, waxy foliage, row of spike, spike density, number of flowers, waxy spike,

Cereal Research Programme, Universidad Nacional Agraria de La Molina, Lima, Peru

* Corresponding author. E-mail: luzgomez@lamolina.edu.pe

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anwless spike and naked grains. **Table 2** shows the different frequencies and types of mutations. There were more kinds of mutations and higher frequencies with the higher doses. The interesting aspects of these results were the significant genetic variability created in different plant characters. This is an indication of the possibility for improvement of many characters through induced mutations [5, 18]. Rice research work, in Peru, using induction mutation reported changes in characters as heading date, maturity date and plant height [8, 16,].

Table 2. Frequency of different kinds of mutations in Buenavista Cultivar irradiated with Gamma-rays

Mutations	Gamma-rays (Grays)	
	200	300
Heading date (Very late-super early)	0,02	0,13
Maturity date (Very late-super early)	0,01	0,04
Height reduction	0,01	-
Height increment	0,05	0,08
Prostrate habit of growth	0,006	0,09
Narrower leaves	-	0,014
Waxy foliage	-	0,014
Six rows	0,034	0,044
Irregular spike	0,095	0,106
Raquis internodes reduction	0,017	-
Raquis internodes increment	-	0,014
Increment of number of flowers	-	0,014
Awnless spike	-	0,014
Waxy spike	0,022	-
Naked grain	0,091	0,071

Table 3 presents some agronomic characteristics and the response to yellow rust (*Puccinia striiformis* f *sp hordei*) of the mutant line Centenario (Mbv-earlier), Buenavista (parent line) and modern and traditional commercial cultivars. Centenario had a significant higher yield than the parental line and the traditional cultivars and slightly lower yield than the modern cultivars; however this difference was not significantly high. The yield of Centenario improved by 37% over the parent cultivar. For the duration of the life cycle (heading and maturity data), Centenario mutant was the earliest cultivar, 18 days earlier than the parental cultivar. The plant height of Centenario was similar to that of the parent and shorter than the other commercial cultivars. All genotypes including Centenario were resistant to yellow rust with the exception of Yanamuclo and Zapata. In reference [13] it is reported that the significant increase in cotton yield achieved with the mutant NIAB 78 in Czechoslovakia and mutant barley Diamant exceeded 12% return on the original variety. Reference [18] reports that mutation in productivity, grain size, protein content, and duration of vegetation period in barley. Reference [15] reports mutations in precocity in rice.

Among the characteristics related to barley quality presented in **Table 4**, the greatest statistically significant difference between parental cultivar Buenavista and Centenario mutant cultivar was in protein content (%) and test weight (kg/Hl). Centenario Mutant had better values of protein content, thousand-kernel weight and test weight than the traditional and modern cultivars. Reference [1] reported valuable progress on the improvement of nutritional quality of cowpea "IT84S 2246 D" mutants.

Centenario mutant cultivar shows four principal differences with the parental cultivar Buenavista, better yield, earlier life cycle date, better protein content and better test weight. The combination of these characters with a good plant weight and resistance to yellow rust made the cultivar Centenario very valuable for the highland farmers.

Centenario is replacing the traditional and modern cultivar at the central highlands of Peru. The replacement means self-sufficiency and improvement of household income through the sale of surplus (**Table 5**). Improvement of the quality implies an improvement of the nutritional value and increased price of the product. Current prices of traditional and modern cultivars per ton varies from 185 to 296 US dollars, and those obtained with Centenario can reach 488 to 533 US dollars, because of higher grain quality (**Table 6**).

Table 3. Mean yield, heading date, maturity date, plant height and response to yellow rust of Centenario mutant cultivar compared with the parent cultivar Buenavista and commercial barley cultivars

Genotypes	Yield (kg/ha)	Heating date (days)	Maturity date (days)	Plant height (cm)	Yellow rust
UNALM 96	5812.7a	73.7cd	160.0b	108.3bc	0
UNALM 94	5697.7a	81.3a	172.0a	112.3b	0
Centenario (Mutant Cultivar)	5552.3a	64.7e	141.0d	106.3c	0
UNALM 95	5114.7b	68.0de	143.67d	109.3bc	0
Buenavista (Parent cultivar)	4041.7c	72.7cd	159.7b	107.0c	0
Yanamuclo (Traditional Cultivar)	3281.0d	78.0ab	149.0c	112.0b	80s
Zapata (Traditional Cultivar)	3198.0d	78.7a	169.7a	119.3a	30s

Table 4. Mean protein content, thousand-kernel weight and test weight of *Centenario* mutant cultivar compared with the parent cultivar Buenavista and commercial barley cultivars

Genotypes	Protein content (%)	Thousand-kernel weight (g)	Test weight Kg/Hl
UNALM 96	9.12c	41.41b	67.1c
UNALM 94	9.49b	39.62b	59.1d
Centenario (Mbv-Earlier)	10.26a	51.72a	77.2a
UNALM 95	9.54b	51.42a	77.3a
Buenavista (Parent cultivar)	9.33bc	50.44a	71.4b
Yanamuclo (Traditional Cultivar)	8.10d	40.89b	68.4c
Zapata (Traditional Cultivar)	7.71e	37.62c	58.4

Table 5. Mean yield (kg/ha) of Barley Mutant Cultivars compared with old cultivars in farmer field at the highland of Peru

Location	Old Cultivars (Kg/ha)	Mutant Cultivars (Kg/ha)	Increase %
Junín	1600	2400	50
Huancavelica	1200	2640	105
Huanuco	1000	2200	120

Table 6. Commercial value of barley mutant cultivars production per hectare compared with the old cultivars at farmer location

Location	Old Cultivars US $	Mutant Cultivar US $	Increase %
Junín	296	533	80
Huancavelica	200	586	193
Huanuco	185	488	163

Kiwicha

Kiwicha (*Amaranths caudate*) is a native and ancient crop of the Andean region. It has been rediscovered as a promising source of high quality protein food and as a drought-tolerant crop. It has the potential to broaden the diversity of commercially grown crops and make an important contribution to food supplies in the near future with the world's growing water shortage. The aim of this research work was to improve the traditional cultivar Ancash of *Amaranthus caudatus* using Gamma-rays. A mutation induction method is used to improve well-adapted cultivars,through2].

Materials and Methods

Dried seeds of the traditional cultivar "Selection Ancash," previously purified in isolated conditions, were treated with Gamma-rays at doses of 100, 200, 300, 400, 600, 800 and 1,000Gy . A wide range of doses with Gamma-rays was selected because there were no reports of similar work in *Amaranthus caudatus*. The management of generation M_1 to M_7 was similar to that used in barley and described above.

Results and Discussion

In generation M_2, two types of mutations were identified in the treatment with 400Gy (**Table 7**). The other lower doses did not show any kind of mutation and the higher doses at M_1 generation killed the major part of plants. The frequency chlorophyll and plant color mutations are showed in **Table 8**.

Table 7. Frequency of different kinds of mutations in selection Ancash cultivar irradiated with Gamma-rays

Gamma-rays	No. Plants	No. of mutants	Mutation frequency	Type of mutation
400	186 915	3	0.00161	Chlorophyll (Xhanta)
		36	0.0193	Plant and grain colour

In generation M_3 and M_4 mutants with different yield potential were selected among the 36 mutant lines. In **Table 8**, five mutants had more yield than the parental cultivar. From these lines Centenario cultivar was selected and liberated in March 2006 with similar quality, better yield and different plant color than the parental material from the treatment of 400Gy of Gamma-rays. At farmer location in the coast the yield has a variation of 3,500 to 5,500 kg/ha and in the highland from 2,500 to 3,700 kg/ha. Some mutant varieties of rice had considerable economic impact, such as the mutant Yuanfengzao developed through irradiation with Gamma-rays. This variety matures about 45 days earlier than the parent material IR8, and still has high yield potential close to 10t/ha. During 1975-1983 the cultivation acreage reached six million hectares [13].

Table 8. Mean yield of Kiwicha mutant lines compared with the parental line "Selection Ancash" in experimental plot in La Molina

Genotypes	Yield (Kg/Ha)	Duncan Test (5%)
Centenario (MSA 011)	5,541	A
MSA 017	5,337	A
MSA 014	4,898	A
MSA 012	4,834	A
MSA 010	4,804	A
MSA 018	4,354	A B
MSA 013	4,331	A B
MSA 015	4,156	A B
MSA 016	4,147	A B
Selección Ancash (P)	2,764	B

The experiments conducted in different locations of the coasts and the highlands of Peru have permitted us to learn about the wide adaptation, and the tolerance to salinity and to *Sclerotinia sclerotiorum,* as well as the fact that it is earlier than the Oscar Blanco commercial cultivar. The better yield, color and size of its grains have contributed to the preference of Centenario over the other commercial cultivars. The area seeded with Centenario is nearly 40% of the total of Peruvian land dedicated to kiwicha crop.

Conclusions

1. Significant genetic variability was identified, especially in barley, with respect to chlorophyll mutations, morphological and physiological characters after treatment with Gamma-rays.
2. The mutant cultivars Centenario barley and Centenario Amaranhus- Kiwicha have a superior or similar behavior in important agronomic and quality characters compared to parent material and traditional and modern commercial cultivars.
3. In farmer fields the mutant cultivars are widely accepted, improving the amount and quality of the food. Household incomes are improving due to the higher prices reached in the commercialization of the mutants.

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Understanding the Molecular Mechanisms of Disease Resistance Using Rice Mutants

Y Jia

Abstract

The *Pi-ta* gene in rice has been used to prevent the rice blast disease worldwide for several decades. In the US, *Pi-ta* was introgressed from a landrace indica variety, Tetep, into several tropical japonica cultivars including Katy. *Pi-ta* is predicted to encode a cytoplasmic receptor that directly binds to the elicitor produced by the pathogen avirulence gene *AVR-Pita* for initiating resistance. Katy, expressing resistance conditioned by *Pi-ta*, *Pi-ta*^{2}, and *Pi-k*^{s} to the races of *M. oryzae*, IB1, IB45, IB49, IB54, IC17, IH1, IE1, and IG1, was treated with mutagens, fast neutrons and ethyl methyl sulfate (EMS). Six mutants with enhanced resistance or susceptibility were identified by screening M_2 seedlings derived from 15,000 M_1 plants. Among them, M562, induced by EMS, is a lesion mimic mutant (named as LMM1) that produces spontaneous hypersensitive cell death. This rapid cell death was quickly induced using detached leaves at and near the site of inoculation by the virulent race IE1k and more slowly induced when inoculated with the avirulent isolate IB49. Similar hypersensitive cell death was observed after detached leaves were inoculated with the fungus *Rhizoctonia solani*, the causal agent of rice sheath blight disease. Hypersensitive cell death is known to be a form of the defense response. Thus, we suggest that LMM1 has enhanced resistance to both rice blast and sheath blight pathogens. Although the *Pi-ta* gene in rice provides resistance to the races, IB1, IB45, IB49, IC17, IH1, IE1, IB54, and IG1, the mutant M2354 was observed to be susceptible to all races except IB54 although there was no change in the *Pi-ta* DNA sequence. Expression of *Pi-ta* in M2354 was also similar to that of the parent examined by qRT-PCR. Thus, mutations in M2354 likely occurred at a new locus named as *Ptr(t)*. Another four lines were determined to be near isogenic lines at a 9 megabase genomic region spanning the *Pi-ta* locus of Katy. Progress on characterizing these six genetic stocks is presented.

Introduction

In contrast to humans and animals, plants are not able to physically move to escape from pathogens. Over time plants have evolved multifaceted sophisticated mechanisms to cope with the pathogens. Knowledge of mechanisms of interactions between both plant and pathogen genes is important for crop protection. Rice -*Magnaporthe oryzae* is a model system for studying mechanism of host-pathogen interaction. Available resources for this system include the map based genome sequences of rice, draft sequence of a strain of the fungal pathogen, high density integrated physical and genetic maps of rice, and the ability to perform genetic analysis of both rice and the pathogen. To date, over forty race specific blast resistance (*R*) genes have been identified, seven of which have been molecularly characterized. Most cloned blast *R* genes are highly similar to other plant *R* genes that encode putative receptor proteins with nucleotide binding sites and leucine rich repeats. Among 7 blast *R* genes, a single amino acid in three *R* genes was known to determine the pathogen recognition specificity.

We are investigating the genetic mechanism of the host-pathogen interaction using the blast *R* gene *Pi-ta* and the corresponding avirulence gene *AVR-Pita*. *Pi-ta* is a putative cytoplasmic receptor with nucleotide binding sites and leucine rich domain, whereas AVR-Pita is a metalloprotease whose product is predicted to bind to leucine rich domain of the Pi-ta protein [1,2]. Limited surveys of rice germplasm and isolates of the pathogen revealed that both Pi-ta and AVR-Pita may have coevolved. Continued characterization of more *R* genes and other plant factors that are involved in pathogen recognition and transduction pathways should clarify how plant *R* genes have evolved to activate innate immunity to the pathogen. Here we report six new genetic stocks identified after seeds of a *Pi-ta*-containing cultivar were treated with Ethyl Methyl Sulfate (EMS) and fast neutrons.

Materials and Methods

Plant materials, mutagen treatment and M_1 growth-A tropical japonica rice cultivar Katy resistant to the 10 common races of *Magnaporthe oryzae* in the US, was used for this study [8]. Three different concentrations 0.4%, 0.8% and 1.2% of EMS were used to treat Katy following a protocol of Hu and Rutger [6]. Fast neutrons at 7.7, 26.3, 49. 9Gy were used to treat rice seeds at Oakridge Laboratory, TN, USA. Treated M_0 seeds were grown in fields in Stuttgart in 2001 and 2004 respectively. A panicle from each M_1 plant was harvested and was subsequently amplified in a greenhouse.

The fungal isolates and mutant screening-A virulent isolate TM2 of race, IE1k, was used to screen for enhanced resistance and an avirulent isolate ZN57 of race IC17 was used to screen for susceptibility. Seventeen M_1 seeds from each M_1 panicle were grown in a pot, and M_2 rice seedlings at three to four leaf stage were spray-inoculated with spores of *M. oryzae* at 5 X 10^5 spores/mL. Inoculated seedlings were incubated in a dew chamber overnight, and were moved to a greenhouse for an additional six days to allow phenotype development. Rice seedlings with disease or hypersensitive cell death were transplanted into new pots and grown to maturity.

Simple sequence repeat (SSR) marker analysis, PCR amplification, cloning, sequence analysis and controlled inoculations-SSR analysis, PCR amplification, cloning and sequence analysis were performed using procedures described in Jia and Martin [3]. Controlled inoculations with *M. oryzae* followed a procedure by Jia, *et al* [5] and with *R. solani* followed a procedure described by Venu, *et al* [9].

Results

A total of 142 rice seedlings with altered blast disease reactions were transplanted to produce seeds in a greenhouse. Eight SSR markers were used to determine the origin of the putative mutants. Twenty mutants were confirmed to be derived from Katy, and six of these were further characterized in the present study (**Table 1**).

USDA-ARS Dale Bumpers National Rice Research Center, Stuttgart, Arkansas, United States of America

E-mail: yulin.jia@ars.usda.gov

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Table 1. SSR genotyping and disease reactions to rice blast and sheath blight pathogens

The parent and mutant line	SSR marker								Disease reaction	
	RM149	RM190	RM22	RM225	RM481	RM484	RM303	RM489	M. oryzae[c]	R. solani[d]
Katy	241	113	192	143	217	293	144	270	R	MR
2354[a]	241	113	192	143	217	293	144	270	S	MR
2494[a]	241	113	192	143	217	293	144	270	S	MR
4935[a]	241	113	192	143	217	293	144	270	S	MR
2500[a]	241	113	192	143	217	293	144	270	S	MR
5415[a]	241	113	192	143	217	293	144	270	S	MR
562[b]	241	113	192	143	217	293	144	270	R	R

[a] The mutant was derived from a M_0 seed treated with fast neutrons.
[b] The mutant exhibits lesion mimic symptoms derived from a M_0 seed treated with 0.8% ethyl methyl sulfate.
[c] The races, IB1, IB45, IB49, IC17, IH1, IE1, and IG1 of *M. oryaze* were used for inoculation. Disease reaction was scored as a scale 0 to 5, 0-2 is resistant and 3-5 is susceptible. The parent and all mutant lines were resistant to IB54.
[d] Three isolates of *R. solani* were used, and disease lesions were compared with Katy. MR indicates moderate resistance and R indicates enhanced resistance.

Mutant 2354- M2354 has identical alleles for 8 SSR markers and morphological traits (data not shown) to the parent Katy but is susceptible to nine races of rice blast in the US except race IB-54 (**Table 1, Fig. 1**). **Fig. 1A** shows typical symptoms of the susceptibility of rice blast fungus.

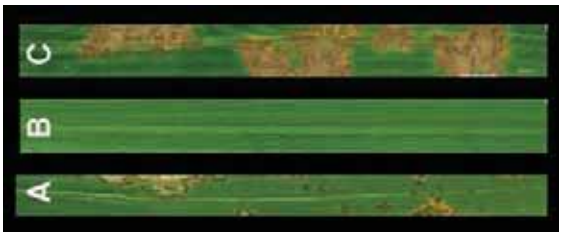

Figure 1 Symptoms of lesion mimic and blast disease. Typical symptoms of rice blast disease observed in mutants 2354, 2500, 2912, 4935 and 5415 (A), healthy non inoculated rice leaf (B) and phenotypes of lesion mimic mutant 1(mutant 562) (C).

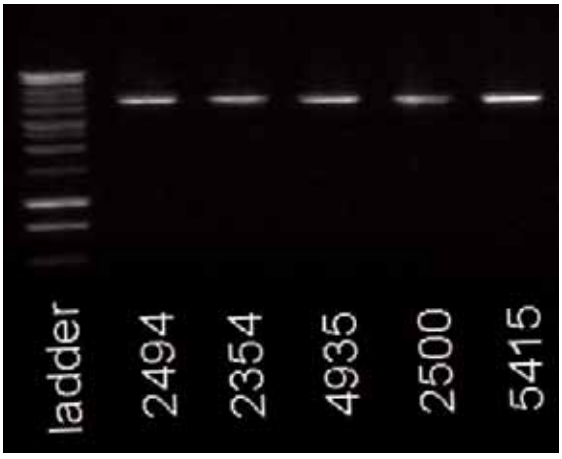

Figure 2 PCR amplification of the Pi-ta alleles from 5 susceptible lines. The same size of the Pi-ta allele was amplified using the Pi-ta gene specific primers.

The *Pi-ta* gene in Katy confers resistance to the races IB1, IB45, IB49, IC17, IH1, IE1, IG1 and IB54 and *Pi-k*^{s} confers resistance to IB54. These results suggest that either *Pi-ta* or a genetic factor required for *Pi-ta* is defective in M2354, and this defect is likely specific to the *Pi-ta*-mediated defense response. The genomic region including 863 bp of 5' untranslated region, open reading frame, intron, and 500 bp of 3' untranslated region of the *Pi-ta* allele in M2354 was amplified (**Fig. 2**) and amplified products were determined to be identical to that of Katy. However, genomic sequences of the *pi-ta* alleles in M2494, M4935, M2500 and M5415 were identical to that of the *pi-ta* allele in one of susceptible parents in Katy's background (data not shown). Further genotyping using 50 SSR markers revealed that these lines were near isogenic lines from the parent Katy but had a 9 megabase region on chromosome 12 comparable to that of susceptible parent (data not shown).

Expression of the *Pi-ta* allele in Katy was identical to that in M2354 as examined by RT-PCR and real time RT-PCR [3]. Taken together, these data suggest that the *Pi-ta* allele was not altered, and hence we predict that an additional component in M2354 was defective rendering disease susceptibility. Further segregation analysis of the progeny of the cross of Katy with M2354 revealed that a nuclear gene had been altered. This gene was designated as *Pi-ta* required (*R*) gene temporary [*Ptr(t)*]. *Ptr(t)* was tentatively mapped at the *Pi-ta* locus, and is being identified using a map based cloning strategy.

Mutant 562-M562 was named as lesion mimic mutant 1 (LMM1) [2]. Lesion mimic plants are plants that produce spontaneous disease lesions similar to hypersensitive cell death in the absence of the pathogen attack (**Fig. 1**). Lesion mimic phenotypes of LMM1 were found to be rapidly induced three days after inoculation with a virulent isolate TM2 of *M. oryzae*, and the same phenotype was found to be induced by an avirulent isolate ZN60 of *M. oryzae* 6 days after inoculation (**Fig. 3**). Rapid occurrence of the cell death was hence predicted to be one plausible mechanism to prevent the invasive growth of the fungal pathogen [11].

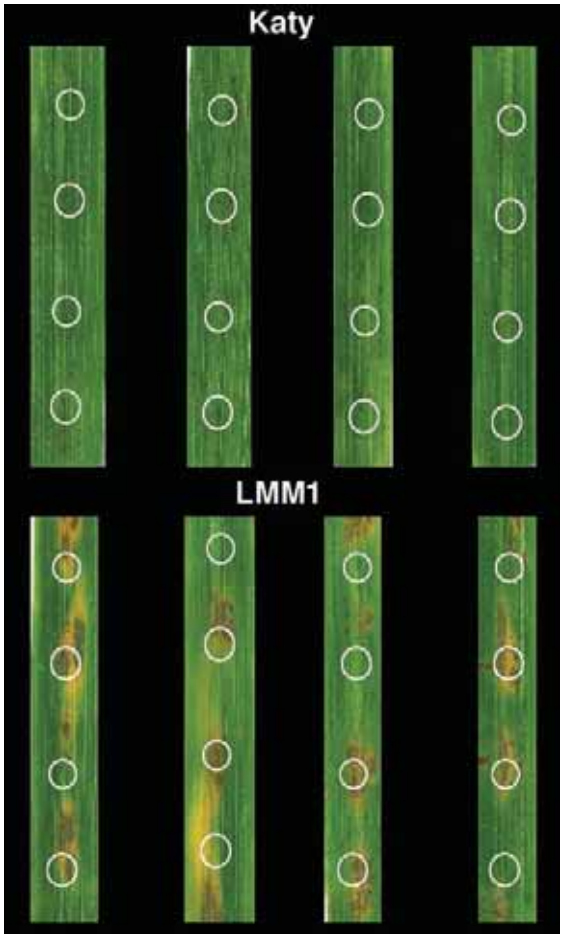

Figure 3 Induction of lesion mimic phenotypes on detached rice leaves. No visible lesions could be seen four days after detached leaves of the parent Katy were inoculated with isolate ZN57(IC-17) of *M. oryzae* (upper panel); however, lesions were induced on the detached leaves of LMM1 at and near the sites of inoculation (lower panel). Five uL of spores at 4.16 X 105 spore/mL were inoculated onto indicated positions on detached leaves.

To determine if enhanced resistance was specific to rice blast pathogen, disease reactions of LMM1 to *R. solani*, the causal agent of rice sheath blight disease, were also determined. As shown in **Fig. 4**, smaller disease lesions were observed four days after inoculation with three field isolates of *R. solani.* This result suggests that LMM1 also has enhanced resistance to *R. solani*.

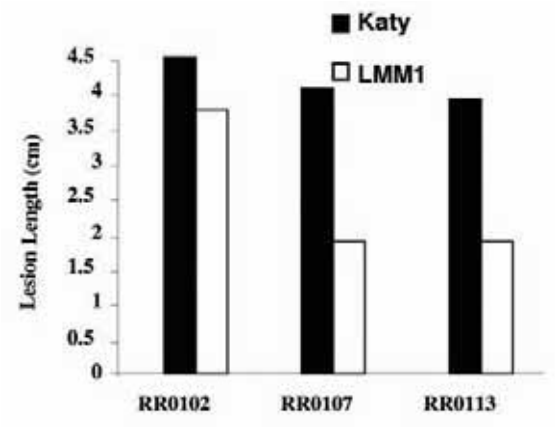

Figure 4 Smaller lesions were observed after detached leaves of LMM1 was inoculated with three field isolates of *R. solani*. Detached leaves of Katy and LMM1 were inoculated with three isolates RR0102, RR0107 and RR0113 of R. solani. Lesion length was measured from the center of the inoculation four days after inoculation.

Discussion

In this study, six genetic stocks for studying the molecular mechanisms of host-pathogen interaction were developed by fast neutrons and EMS. Two mutants were identified with alteration in different genetic components of resistance.

LMM1 is a lesion mimic mutant similar to Sekiguchi sasahi (*sl*) [7]. Both mutants produce spontaneous cell death and are conditioned by single recessive genes [2,7]. Allelism test suggests that LMM1 is not allelic to *sl* (Jia, unpublished data). We observed that LMM1 had enhanced resistance to both rice blast and sheath blight pathogens. Similarly, Yin, *et al* [10] reported that a lesion mimic mutant exhibiting small brown spots had broad resistance to both rice blast and sheath blight diseases. LMM1 identified in this study is another genetic resource that can be used to dissect the components in host-pathogen interactions.

M2354 has a defect at the *ptr(t)* locus resulting in susceptibility to blast. Pathogenicity assays using *M. oryzae* isolates recognizing *Pi-ta* and *Pi-ta*^{2} revealed that M2354 lost resistance mediated by *Pi-ta* and *Pi-ta*^{2} (B. Valent, unpublished data). The *Ptr(t)* gene was recently fine mapped and the candidate genes are being sequenced for confirmation.

M2494, M4925, M2500 and M5415 are near isogenic lines possessing a 9 megabase region from a susceptible parent of Katy. It is believed that these are a result of a heterogeneous seed source of Katy being mutagenized at the onset of this project. Nevertheless, these lines are useful to study biological functions of genes within this region.

To summarize, we have identified six genetic stocks of *Pi-taptr(t)*, *pi-taptr(t),* and *Pi-taPtr(t)* homozygotes with and without lesion mimic phenotypes. These genetic stocks are important materials to study the functions of important plant genes, some of which are involved in *Pi-ta*-mediated signaling recognition and transduction pathways [1,4]. Further manipulation of genetic interactions of these genes should facilitate the development of more effective strategies to control plant diseases.

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An Innovative Way of Developing an Improved Variety Utilizing Both Gamma-ray-induced and Recombinational Variability in Blackgram (*Vigna mungo* L.(Hepper))

S T Kajjidoni*, K Roopalaksmi, S Revanappa & I Nagaral

Abstract

An attempt was made to compare variability generated through different mating schemes and combination of mating and irradiation in *Vigna mungo* L. (Hepper) to improve productivity of recommended varieties. Two locally adapted varieties and two selected complimentary donor lines for high pod number and for seed mass were crossed to generate four single crosses, two three way and one double crosses. Four single crosses were further irradiated with 20 Kr Gamma-rays and advanced to F_2M_2 and F_2 generations for evaluation based on seven agronomic traits. Variability generated by irradiation was more compared to recombination variability for clusters per plant and pod length traits. Irradiated single cross (F_2M_2) progenies produced higher frequency of superior progenies compared to other hybridized progenies involving two or more than two parents. The nature of association between pod length and number of pods per plant under irradiation improved favorably. Selected superior progenies isolated in F_2 and F_2M_2 (112) and in F_3M_3 and F_3 generations (135) were advanced to the F_5 generation and evaluated in progeny row trial with two replications. We found that 29 advance breeding lines were superior. Out of 29 lines, 18 originated from irradiated single crosses and five lines from single crosses without irradiation, and six lines from hybridized progenies involving more than two parents revealing the importance of irradiation in creation of desirable variability. The stability analysis involving 29 advanced breeding lines revealed the stable performance of DBS-14, DBS-16, DBS-24 and DBS-26 genotypes over environments with better mean performance for seed yield. Genotype DBS-15 had highest seed protein content (27.20%), which was followed by DBS-12 (26%) compared to high yielding check TAU-1 (19.68%). The large scale trials in different agro climatic conditions, genotype DBS-14 (DU-1) was the most promising genotype with superior seed yield (22.0%) and seed mass apart from its tolerance to stem fly damage compared to adapted cultivar TAU-1 and the genotype is identified for commercial cultivation in the name of DU-1 in Karnataka state and also registered with NBPGR New Delhi for its novel agronomic traits.

Introduction

Mutagenesis in association with recombination breeding offers a viable option to improve adapted variety by crossing with donors of seed yield components there by releasing variability hidden in the conserved gene blocks. In general improvement of blackgram (*Vigna mungo* L. (Hepper)) is limited by lack of variability for the components of seed yield particularly pod length, pod number and seed mass. There are no reports available in the literature on the role of irradiation and recombination in creation of desirable variability in blackgram. Hence, an attempt was made to compare variability generated through different mating schemes and combination of mating and irradiation to improve productivity of recommended varieties in the state of Karnataka.

Department of Genetics and Plant Breeding, College of Agriculture, U.A.S. Dharwad–580 005 India

* Corresponding author. E-mail: stkajjidoni@yahoo.co.in. Present address: Principal Scientist (plant breeding) Agricultural Research Station, Nipani-591 237 India

Materials and Methods

The experimental material comprising of eleven populations which were generated by involving four selected lines in different mating schemes in combination with gamma irradiation. Four genotypes included were Manikya and TAU-1 locally adapted varieties which lack optimum seed size and number of pods per plant respectively. Two selected complimentary donor lines viz., No.169, a line for high pod number (50-55) and No. 216 for seed mass (6.5g) were crossed to generate four single crosses, two three way and one double crosses. Four single crosses were further irradiated with 20 Kr Gamma-rays and advanced to F_2M_2 and F_2 generation. These were evaluated in F_2M_2 and F_2 generation for assessment of desirable variability for seven agronomic traits.

Results and Discussion.

Irradiated populations of single crosses exhibited higher phenotypic coefficient of variability (PCV) and genotypic coefficient of variability (GCV) values for clusters per plant and pods per plant traits. Variability generated by irradiation appeared to add to the recombination variability for two traits such as clusters per plant and pod length [1]. Irradiated single cross (F_2M_2) progenies had higher frequency of superior progenies for pods per plant, 100 seed weight and seed yield per plant compared to other hybridized populations involving two or more than two parents (**Table 1**).

Table 1. Frequency of superior progenies in F_2 and F_2M_2 generations of blackgram

Progenies	Generation	N° of plants	N° and % superior plants for pods*	Range		
				Pod number	Seed mass (g)	Seed yield / plant (g)
Single cross	F_2	790	142 (18 %)	20-84	3.93-5.64	10.4-27.94
Irradiated single cross	F_2M_2	1100	231 (21%)	20-87	4.03-5.79	10.37-38.23
Three way cross	F_2	740	141 (19%)	22-79	4.01-5.28	11.22-34.60
Double cross	F_2	500	85 (17%)	19-60	4.36-5.43	11.28-29.71

* 100 seed weight and seed yield per plant

Nature of association between pod length and number of pods per plant under irradiation was improved favorably and even it was changed from non significant negative to positive significant in F_2M_2 progenies of a cross TAU-1x169 [2].

Selected superior progenies isolated in the F_2 and F_2M_2 (112) and in the F_3M_3 and F_3 generations (135), which were separately advanced from F_2, F_2M_2 to F_3, F_3M_3 by following three different selection methods. All these selected individual progenies were advanced to F_5 generation and evaluated in progeny row trial with two replications, based on their seed yield which yielded more than mean + two standard deviation values, we found that 29 advanced breeding lines were superior. It is interesting to note that when the pedigree of 29 advanced breeding lines was traced

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Table 2. Performance of DU-1 (DBS-14) across locations and seasons (Kharif) (Seed yield kg/ha)

Genotype	Dharwad			Bailhongal		Bidar				B'gudi	Gulbarga		Avg.
	K-03	K-04	K-05	K-02	K-05	K-02	K-03	K-04	K-05	K-06	K-05	K-06	
DU-1	1113*	1736*	1875*	805*	464	1107*	1542	986	1020*	826	927*	527	1077.33
TAU-1	825	1357	1424	507	570	754	1381	868	910	833	694	505	885.66
Manikya	790	1083	1179	430	385	684	1104	792	819	778	721	325	757.50
CV %	15.01	12.52	12.10	18	10.6	15.9	10.4	12	8.5	10.18	13.0	18.9	
CD 5%	208	293	310	126	NS	178	173	NS	96.70	NS	156	153.2	

*significant at 5% level of probability

Table 3. Score for insect pests and diseases of promising blackgram genotype

Sl. No.	Entry	Stem fly (%)	Thrips	Aphion infestation		Cercospora leaf spot (0-9)	Powdery mildew (1-5)
				Seeds (%)	Pods (%)		
1	DU-1	30	4.50	22.1	26.0	7	5
2	TAU-1	65	6.25	26.7	31.0	9	5

back, we found that 18 lines originated from irradiated single crosses and five lines from single crosses without irradiation and six lines from hybridized populations involving more than two parents revealing the importance of irradiation in creation of desirable variability.

Further stability analysis of advance breeding lines (29) revealed stable performance of DBS-14, DBS-16, DBS-24 and DBS-26 genotypes over environments with higher mean performance for seed yield and its component traits [3]. The derived advanced breeding lines were evaluated for seed protein content and sugar content across environments. Genotypes exhibited variation for protein content which ranged from 17.9 to 27.2 % when grown at Dharwad location. Genotype DBS-15 had highest seed protein content followed by DBS-12 (26%), compared to high-yielding check TAU-1 (19.68%). Two genotypes DBS-7 and DBS-21 recorded low sugar content in three test environments which can be considered as low flatulence causing lines [3].

Multilocation tests involving these genotypes in different agroclimatic conditions has lead to identification of DBS-14 as most promising genotype (**Table 2 and 3**) with superior seed yield (22.0%) apart from its tolerance to stem fly damage compared to adaptive cultivar TAU-1. Based on the merits (**Fig. 1**), the genotype was identified for commercial cultivation in the name of DU 1 in Karnataka and also registered with NBPGR New Delhi for its novel agronomic traits.

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Induced Mutations Affecting Root Architecture and Mineral Acquisition in Barley

P J White[1,*], A G Bengough[1], I J Bingham[2], T S George[1], A J Karley[1] & T A Valentine[1]

Abstract

Root architecture influences the acquisition of mineral elements required by plants. In general, plants with a greater root/shoot biomass quotient and a more extensive root system acquire mineral elements most effectively. In barley (*Hordeum vulgare* L.) induced mutation has produced commercial cultivars with greater root system size, and genotypes with greater root spread, longer roots and roots with denser root hairs. Work is in progress investigating whether these phenotypes improve the acquisition of mineral elements and, thereby plant growth and grain yield.

Root architectural requirements for mineral acquisition by plants

Plants require at least 14 mineral elements to complete their life cycles. These include six macronutrients (N, K, P, Mg, Ca, S), which are present in relatively large concentrations in plant tissues (g kg^{-1} dry weight) and several micronutrients (Fe, Zn, Mn, Co, Cu, B, Cl, Mo), which are present in smaller amounts (mg kg^{-1} dry weight). Tissue concentrations of these elements must be maintained within a certain range, since any deficiency can limit plant growth, and an excess can be toxic. In most areas of the world, agricultural production requires the application of fertilizers to supply a crop's requirement for essential mineral elements. However, commercially viable sources of most mineral elements are diminishing, and the unbalanced or excessive application of fertilizers can lead to environmental problems. Therefore, it is important to develop management strategies and crops that utilize our mineral resources most efficiently.

Mineral elements are acquired from the soil solution by the plant root system, and the acquisition of each mineral element has its own challenges. Root architecture influences most the acquisition of mineral elements that are required by plants more rapidly than they arrive at the root's surface, and for which the root system must forage in the soil [1], [2]. These mineral elements include P, K, Fe, Zn, Mn and Cu [3]. The ability of the root system to proliferate rapidly throughout the soil, especially in areas where these elements are locally abundant, is an advantage, as are the release of exudates and enzymes that solubilize essential mineral elements and the fostering of beneficial associations with symbiotic fungi and microbes [1], [4], [5], [6].

Barley (*Hordeum vulgare* L.) is a high yielding cereal crop that is cultivated worldwide for animal feed, human food, malting, brewing, and distillation. Its root system is comprised of between three to eight seminal roots, which arise from the embryo, and a greater number of adventitious (nodal) roots that originate from the base of the main stem and tillers during development [7]. It is thought that the ability of a barley plant to acquire mineral elements might be improved by it having a greater root/shoot biomass quotient [8], [9], a more extensive root system [1], [8], longer, thinner roots with more root hairs [10], [11], [12], [13], a greater number and even spread of seminal roots [14], [15] and the ability to proliferate lateral roots in mineral-rich patches [16]. It has been demonstrated that there is considerable variation in these parameters between barley genotypes. For example, wild barley has fewer seminal roots with a narrower spread [15], and invests less biomass in its root system [17] than cultivated genotypes. These observations suggest that root traits have been selected, albeit inadvertently, for particular environmental conditions during the domestication and improvement of the barley crop.

Root traits associated with the *sdw1* and *ari-e*.GP mutations

Induced mutation increases the genetic variation within a species and this technique has a long history of producing barley genotypes with agronomically beneficial traits. The FAO/IAEA Mutant Varieties Database lists 303 barley cultivars that have been produced through induced mutation (http://www-mvd.iaea.org/MVD/default.htm, accessed 21st May 2008). Among these cultivars are the semi-dwarf genotypes Diamant (sdw1 = denso, chromosome 3H), which was generated from the Czech cultivar Valticky using X-ray irradiation and released in 1965 [18], and Golden Promise (ari-e.GP = GPert, chromosome 5H), which was generated from the Maythorpe cultivar using Gamma-ray irradiation and released in 1966 [19], [20]. Other important cultivars bearing mutations in these genes include Triumph (sdw1), Prisma (sdw1), Derkado (sdw1), Optic (sdw1), Tocada (sdw1), Westminster (sdw1) and B83-12/21/5 (ari-e.GP).

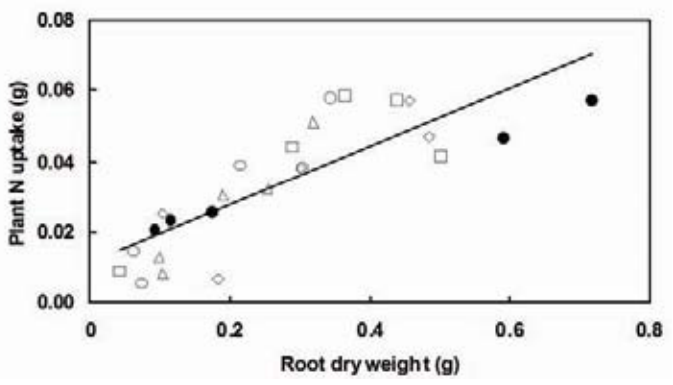

Figure 1 The significant positive relationships (R^2=0.685, P<0.0001, n=25 individual plants) between plant N uptake and root system size at grain maturity among five barley cultivars grown in the glasshouse at SCRI in 1m long tubes filled with a grit-sand-gravel mixture and irrigated daily with a water flush followed by a standard liquid nutrient application. Genotypes included a tall cultivar (Kenia, filled circles), and cultivars bearing the dwarfing gene *sdw1* (Derkado and Westminster, open circles and triangles, respectively) or *ari-e*.GP (Golden Promise and B83-1212/21/5, open squares and diamonds, respectively). In this experiment, grain yield was positively correlated with both plant N uptake and root dry weight (A.J. Karley, data not shown).

The *sdw1* and *ari-e*.GP mutations were originally selected because they reduce plant height and increase grain yield, but they also affect root traits such as root length, root weight and nitrogen isotope dis-

[1] Scottish Crop Research Institute, Dundee, United Kingdom
[2] Scottish Agricultural College, Edinburgh, United Kingdom

* Corresponding author. E-mail: philip.white@scri.ac.uk

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crimination [21], although the manifestation of their effects on root traits is influenced greatly by the environment. It has been observed that genotypes bearing the *sdw1* or *ari-e*.GP mutations often have larger root systems than non-dwarf genotypes when grown in the field [17] and that both mutations are present in chromosomal regions (QTL) affecting root system size among genotypes of a Derkado x B83-12/21/5 double haploid mapping population [17], [22]. The *sdw1* mutation was associated with increased size of both seminal and adventitious root systems in the Derkado x B83-12/21/5 population, particularly during the early stages of plant development [17], [22]. However the *ari-e*.GP mutation was associated with fewer roots, shorter roots and lesser root spread in 10-day-old seedlings [22], lower root mass of six-week-old seedlings, and reduced size of the root system of field grown plants at grain filling [17]. Other chromosomal regions associated with total root length, root number and root spread have been identified in the Derkado x B83-12/21/5 mapping population [17], [22] and in a collection of mutants backcrossed into the Bowman cultivar [14].

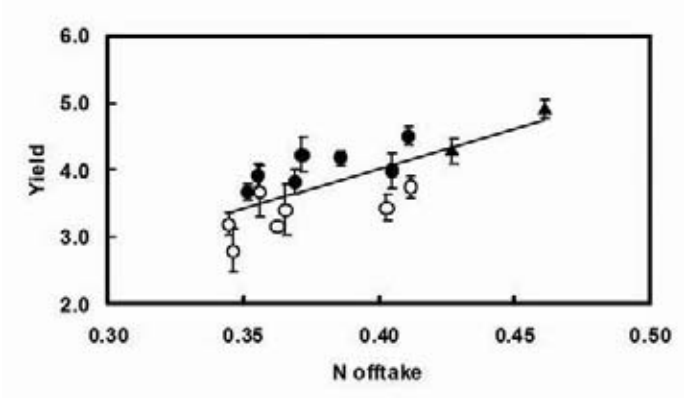

Figure 2 The significant positive relationship (R^2=0.549, P=0.001, n=16 genotypes) between grain yield and N offtake (kg kg^{-1} supplied) among 16 barley cultivars grown in the field at SCRI in 2006. Genotypes included tall cultivars (open circles) and cultivars bearing the dwarfing gene *sdw1* (filled circles) or *ari-e*.GP (filled triangles). Yield data are shown as means ± standard error of four replicates.

In glasshouse (**Fig. 1**) and field experiments (**Fig. 2**, [17]), N uptake and grain yield have been positively correlated with root system size. When grown in field plots, and supplied with ample N fertilizer, cultivars bearing the *sdw1* or *ari-e*.GP mutations generally have higher grain yields than taller genotypes with a comparable N offtake, and cultivars bearing *ari-e*.GP mutation often have higher offtakes of N and other mineral elements (**Fig. 2**). However, cultivars bearing the *sdw1* or *ari-e*. GP mutations can have lower concentrations of mineral elements in their grain, despite their greater offtake of mineral elements, which is possibly a consequence of yield dilution ([21] and I.J. Bingham and P.J. White, unpublished data). The *ari-e*.GP mutation has also been found to decrease shoot Na concentrations and increase salt tolerance [19], [23].

Screening a collection of induced mutants in the Optic cultivar

A population of induced mutants was produced in the Optic cultivar by the Scottish Crop Research Institute using ethyl methane sulphonate [24], [25]. A structured mutation grid for exploiting TILLING (Targeted Induced Local Lesions IN Genomes) has been developed for this population, which consists of >20,000 M_3 families. This collection can be used in different ways to identify genotypes with increased efficiency to acquire mineral elements from the soil. One approach (forward genetics) is to screen these collections directly for an improved phenotype and, subsequently, to identify the genetic origin of the phenotype. The other approach (reverse genetics) is to use the techniques of molecular biology to identify accessions with mutations in target genes known to influence the acquisition of mineral elements beneficially. This population has been screened for rooting phenotypes and their consequences for the acquisition of mineral elements have been investigated.

Early seedling root growth is an important agronomic characteristic, since it accounts for much of the N and P absorbed by a barley plant. The Optic collection was screened for differences in root traits from wildtype plants three days after germination (**Fig. 3**). The frequency of occurrence of mutant lines with altered root traits was high, but comparable to other collections [26], [27], [28]. The root phenotypes of several mutant lines were validated using a two dimensional root observation chamber specifically designed to measure rooting traits of young barley seedlings, such as root length, elongation rate, longest root, deepest root, seminal root number, and angular spread of roots (T.A. Valentine, unpublished data). These lines will be used for future physiological studies.

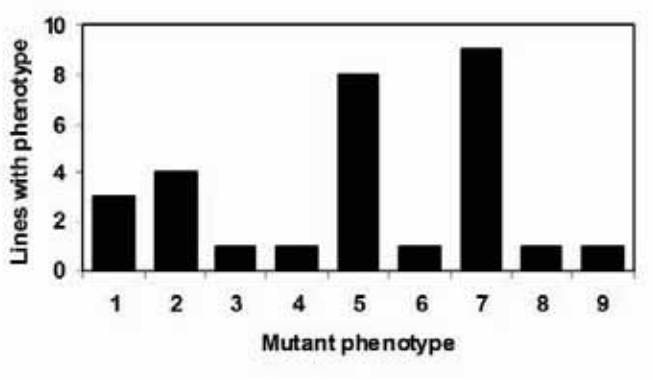

Figure 3 The frequency of specific root phenotypes among mutant lines of the Optic collection. The root phenotypes scored in comparison with the wild type were (1) long roots, (2) short roots, (3) reduced number of roots, (4) highly gravitropic roots, (5) agravitropic or curly roots, (6) more dense root hairs, (7) fewer or shorter root hairs, (8) presence of pigmentation, and (9) visible necrosis. Seeds were germinated on moistened filter paper and seminal roots were observed after three days. Thirty mutant lines with altered root characteristics were recorded from a screen of about 500 lines. (Data courtesy of W.T.B. Thomas, B.P. Forster and J. Lyons, SCRI)

Selected mutants from the Optic collection have also been screened in glasshouse and field trials. Accessions from the Optic collection with contrasting rooting traits (wild type, hairless, dense haired, long rooted and highly geotropic phenotypes) were grown to maturity in the glasshouse in 1 m long tubes filled with a grit-sand-gravel mixture and irrigated daily with a water flush followed by a standard liquid nutrient application. No significant differences in plant dry matter allocation were observed between these genotypes except for seed dry weight, which was significantly smaller in the hairless mutant compared with the wild type. However, the ability to acquire mineral elements was again correlated with the size of the root system. Root dry mass tended to be smaller in the mutant lines, although this was not significant.

Conclusions and Perspectives

The available evidence suggests that the acquisition of mineral elements by plants is related to the ability of their root systems to explore the soil. Mutants can be generated with root systems that exploit the soil better, acquire greater quantities of mineral elements, and produce greater yields on impoverished soils. In the coming years it is planned to screen the SCRI Optic mutant collection for multiple efficiencies in the acquisition and utilization of mineral elements and water. Current projects include screening for traits, and identifying genes, to improve the acquisition of N, P, Zn and Mn, improving the uptake and efficient use of water, and reducing the entry of toxic elements to the food chain. Knowledge of the genes impacting the acquisition of mineral elements can be used to develop genotypes of other common crops that can be

deployed in extreme environments: to increase their ability to grow on resource poor soils, to increase their accumulation of minerals required for animal nutrition and to reduce their accumulation of toxic elements. These outcomes should increase the sustainability of agriculture both at a subsistence and industrial level, and improve the health of populations by increasing the nutritional content and reducing the content of toxic elements in food consumed.

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Current Status and Research Directions of Induced Mutation Application to Seed Crops Improvement in Vietnam

M Q Vinh[1,3,*], D K Thinh[2], D T Bang[1], D H At[1] & L H Ham[1]

Abstract

Nuclear techniques and chemical mutagens have been applied in Vietnam since the 1970's in order to improve seed crops as rice, soybean, maize, groundnut, many mutant varieties were approved as national varieties and some promising regional lines. Main direction and methods using in varietal improvement in Vietnam were exploitation of gene resources, using genetic methods consisting of hybridization, mutation, gene transformation to create crops having high yield, good quality, tolerance to diseases and adverse conditions. Up to the year 2007, according to preliminary statistics, in Vietnam 50 mutant varieties were created (as IAEA database, having 43 mutant varieties created, Vietnam is being the ninth of mutant breeding' achievement record in the world). Among of those, seed crops occupied 47 varieties: rice was 32 varieties, soybean was 11, maize was 2, and peanut was 2. At AGI 17 rice mutant varieties, 11 mutant varieties of soybean were bred and approved by Ministry of Agriculture and RD as national and regional varieties. At present, about 15 % of Vietnam rice area annually cultivated by mutant varieties, some best mutant varieties become one of the top 5 varieties for export and grown recently more than 300,000 ha per year in South, more than 50% of soybean cultivated area occupies by mutants contributing worthily to increasing cereal productivity of Vietnam.

Introduction

For a long history, Vietnam still had lacked a food, in period of 1970 – 1980, every year Vietnam had to import 2 – 3 million tons of cereals for the need of domestic consumptions.

After 20 years of Renovation (1988 – 2007), production of major cereals (rice, corn, soybean, groundnut) have been increased by 2.5 – 4.0 times, yield – 2 times, consequently national food security has been already established in the whole country. In 2007 Vietnam exported 4.3 million tons of rice keeping the world's second-largest exporter. However Vietnam still had to import about 0.7 million tons of maize, 2.5 million tons of soybean. (**Table 1**). To solve with this problem Vietnam has many kinds of means to promote cereals production, in that genetic improvement is considered as a first priority.

Brief history of mutation breeding in Vietnam

Nuclear techniques and chemical mutagens have been applied in Vietnam since the 1970's in order to improve crops. Many mutant varieties have been planted in field of large area. A lot of agriculture research institutes have cooperated with Vietnam Atomic Energy Commission (VAEC), International Atomic Energy Agency (IAEA) and other organizations to conduct mutation breeding in varieties such as rice, maize, soybean, groundnut and other ornamental and fruit plants, many of which were approved as national varieties and some promising regional lines. Among them Agricultural Genetics Institute (AGI) is a specific research center, which used to be one of the earliest institutes applying nuclear techniques to create new mutant varieties by gamma rays, X rays, and the other mutagenic chemicals and had many successes in this field. Main methods using in varietal improvement in Vietnam were exploitation of gene resources, using and genetic methods consisting of hybridization, mutation, gene transformation to create crops having high yield, good quality, tolerance to diseases and unsuitable climate conditions.

Up to the year 2007, according to preliminary statistic, in Vietnam 50 mutant varieties were created (as IAEA database, having 43 mutant varieties created in 2007 Vietnam is being the ninth of mutant breeding achievement record in the world), among of those seed crops occupied 47 varieties, rice occupied 32 varieties, soybean 11, maize 2 and peanut 2 (**Table 2**, **Appendix 1**).

Table 1. Some achievements in seed crops production after 20-years of Renovation (1988 – 2007) in Vietnam (*)

Seed Crops	Years	Total area (Thous. Ha)	Production (mill. tons)	Yiel (tons/ha)
Paddy rice	1985	5,603.9	15.8	2.78
	2007	7,210.0	35.994	4.99
Corn	1985	587.1	0.86	1.47
	2007	1,067.9	4.11	3.58
Soybean	1985	102.1	0.0791	0.78
	2007	190.1	0.2755	1.47
Groundnut	1985	212.7	0.2024	0.95
	2007	254.6	0.5051	1.98

(*) Source: State staticstical office 1985 - 2007

Table 2. Mutation derived varieties in the world (FAO-IAEA Mutant Variety Database, 2007, Apr.)

Country	Var. No	Rank	Country	Var. No	Rank
China	638	1	Brazil	36	13
India	272	2	Slovakia	35	14
Japan	232	3	UK	34	15
Russia+USSR	214	4	Bangladesh	27	16
Netherland	176	5	Sweden	26	17
Germany	176	6	Cote d'voi	26	17
USA	128	7	Guyana	26	17
France	43	8	Belgium	23	20
Vietnam	42 (50*)	9	Iraq	23	20
Pakistan	42	9	Denmark	22	22
Bulgaria	38	11	Austria	21	23
Canada	37	12	Rep/of Korea	19	24

(*) Remark: By Vietnam primary data

[1] Agricultural Genetics Institute (AGI), Hanoi, Vietnam
[2] Institute of Agricultural Sciences for Southern Vietnam (IASSV), Ho Chi Minh City
[3] Vietnam Radiation Medical and Biology Association (VRMBA)

* Corresponding author. E-mail: maiquangvinh@gmail.com

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Main achievements of nuclear application on mutation breeding in rice in Vietnam

Agricultural Genetics Institute (AGI) is a specific research center, one of the institutes apply early nuclear techniques to create new mutant varieties by gamma rays, X rays, and the other mutagenic chemicals and we had many successes in this field (**Appendix 1**)

Rice is the major cereal and the staple food in Vietnam 75% starch quantities for daily meals of almost people. At present, 4.5-5 million tones of normal white rice from the total production 36 million tons are exported, reached up to 1.5 billion USD (2007), mainly for Asian market with competitive price (ca. ~260-275 USD/t), and very limited quantities of rice with high quality and glutinous rice (<100.000 t) are included, but mainly for Japan market with increasing price (>420 USD/T).

The National Strategy Project on Rice Breeding is identified for yield and quality improvement to increase the income of rice producing farmers and export price to support the food security of the country with the increasing population (ca. 90 mil. persons) and to meet the higher requirements of many markets opened in Europe, Middle East and Africa.

Recent development of combination of nuclear techniques with biotechnology, known as *in vitro* mutagenesis and manipulation, and molecular markers has been effectively introduced for the induction of new and novel types of crop varieties.

The used materials were local lines (Cuom, Chiem bau, Tam thom, Nang thom, Nang huong, Te etc.) and the varieties presently used in the crop productivity (C4-63, A8, CR203, Khang dan, IR64, IR50404). The treatment methods were dry seeds with different radiation doses (Gamma ray, X-ray with 80, 100, 150, 200, 250Gy, useful doses were 100, 120, 200Gy); the others were germinated seeds with radiation doses of 20, 30, 40, 80Gy, useful doses were 30, 40, 60Gy).

In the decades of 1980-1990 of last century, AGI has created 17 national, regional mutant varieties, particularly the varieties having high yield and good resistance. DT17 and DT18 are submerged tolerance, salinity tolerance varieties comprising CM-1, CM-2, CM-3, cold tolerance varieties including DT-10, DT11, DT13, Khang dan Dot bien, DT37, DT3 having short growth, good quality, high yield and good resistance. In 2007, licence of mutant variety Khang Dan Dot bien (origin is KD18) was successfully transferred on Centural Seed Company. These varieties are preponderance in the Northern provinces and every year occupies about 40% of cultivated area (about (0.4 million ha per year).

In present, some of the best mutant varieties such as VND95-19, VND95-20, VND404, VND99-3, TNDB-100, THDB created by Institute of Agricultural Sciences for Southern Vietnam (IAS) and Cuu Long Delta Rice Research Institute (CLRRI) have been released for large-scale production in Mekong River Delta. Nowadays, these varieties were planted in 3.0 million hectares , counted for 10 – 15 per cent annual area. Among of those, VND95-20 has become one of the top 5 varieties for export and grown recently more than 300,000 ha per year in Southern Vietnam. Due to significant contribution for socio-economic development, **VND95-20** was awarded National Prize of Science & Technology by Vietnam Government.

In combination with hybridization method, some mutants gave promising recombinants in aroma, tolerance to BPH, Grassy Stunt Virus (GSV) & Ragged Stunt Virus (RSV) diseases. Selected varieties as VN 121, VN 124, VN24-4 are released into production in recent time [5,6].

Problems need to be stressed for research directions on rice

In Vietnam, the most constraints to the crop production, especially rice, fruits, legumes by far caused by biotic, particularly brown plant hopper, and abiotic stresses such as salinity, drought, acid-sulphate and other adversely environmental factors.

The elite rice varieties such as Khang Dan, Q5, Tam thom, Basmati have been popularly cultivated in Vietnam because of their stability of high yields, very wide adaptability. But the major constrains facing production of the varieties for both domestic and export demand are their low quality not only in cooking, edibility and nutrition but also in grain appearance.

To raise grain quality it is necessary to improve related characteristics, such as: cooking and edibility relating to amylose content, gelatinization temperature; nutrition relating to protein content; grain appearance relating to grain length, grain width, width-length ratio and translucency of endosperm.

The major constrains of grain quality can be overcome by radiation-induced mutation.

To improve plant type of traditional aromatic rice varieties it is mainly necessary to reduce their long duration of growth and responses to N-fertilizers, to increase grain yield. To remove their photoperiod-sensitivity, that partly relates to so long durations of growth, going along with maintain and improve their characteristics in aroma and grain quality both for domestic consumptions and export demands.

The Programme entitled "*Radiation-induced aromatic rice varieties for high yield and good quality*" has been started June 2000, applying irradiation treatments with γ rays of ^{60}Co on traditional aromatic rice cultivars with some special characters, e.g. grains translucent, fragrant, fine, rich in protein but short and small; good tolerance, but without improved plant type, e.g. over 160 cm in height, lodging, 160-180 days in growth duration and low yield (<3 t/ha),… Moreover, the conventional crossings are quite limited for this group of rice varieties.

Therefore in this cycle our programme during 5 years to come is focused on some following pivotal tasks as following:

- Development of rice varieties with high yield (>5.5 t/ha), short growth duration (ca. 90 - 120 days), good quality for export and domestic consumptions at high grades.
- Improvement of drought, salinity and disease tolerance of rice varieties.

The doses of irradiation of 60-90Gy were applied for germinated seeds of Aromatic Tam and Basmati 370 (from Pakistan), respectively, since 2001-2002 from broad spectra of variants a lot of novel mutants have successfully been selected for improved criteria, such as: no photoperiodic sensitivity (suitable with any crop seasons in the year), short growth duration (90-100 days), stiff and short plant type (90-110 cm),… and yields more than twice higher than original variety (6-7,5 t/ha).

During 5 years of selections and multi-regional trials, some pure line mutants (at M8-M10) with high quality grains meeting export demands of aromatic rice. Over 4 crop seasons in many provinces both in Mekong and Red River Deltas, their consistency has been established.

In Northern Vietnam, some pure mutant lines of Tam Aromatic rice has been isolated and tested in National Programme as follows : HP-101 (HN-PN-103-1), HN-PN-103-3, HN-PN-103-4 (selected in Institute of Food & Cereal Crops) and TL4 (selected in Institute of Agricultural Genetics); produced at large scales in 3 provinces.

In Southern Vietnam, 5 pure mutantlines: TDS3 (Tám 28-9-4), E 4, E 6 and BDS have been enlarged areas of cultivation in provinces: Soc trang, Dong nai, Long an, Daknong, An giang,… They are well improved in grain quality, e.g. length (>7-7,5 mm), quite translucent and their fragrance is still maintained as required for export. Highest yields in large scaled-production are recorded in An Giang (7,5-10 t/ha) and approved by almost rice farmers at Field Symposia (9/2004, 1/2005, particularly in January and June 2006), and appreciated by breeding experts.

Main achievements of nuclear application on soybean mutation breeding in Vietnam

In Vietnam, soybean (*Glycine max* (L.) Merr.), an important food and industrial crop, provides the protein need and oil for human being, the food for animals and the materials for industry. Although spreading of soybean cultivated area in Vietnam still has a large potency, but it increases quite slowly. In 2007, the soybean planted area was reached

only to 190,100 ha with the yield of 1,47 ton/ha (63.2% average world yield), and the soybean produce was 275.5 thousand tons, meanwhile in 2007, Vietnam had to import 2.5 million tons (equivalent of dried seed) from foreign countries. Up to 2015, Vietnam intends to import 3.5 – 4.0 million tons/year of soybean.

Thus the problem, which made by the fact of production and market to Vietnamese soybean breeders was selecting and creating soybean varieties with short growth duration (75-100 days), high yield (2.0 -3.5 tons/ha), good seed quality, tolerant to drought, resistant to diseases, adapted to crop pattern and ecological regions in the whole country.

In Vietnam, Tran Dinh Long (1990) selected M-103 soybean variety by using EI. At AGI, the researcher group, leaded by Mai Quang Vinh et al (1985, 1987, 1990, 1995, 1998, 2000, 2002, 2004, 2007), has been carrying out the research on the application of induced mutation and bred 9 mutant soybean varieties and 10 other promising varieties and lines.

From 1980 to 2006, 31 varieties and lines were used, consisting 6 local cultivars: Coc chum, Quang hoa, Dau lang, Cuc luc ngan, Xanh bac ha, Cuu long Delta. 7 bred or introduced varieties: DT-70, DT-76, DT-94, K7002, K6871, IS-011, DT-80. 11 mutant varieties and lines: M-103, DT-83, DT-84, DT-90, DT-94, DT-95, DT-96, DT-99, MV1, MV4, AK04… Treatment Methods: Dried seeds treated with Gamma ray Co^{60} with doses of 70, 100, 150, 180, 200, 220Gy, Chemical mutagens: EI, DES, EMI, NMU, DNMU, DEU with concentrations of 0.02, 0.04, 0.06, 0.08% in 2, 4, 6 and 8 hours; combining treatment by Gamma ray Co^{60} with dose of 100Gy and chemical mutagen Ethyleninine 0.008, 0.02 and 0.05%. Treated in *pre-embryo phase and zygote cell in flowering phase* by Gamma ray Co^{60} with the doses of 100, 200 300Gy.

In twenty years (1987 – 2007), 4 National, 5 regional production, 10 promising mutant and cross-mutant soybean varieties and many other valuable soybean lines, selected by AGI, were adopted by Scientific Committee of MARD as national varieties, among of those DT84, DT99, DT96 are 3 varieties occupied the largest-scale of soybean cultivated area thanks to their grown ability of three crops per year, broad adaptation, good tolerance to hot, cold temperature and good resistance to diseases. At present Vietnamese mutant soybean varieties occupy more than 50% of soybean cultivated area in the whole country (more than 100 thousands ha per year), contributing worthily to increasing soybean productivity of Vietnam from 0.78 tons/ha (1985) to 1.47 tons/ha (2007), cultivated area from 102.1 thousands ha (1985) to 190.1 thousands ha (2007), production increased three times, resulting in the productivity of Vietnamese soybean is ranked as the highest in South-East Asian countries (**Table 3**).

In Vietnam, after the results of using induced mutation combined with crossing for soybean varietal improvement, the mutation breeding works could:

1) Improve yield component factors:

Mutant variety DT-83 have the yield higher than that of the original variety Coc chum 70%, plant higher than 50%.

Mutant variety DT-84 have the yield higher than that of its parent 30-40%.

Mutant variety DT-95 have the yield higher than that of original variety AK-04 15-20%.

2) Improve seed quality and color.

Change from blue seed to yellow seed: DT-83 and DT-95 varieties have yellow seed meanwhile original varieties Coc chum and AK-04 have blue seed.

Seed size: P1000 seeds of DT-83 variety heavier than that of Coc chum 60% (86 gr. to 138 gr) the mutant variety DT2003 (Line NC12, improved from DT-83) has P1000 seeds of 160gr. The DT-90 variety has P1000 seed heavier than that of its parent 20-30%.

Protein content increased +6.46 to -7.7% in mutant lines from Coc chum, 41.15% instead 39.50%.

Improve the cracked seed coat character in D.3/33 (DT-80 x DT-76) and breed DT-84 with less cracked seed coat character.

Table 3. The characteristics of mutant soybean varieties and hybrid recognized in 1987-2008

Variety & line	Growth duration (days)	Plant height (cm)	Flower color	Average yield (ton/ha)	P1000 seed (gr.)	Seed color	Crop
DT-83 (*)	90	40-50	Violet	13-27	138	Yellow	Sp-Sm
Cochum(origin)	88		Violet	8-18	86	Blue	Sp-Sm
DT-84 (*)	85-90	45-50	Violet	15-35	160-180	Yellow	Sp-Sm-W
ĐT-80 (origin)	85-105	40-60	Violet	14-27	120-140	Yellow	Sp-W
ĐT-76 (ĐH4)(Con)	89	40-45	Violet	12-25	180-200	Yellow	Sm
DT-90 (*)	95-100	45-50	White	15-30	180-200	Yellow	Sp-Sm-W
K.7002x	90-95	45-50	White	15-30	150-180	Yellow	Sp-W
Coc chum	88	45-50	Violet	8-18	90	Blue	Sp-Sm
DT94 (**) (DT83 x DT84)	90-95	45-55	Violet	15-30	150-160	Yellow	Sp-Sm-W
DT-95 (*)	90-103	50-80	Violet	15-30	160	Yellow	Sp-Sm-W
AK-04 (origin)	95-100	40-55	Violet	15-20	150-180	Blue	Sp-Sm-W
DT-96 (**) (DT84 x DT90)	95-98	45-50	Violet	15-30	180	Yellow	Sp-Sm-W
DT-99 (*) (IS-011 x Cuc)	70-80	35-45	Violet	13-24	150	Yellow	Sp-Sm-W
AK-06 (DT.55)	85-95	40-60	White	17-25	165-180	Yellow	Sp-Sm-W
ĐT-74 (origin)	95-100	40-60	White	15-23	160-170	Yellow	Sp-W
DT2001(**) (DT84 x DT83)	88-100	45-70	Violet	18-40	170	Yellow	Sp-Sm-W
DT2003(*) DT83 (origin)	88-98	40-60	Violet	18-35	160	Yellow	Sp-Sm-W
DT2008 (*) D.158 (origin)	100-120	50-80	Violet	25-40	160	Yellow	Sp-Sm-W

Note: (*) Variety acquired directly from mutation - (**) Hybrid variety from mutant parent
Origin: Original variety; Con: Control variety; Sp: Spring; Sm: Summer; W: Winter

3) Improve temperature and disease resistance:

The varieties DT-84, DT-90, DT-94, DT-95, DT-99, AK06 (DT55): can be cultivated in 3 crops/year in Northern provinces of Vietnam by combining heat and cold tolerance of their parent.

AK-06 variety can be planted in 3 crops in North Vietnam after improving non-tolerance to heat character of the original variety V-74.

Mutant DT95 variety showed resistance to 7/10 trains of rush (*Phakopsora pachirhizi* Sydow), it's AK04 (origine) are susceptible to rush [7].

Mutant DT2008 showed high resistance to 3 kinds of diseases: rush, downy mildew, bacterial posture and drought tolerance.

4) Improve growth duration:

Mutant line DT95/049 (DT95B) of DT-95 variety shortened 8 days of growth duration.

From the practical research on mutation process and mutant soybean variety breeding at AGI, we come to some conclusions as following [3,4]:

The dry seeds newly harvested, stored less 3 months, having high survival rate, can tolerate to high dose or concentration of mutagens treated. They can generate more variations/mutants than the long-term stored seeds.

The genetic sensibility ability of the local varieties is higher than that of the selected and introduced varieties. Mutant frequency of local varieties usually was lower.

It is possible to use cytological methods, meioses index, chromosomal aberration frequency combined with physiological methods (germination rate, survival rate, chlorophyll variation frequency, optimal concentration and dose of treatment) to obtain the most useful mutation spectrum.

The effective concentrations of mutagen: EI-0.02-0.04%; NMU, NDMU: 0.06%; EMS, DEU, DES: 0.02-0.06% in 6-8 hours pH: 6 or 7, doses of radiation: 15-18Gy. Especially the treatment with EI (concentration 0.02-0.04%) combined with gamma ray 10Gy can give many valuable mutations in soybean.

The use of induced mutation can improve some economic and morphological characters in soybean, and improve economic characters of local varieties in keeping valuable characters of the original varieties.

Some mutant soybean varieties selected by VAGI which are widely applied in Vietnam:

DT-84: National, famous Vietnam soybean mutant, created by Gamma rays Co^{60}- 18Gy + F3 (DT80/DH4) adopted by MARD in 1994, Nowaday, DT84 have occupied 40% of 180,000 ha soybean areas of Vietnam and 80 - 90% soybean areas of many north provinces. Grow duration: 84 – 90 days, Yield: 1.8 – 3.5 tons/ha, protein content: 41%, wide adaptability, hot and cold tolerance, can be cultivated in 3 crops/year. DT84 was awarded national prize of Science & Technology VIFOTEC – 2005.

DT96: National variety, hybrid between two mutants (DT90/DT84), moderate drought tolerant, rush resistance, wide adaptability, suitable cultivated in 3 crops/year, growth duration: 88 – 100 days, yield: 1.8 – 3.6 tons/ha, high protein content (43 – 45%), DT96 was adopted by MARD in 2004.

DT2008: Newly selected, prospective variety, created by gamma rays Co^{60}- 18Gy + F4 (DT2001/IS10), drought tolerant, thermo-tolerant, resistant to rush, rush, downy mildew, bacterial posture. wide adaptability, suitable cultivated in 3 crops/year, grow duration: 110 - 120 days, high-yielding: 2.5 – 4.0 tons/ha.

Conclusion and Suggestions

After more than 30 years of close cooperation with IAEA, FAO and other foreign organizations, Vietnam recorded remarkable achievements in application of induced mutation breeding for seed crops improvement. About a 50 mutant varieties were created, among of those seed crops occupied 47 varieties (rice was 32 varieties, soybean - 11, maize - 2 and peanut - 2). These varieties occupy more than 50% of cultivated area of Vietnam mutant soybean and 15 % by mutant rice, contributing significantly to increasing cereal productivity of Vietnam.

Our research experienced that improvement of seed crops by inducing mutation, especially by radiation mutation breeding was effective method. In the coming years plant breeding directions will be close combination between conventional (mutation, hybridization) with modern biotechnology methods to reach the goal increase of plant breeding effective, to serve a The National Strategy Project on Food Security.

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APPENDIX

Apendix 1. Mutant varieties released by Agricultural Genetics Institute (AGI) and other Organization up to 2007

Plant species	Mutation breeding cultivars		
	AGI	AGI +other	Other
Rice (Oryza sativa L.) 32 cultivars	DT10, DT11, DT33, DT17, DT50, CL8, CL9, DT21, DT22, Mutant Khang dan , DT38, CM1, CM6, MT4, MT6, DB250, DB2 (17)	A20, Mutant Tam thom (2)	VND95-20, VND95-19, VND99-3, VND95-26, VN4, TNDB, 6B, OM2118, NN22-98, TNDB100, THDB, ST3 Luc do (Red rice), ST3 Luc tim (Violet rice) (13)
Soybean (Glycine max.) 11 cultivars	DT84, DT90, DT96, DT99, DT94, DT95, DT83, DT2001, S-31 (9)	DT55 (AK06) (1)	M103 (1)
Maize (Zea mays L.)	DT6, DT8 (2)		
Groundnut (Arachis hypogea L.) 2 cultivars	DT332 (1)		B5000 (1)
Indian Jujube (Ziziphus manritiana L.) 2 cultivars			Dao tien, Ma hong (2)
Peppermint (Mentha varvensis L.) 1 cultivars	TN8 (1)		
Total: 50 cultivars	30	3	17

Appendix 2. Vietnam new mutant varieties and their cultural area (ha)

No	Variety	Organization	Origin	Mutagen	Certificate	Cultural area (ha)
Rice						
1	DT10	(AGI)	C4-63	200Gy + 0,025% NEU	NV, 1990	1,000,000 (1990 to present)
2	DT11	(AGI)	C4-63	20Kr + 0,025% NEU	NV, 1995	100,000(1995 to 2000)
3	A20	(AGI)	A8	0,015% NMU	NV, 1993	100,000 (1993 to present)
4	CM1	(AGI)	Chiem bau	200Gy	NV, 1999	1,000 ha/5 years
5	CM6	(AGI)	Chiem bau	200Gy	RV, 2000	1,000 ha/5 years
6	DT33	(AGI)	CR 203	200Gy	NV, 1994	200,000 ha (1994 to present)
8	DT38	(AGI)	KD 18	200Gy	RV,2007	500 ha/year
9	Khang dan mutant	(AGI)	KD 18	100-200Gy	NV, 2007	20,000 ha/year
10	Tam thom mutant	(AGI)	Tam thom	100-200Gy	NV, 2000	5,000ha/5 years
11	CL9	(AGI)	IR64/KD18	150Gy	NV, 2006	10,000 ha/2 years
12	CL8	(AGI)	DT20	150Gy	PV	5 000 ha
13	VND95-20	(IASS)	IR64	GI	NV,	900,000 ha/3 years
14	TNDB-100	(IASS)	Nang huong	GI	NV,	300,000 ha/3 years
15	VND 99-3	(IASS)	-	GI	NV,	45,000 ha, 60 bill. VND /3 years
16	OM 2118	(IASS)	-	200Gy	NV,	50,000 ha/3 years
17	ST3 red	CNT, HCM city	-	GI +H	PV	-
18	ST3 purple	CNT, HCM city	-	GI + H	PV	-
Maizes						
1	DT6	(AGI)	Mehico variety	GI + chemical	NV, 1990	50,000 ha (1990-2000)
2	DT8	(AGI)	DT6	GI + chemical	RV,1996	5,000 ha (1994-2004)
Soybeans						
1	DT84	(AGI)	DT80/ DH4	GI + H	NV, 1995	70,000 ha/year
2	DT90	(AGI)	K7002/Cuc	GI + H	NV, 2002	3,000 ha/year
3	DT96	(AGI)	DT84/DT90	GI + H	NV, 2004	5,000 ha/year
4	M103	(VASI)	DH4	EI	NV, 1990	1,000 ha/year
5	AK06 (DT55)	AGI, HAU, VASI	V74	GI	NV, 2000	1,800 ha/year
6	DT99	(AGI)	IS-011/Cuc	GI + H	RV,2003	11,000 ha/year
7	DT94	(AGI)	DT83/DT84	GI + H	RV	400 ha/year
8	DT95	(AGI)	AK04	GI	RV,1995	800 ha/year
9	DT83	(AGI)	coc chum variety	EI – 0.04%	RV,1990	50 ha/year
10	DT2001	(AGI)	DT84/DT83	GI + H	RV, 2007	500 ha/year
Peanut						
1	DT332	(AGI)	-	GI	RV, 1998	200 ha/year

Note: NV- National variety; RV- regional variety; PV- potential variety
Gamma irradiation – GI; hybridization – H

Induced Genetic Variability for Yield and Yield Components in Peanut (*Arachis hypogaea* L.)

H L Nadaf, S B Kaveri*, K Madhusudan & B N Motagi

Abstract

An experiment was conducted during 2005-07 to induce polygenic variability for yield and its components in peanut (*Arachis hypogaea* L.). Two cultivars of peanut, 'GPBD-4' and 'TPG-41' were treated with γ-radiation (200Gy & 300Gy) and ethyl methane sulphonate (EMS- 0.5 %). The mutagenized populations showed significantly higher variability in the M_2 generation. Mutant lines showing higher yield per plant than the respective parents and checks were isolated in M_2 and subsequent generation. The evaluation of 10 superior mutants isolated in M_4 over three successive generation yielded few mutants performing better over the parents and checks. In both the genotypes, superior mutants were isolated from 200Gy treatment, indicating effectiveness of the mutagen in obtaining the desired trait. Two of the mutant lines, G2-52 and TG2-30, gave significantly more pod yield (3,315 and 2,647 kg/ha respectively) than the parents and checks. One of the most interesting features of these mutant lines was the significant increase in hundred seed weight over the parent, contributing to higher yield. The mutant G2-82 recorded highest 100-seed weight of 40.28 g among GPBD-4 mutant population and T2-30 had 67.24 g as against parental value of (62.43 g). Mutants were found to be on par with respective parents for oil content, but had improved oil quality with their parental character of disease resistance/susceptible reaction. Magnitude of induced variation was found to depend upon the mutagen used, character under study and the genotypic background of the genotype. These promising mutant lines need to be further tested for their adaptability and stability. These can be further utilized in recombination breeding with other mutants and/or cultivars to derive distinct lines with improved agronomic traits.

Introduction

Peanut is an important food, feed and principal oilseed crop which is cultivated on a large scale throughout the world. Recently, peanut has been gaining importance as a food crop, due to its high content of digestible proteins, vitamins, minerals, and phytosterols, and to increased consumer preference after value addition. In India, 80% of the peanut produce is crushed for extraction of oil and accounts for 36.10% of the total oil production. Although India has achieved great success in cereal production, there is a large gap between the demand and supply of edible oils. It is estimated that more than 32 million tons of oilseeds are needed every year [1]. One possible way to increase oilseed production is to include efficient oilseed crops in existing cropping system and the second way is to develop high-yielding cultivars. Though the peanut crop has morphological, biochemical, and physiological variability, it has a narrow genetic base because of its monophyletic origin, and a lack of gene flow due to ploidy barrier and self-pollination. Consequently, the extent to which peanut cultivars may be improved through conventional breeding methods is limited. Hence, there is an urgent need to produce and identify new cultivars combining high level of disease resistance, early maturity, besides increased yield and oil content in peanut. Mutation breeding supplements conventional plant breeding as a source of increasing variability and could confer specific improvement without significantly altering its acceptable phenotype [2]. The most popular mutagen used for creating genetic variability is gamma irradiation [3]. Besides gamma irradiation, chemical mutagens like ethyl-nitroso-urea, ethyl methane sulphonate and sodium azide are also used for mutation-assisted breeding. Genetic improvement of peanut through induced mutations alone or their use in recombination breeding has been in progress in our country since the late 1950s. Therefore, an attempt was made to induce polygenic variability in peanut and to isolate mutant genotypes with improved yields.

National Seed Project, Seed Unit, University of Agricultural Sciences, Dharwad, Karnataka, India

* Corresponding author. E-mail: sosuasd@yahoo.com; kaveri_pnut@rediffmail.com

Materials and Methods

Material

Two Spanish Bunch genotypes viz., GPBD-4 and TPG-41 were used for mutagenic treatments.

Mutagen treatments

Seeds of peanut cultivars GPBD-4 and TPG-41 were treated with γ-radiation and ethyl methane sulphonate (EMS). Uniform size seeds of each cultivar were used for treatment. Treatments (500 seeds per treatment) consisted of two different doses of γ-radiation (200 and 300Gy) and EMS (0.5%). Untreated seed stock of the respective cultivars was used as a control. Seeds were irradiated with γ-radiation at Bhabha Atomic Research Center (BARC) Mumbai, India. EMS solution was prepared in 0.1 M phosphate buffer (pH = 7.0). Seeds were presoaked in distilled water for eight hours to allow uptake of EMS. Presoaked seeds were than treated with EMS for two hours at room temperature in cloth bags. Treated seeds were then rinsed in running tap water for four hours and sown in the field plots along with untreated control. The seeds were sown in a randomized complete block design in five replications with spacing of 30 cm between the rows and between plants. The recommended package of practice for the crop was followed. The M_1 plants were harvested on a single plant basis. In M_2, 90 mutant lines/progenies, yielding more than respective controls viz., GBD-4 and TPG-41 for kernel yield per plant were selected and further evaluated in replicated trial to access the performance over different generations. Segregating mutant lines based on visual observations and low performing were discarded in the initial stages of evaluation and progenies were advanced on the basis of superiority of their yield performance over the respective controls, finally ending up with 10 superior mutant lines in M_4 generation. The selected 10 mutant progenies were evaluated during the summer of 2005 (M_5) and kharif 2006 and kharif 2007 (M_6-M_7) in a replicated trial to assess their performance and identify high-yielding mutants. The 10 mutants, untreated controls (parents) and two checks were grown in randomized complete block design with three replications in a plot of 4.0 m x 2.4 m with spacing of 30 cm x 10 cm over three successive generations. From each entry, 10 plants were randomly selected for recording observations

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on important yield attributing characters, plant height (cm), number of branches, number of pods, pod yield (g), kernel yield (g), pod yield/plot, shelling percent and sound mature kernel percentage.

Fatty acid analysis

Fatty acid analysis of superior mutants for yield selected over three generations was accomplished in M_7 following the extraction and esterification method [4]. The oil content of the selected mutants based on fatty acid profile was determined by the nuclear magnetic resonance (NMR) technique [5]. The modified nine-point scale for rust and late leaf spot [6] was used for assessing genotypes for the reaction to late leaf spot and rust diseases.

Gas chromatography analysis

A gas chromatograph, model GC-2010 equipped with automatic sample injector AOC-20i, flame ionization detector (Shimadzu, Kyoto, Japan) and fitted with a narrow bore capillary column; Rtx-wax (film thickness-0.25µm; I. D.-0.25 mm ; length-30 m) was used to separate methyl esters. The initial column temperature was set at 170°C and held for three minutes, then programmed at an increase of 10 C per minute to a final temperature of 230°C, at which it was held for 1 minute. Injector and detector temperatures were both set at 250°C. The flow rates for nitrogen (carrier gas), hydrogen and air were 45, 40 and 400 ml per minute respectively. The fatty acid methyl esters were compared by a comparison of retention time to a standard methyl ester fatty acid mixture (Sigma, Aldrich). Concentration of each fatty acid were recorded by normalization of peak areas and reported as percent of particular fatty acid.

Statistical analysis

In each generation the parent and mutants were compared for yield and yield components by Student 't' test' using MstatC software. The data of 10 mutant lines evaluated in M_5, M_6 and M_7 were subjected to pooled analysis using SPAR1. Two-way analysis of variance was computed to ascertain differences in the treatments and genotypes and their interaction. Significant differences between and within treatments means were determined using critical difference (CD) values. The oil quality parameters viz., O/L ratio, unsaturated to saturated ratio (U/S) and iodine value (IV) were computed as follows.

O/L ratio = % oleic (C18:1)/% linoleic (C18:2)

U/S ratio = % (oleic + linoleic + ecosenoic)/% (palmitic +srearic + arachidic + behenic + lignoceric).

Iodine value = (% oleic x 0.8601) + (% linoleic x 1.7321) + (% ecosenoic x 0.7854) [7]

Results and Discussion

As yield increment has been the prime objective in most of the plant breeding programmes, mutation breeding had played a key role in achieving the goal. Induced mutants or their utilization in recombination breeding with other mutants and/or cultivars evolved several distinct Trombay groundnut (TG) lines, which had improved agronomic traits.

Performance of the entries

Results obtained from the analysis of variance are shown in **Table 1**. There were significant differences among entries for pod yield, shelling

Table 1. Mean squares in the analysis of variance for characteristics measured on 17 peanut mutant lines evaluated over three seasons.

Source of variation	d.f.	Mean squares					
		Pod yield (kg/ha)	Shelling %	100-seed weight (g)	Sound mature kernels %	Pod no/plant	Oil content %
Factor A(generation)	2	546.30**	504.10**	1294.40**	27.93**	903.0**	14.25**
Factor B (genotype)	16	133.56**	63.40**	989.70**	16.60**	235.30**	8.20**
Interaction	32	12.38**	20.09**	30.50**	14.09**	29.10**	1.59**
Error	96	5.98	8.80	5.23	1.80	3.64	0.55

** significance at p<0.01

Table 2. Mutants with enhanced yield and component traits in peanut over three generations

Mutants	Pod yield (kg/ha)	Shelling %	100 SW (g)	SMK %	NP/plant	Oil content %	% yield increase over parent	O/L ratio	LLS	Rust
G2-52[a]	3315	69.32	38.71	86.90	33.28	47.90	25.32	2.14	2.0	2.0
G2-49	3120	68.82	36.11	86.67	30.71	48.00	17.91	1.71	2.0	2.0
G2-58	3100	67.20	39.51	88.33	33.08	47.21	17.20	1.88	2.0	2.0
G2-29	3026	70.63	38.91	88.00	30.70	47.62	14.36	1.59	2.0	2.0
G2-82	2930	70.77	40.28	85.67	32.02	47.68	10.70	1.67	2.0	2.0
TG2-30	2647	65.70	67.24	86.00	22.76	45.88	17.64	2.67	8.0	7.0
Parents										
GPBD-4	2646	67.43	35.00	86.44	34.10	47.32		1.65	2.0	2.0
TPG-41	2250	65.57	62.43	83.00	20.60	45.20		2.56	8.0	8.0
Checks										
TMV-2	2298	64.56	34.18	85.67	24.25	45.78		1.08	8.0	7
JL-24	2310	63.83	48.24	85.56	23.03	45.86		0.98	8.0	8.0
CD (1%)	4.24	5.17	3.98	2.32	3.32	1.30				
CD (5%)	3.20	3.90	3.01	1.76	2.51	0.97				

[a] First and second alphabet indicates the genotype and mutagen treatment respectively (Ex. G2-52: GPBD-4, 200 Gy, 52 progeny)

percent, 100-seed weight, number of pods/plant and oil content ($p<0.01$) averaged over the three generations. The traits also showed significant generation and interaction effects. However the proportion of variation due to generation is more followed by genotypic variation.

Mean values of the top six out of 10 mutants for yield and yield components averaged over three generations are presented in **Table 2**. The mutants showed significant differences for most of the traits studied. All the mutants derived from GPBD-4 were significantly higher yielding than the parent and checks and some of the mutants revealed significant differences for shelling percent (S%), pod number/plant and sound mature kernel percent (SMK%). On average over three generations, the mutant line G2-52 recorded the highest yield (3315 kg/ha). This was followed by G2-49 (3120 kg/ha), G2-58 (3100 kg/ha) and G2-29 (3026 kg/ha). Mutant lineT2-30 derived from TPG-41 exhibited higher yield (2647 kg/ha) compared to its parent (2250 kg/ha) and checks (2304 kg/ha). Shelling percent showed significant differences ($p<0.01$), where G2-82 gave the highest result (70.77 %), followed by G2-29 (70.63 %). Mutants recorded significant differences for 100-seed weight over the respective parents and revealed non-significant differences compared to checks. Among the GPBD-4 mutants, G2-82 recorded highest 100-seed weight of 40.28 g and T2-30 had 67.24 g. Mutant G2-52 had the maximum number of pods per plant (33.28) and it was closely followed by G2-58 (33.08). Two of the mutants of GPBD-4 viz., G2-58 (88.33%) and G2-29 (88.0%) recorded significant differences for SMK%. The mutants were superior for oil content compared to checks but on par with the parental values. Percent increase for pod yield over the respective parents clearly indicates the superiority of the mutants lines identified over three generations, ranging from 10.70% (G2-82) to 25.32% (G2-52). The superior mutants identified in both generations were from 200Gy treatment, indicating the effectiveness of mutagen in obtaining the desired traits.

From these varied performances of mutants, it can be inferred that the mutants and cultivars evaluated in this study represented a wide range of performance. This is supported by the fact that the two genotypes differed in their response to mutagenic treatment. One of the most interesting features of these mutant lines was the significant increase in 100-seed weight over the parental value contributing to higher yield. In peanut, large seeds have consumer and market preference, particularly for confectionery and value addition, in turn fetching premium prices in domestic and international markets. High-yielding, large-seeded varieties with earliness would fit into a diverse cropping pattern. Several large-seeded cultivars have been released [8, 9]. High-yielding mutants of peanut in different generations have been reported by earlier scientists [10, 11].

The mutants were evaluated for fatty acid profile and disease resistance in the M_7 generation. Interestingly they had high O/L ratio compared to parents and checks and the genetic background of the parents for disease resistance was found unaltered. Based on yield and yield contributing parameters, the selected mutants were found to be significantly superior for pod yield accompanied with significant improvement in O/L ratio and parental character of disease resistance/susceptible reaction. These promising mutant lines need to be further tested for their adaptability and stability. These can be further utilized in recombination breeding with other mutants and/or cultivars to derive distinct lines with improved agronomic traits.

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Selection for Resistance to Yellow Vein Mosaic Virus Disease of Okra by Induced Mutation

V Phadvibulya[1,*], K Boonsirichai[1], A Adthalungrong[2] & W Srithongchai[3]

Abstract

Yellow vein mosaic virus disease (YVMD) caused by a begomovirus is the most serious factor affecting okra (*Abelmochus esculentus*) production for both exporting and domestic consumption in Thailand. Seeds of two okra varieties, Annie and Okura, were irradiated with Gamma-rays at doses of 400 and 600Gy. Screening of YVMD resistant plants was conducted for M_3 and M_4 plants under field conditions in Petchaburi and Phichit provinces, and greenhouse conditions using whitefly transmission in Bangkok. One M_4 plant of Okura (B-21) irradiated at 400Gy was found to be highly resistant, but none of Annie. M_5 plants of B-21 were screened further for YVMD resistance under both greenhouse and field conditions. Ten resistant lines obtained by screening for YVMD resistance up to the M_7 generation were selected for yield trial observations at Phichit Horticultural Research Center (PHRC) and Chiengmai Horticultural Research Station (CHRS), both located in the northern Thailand. Three of the mutant lines were further tested at Kanchanaburi Horticultural Research Center (KHRC) in Kanchanaburi province, an okra growing area in the west of central Thailand where YVMD was seriously widespread. At the KHRC, all tested mutant lines showed resistance up to a month, when the susceptible check variety already showed symptoms of the disease. However, only a small portion of the plants of the mutant lines appeared to be resistant throughout the whole growth duration; others eventually exhibited the yellow vein symptom. Plants were further screened in two growers' fields. Growers were satisfied with the plant stature and fruit shape of the mutants and their delayed disease development, and further screening is underway to select uniformly YVMD resistant lines for okra production in Kanchanaburi.

Introduction

Okra breeding programmes for the yellow vein mosaic virus disease (YVMD) in Thailand involve selection breeding [1], cross breeding [2] and mutation breeding [3]. Our programme focuses on mutation breeding and utilizes whitefly transmission of YVMD under greenhouse conditions, as well as field screenings at Phichit Horticultural Research Center (PHRC) and Chiengmai Horticultural Research Station (CHRS) to identify resistant individuals. Later on, some of the resistant lines obtained from these locations were field-tested at Kanchanaburi Horticultural Research Center (KHRC). It was observed that YVMD manifested itself more aggressively on the mutants tested there than at PHRC and CHRS. Nonetheless, mutant lines from our breeding programme exhibited a higher percentage of resistant individuals than okra lines obtained via other breeding techniques being tested at the same time. As a result, further screenings of the mutants were conducted in the fields of commercial okra growers in Kanchanaburi.

[1] Research and Development Division, Thailand Institute of Nuclear Technology (Public Organization), Nakornnayok, Thailand
[2] Horticultural Research Institute, Department of Agriculture, Bangkok, Thailand
[3] Crop Protection Research and Development Office, Department of Agriculture, Bangkok, Thailand

* Corresponding author. E-mail: valailak@tint.or.th

Materials And Methods

Seeds of two okra varieties, Annie and Okura, were irradiated at doses of 400 and 600Gy and planted with non-irradiated ones at Huaysai King's Project, Petchaburi province. M_2 seeds from plants with good phenotype were planted. M_3 seeds were collected from selfed flowers of healthy plants and grown at PHRC and Huaysai King's Project for YVMD screening under field conditions. M_3 plants without the disease symptoms were selected to collect M_4 seeds (each plant as a line). About 40 M_4 seeds of each line were planted and screened for YVMD resistance by using whitefly transmission under greenhouse conditions at Crop Protection Research and Development Office. Plants without disease symptoms were transplanted to the field at PHRC, and M_5 seeds were collected. The same selection techniques as in the M_4 generation were repeated from the M_5 up to the M_7 generation. The resistant lines were selected for yield trials and resistance screening at CHRC and PHRC. Three of them were tested for YVMD resistance at the Kanchanaburi Horticultural Research Center (KHRC) in Kanchanaburi province, west of central Thailand, where YVMD was seriously widespread. Seeds of resistant individuals were collected and planted in the fields of two okra growers in Kanchanauri for further screening in order to obtain uniform okra lines with good fruit quality as well as YVMD resistance.

Results

Development of YVMD resistant mutant lines

A single YVMD resistant mutant plant, B-21, was identified in the M_4 generation from 400Gy gamma-irradiation of Okura variety only. Resistant individuals that were derived from this plant were further screened up to the M_7 generation. Ten resistant lines were selected for yield trials and resistance screening at CHRS and PHRC (**Fig. 1** and **Table 1**). Spines were observed on the skin of the fruits in some of the lines. Seven lines exhibiting uniformed resistance to YVMD and spineless pods were later selected.

Figure 1 YVMD symptoms in a susceptible okra plant (Left panel). Resistant B-21 mutant plants are shown in the right panel with the susceptible Phichit 03 in the middle. Location: Phichit Horticultural Research Center.

Performance of YVMD resistant mutants in Kanchanaburi

Three of the B-21-derived, spineless mutant lines, including B4606, B4609 and B4610, were tested at the KHRC and at two okra grower's fields . It was observed that the disease symptoms were more severe in Kanchanaburi than in Phichit. All tested mutant lines showed resistance

to YVMD up to a month, when the susceptible check variety already showed symptoms of the disease. However, only a small portion of the plants of mutant lines appeared to be resistant in the whole growth duration, others eventually exhibited the yellow vein symptom. (**Fig. 2, Table 2**).

Table 1. Yield trial of 10 B-21-derived mutant lines at Phichit Horticultural Research Center (PHRC) and Chiengmai Horticultural Research Station (CHRS).

Mutant line	PHRC		CHRS	
	Yield[a] (kg/rai[b])	% resistant plants	Yield (kg/rai)	% resistant plants
B4601	8167	99.2	10140	75.2
B4602	6169	94.8	8671	66.3
B4603	8309	81.0	9090	62.8
B4604	9716	94.1	10127	62.8
B4605	7746	77.1	9791	55.9
B4606	7355	65.6	8192	48.2
B4607	7997	95.6	9488	60.7
B4608	7318	94.0	9493	55.6
B4609	9224	86.7	10896	58.3
B4610	6379	55.4	6743	29.9
Resistant check Hit 9701	5966	100.0	5329	72.8
Susceptible check Phichit 03	454	0.0	2849	0.0

[a] Measured as fresh weight of fruits per growing area
[b] 1 rai = 6.25 ha

Figure 2 Performance of three mutant lines (B4606, B4609 and B4610) and one YVMD susceptible check variety (Phichit 03) in Kanchanaburi field trial. YVMD susceptible plants showed yellow leaves, while resistant individuals (marked with a red tie) were with normal leaves (two bottom right panels).

Breeding YVMD resistant lines for Kanchanaburi

Progenies of the plants without YVMD are being screened further in order to obtain uniform YVMD resistant okra lines for production in Kanchanaburi. Attention is also paid to the selection of other agronomic and quality related traits. Compared to a commercial variety grown by both growers, these mutant lines yielded plumper, fleshier, green fresh pods, which satisfied the growers (**Fig. 3**).

Table 2. Segregation of YVMD resistance in mutant lines in different experiments in Kanchanaburi, Thailand

Material	KHRC			Grower's field 1			Grower's field 2		
	Diseased	Not diseased	Total	Diseased	Not diseased	Total	Diseased	Not diseased	Total
B4606	13	27	40	220	22	242	253	35	288
B4609	25	15	40	245	19	264	433	39	472
B4610	26	14	40	325	40	365	422	27	449
Phichit 03	40	0	40	296	0	296	113	0	113

Figure 3 Fruit characteristics of three mutant lines (B4606, B4609 and B4610), compared to that of a commercial variety. All four plant lines were grown in a grower's field in Kanchanaburi.

Discussion

Gamma radiation was successfully applied to induce a mutation that conferred YVMD resistance in the okra variety 'Okura'. However, the resistant mutant lines that were obtained through selection at PHRC and CHRS failed to exhibit uniform resistance to YVMD when grown at KHRC and in okra growers' fields in Kanchanaburi, the major okra growing area. The disease symptoms of the susceptible Phichit 03 appeared more severe in Kanchanaburi than in Phichit and Chiengmai provinces. Both Phichit and Chiengmai are located in northern Thailand, while Kanchanaburi is located to the west of central Thailand. Therefore, it was possible that the strain of YVMV in Kanchanburi differed from that found in Phichit and Chiengmai. It would be interesting to investigate the differences of their viral DNA sequence.

Although the symptoms were severe, the majority of the mutant individuals yielded plump, green fruit pods and developed YVMD symptoms later than the susceptible Phichit 03, allowing for additional yield collections in Kanchanburi. The growers were satisfied with the fruit characteristics of these mutant lines and took part in the selection for YVMD resistant individuals.

Since the molecular basis of YVMD resistance in general is not yet understood, the mutant lines would provide excellent materials for further study on the genetics of YVMD resistance in okra and to identify DNA markers for the YVMD resistance, and eventually to determine the nature of the obtained mutation in the future.

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Genetics of the Radiation-Induced Yellow Vein Mosaic Disease Resistance Mutation in Okra

K Boonsirichai[1,*], V Phadvibulya[1], A Adthalungrong[2], W Srithongchai[3], V Puripunyavanich[1] & S Chookaew[1]

Abstract

Yellow vein mosaic disease (YVMD) is one of the major diseases affecting okra production in Thailand. YVMD-resistant B4610 mutant was generated through gamma irradiation of the Okura variety of okra. In an attempt to develop a DNA marker for YVMD-resistance, a BC_1F_1 and an F_2 mapping population were generated from the cross between B4610 and Pichit 03, a YVMD-susceptible variety. The populations were naturally inoculated with YVMD virus in the field at Pichit Horticultural Research Center, Pichit province, where the disease is widespread. Analysis of F_1 and F_2 progeny revealed the semi-dominant nature of the resistance which appeared to be caused by a single-locus mutation. MFLP fingerprintings of the F_2 and the BC_1F_1 populations revealed a DNA fragment that is potentially linked to the mutation. In addition to the visual assessment of YVMD, a PCR method was developed for the assay of the presence of YVMD virus in leaf tissues. Sequencing of the amplified DNA fragments confirmed the presence of okra YVMD virus in the infected leaf tissues in susceptible plants.

Introduction

The yellow vein mosaic disease (YVMD) can cause yellowness in leaf veins, shoots and fruits, affecting plant growth and yield. Resistant germplasm originally isolated in India produces long and skinny pods, in contrast to the plump but shorter pods of Japanese-derived varieties, such as Okura, grown in Thailand for export. Gamma radiation has been used to induce YVMD resistance mutation in the Okura variety of okra. B-21 mutant was identified in M4 generation as a resistant individual [1]. The line B4610, derived from B-21, showing uniform resistance, was later identified in subsequent generations.

YVMD is caused by a geminivirus and can be transmitted through white fly *Besimia tabaci* Gen. and also through cuttings and grafts. However, it cannot be transmitted through seeds. The mechanism of YVMD resistance in B-21-derived plants is not understood. In order to identify genes responsible for YVMD resistance in the line B4610, we have generated mapping population and initially identified linked DNA fragments as a basis for future identification of the nature of the mutation.

Materials And Methods

YVMD resistant mutant seeds and growing conditions

YVMD resistant mutant seeds belonged to the line B4610 of the M_8 and M_9 generations derived from B-21 mutant. Crosses between the mutant and Okura or between the mutant and Pichit 03 were conducted and their progeny planted in the field at the Pichit Horticultural Research Center, Pichit province, where YVMD had been widespread.

[1] Research and Development Division, Thailand Institute of Nuclear Technology (Public Organization), Bangkok, Thailand
[2] Horticultural Research Institute, Department of Agriculture, Bangkok, Thailand
[3] Crop Protection Research and Development Office, Department of Agriculture, Bangkok, Thailand

* Corresponding author. E-mail: kboonsir@yahoo.com

Greenhouse inoculations using the white fly carrier were conducted on okra seedlings at the nursery belonging to the Crop Protection Research and Development Office, Department of Agriculture, Bangkok. YVMV-carrying white flies were captured from Pichit province for inoculations of F_1, F_2, BC_1F_1 individuals. Inoculations of Pichit03 and B4610 for the development of a PCR method for YVMV detection were carried out with YVMV-carrying white flies from Pichit and Kanchanburi provinces.

DNA extraction

DNA was extracted from leaf tissues using a method modified from Dellaporta, *et al.* [2] and Boonsirichai, *et al.* [3]. Briefly, frozen leaf tissues were ground in 100mM Tris-HCl, pH 8.0, 50mM EDTA, 500mM NaCl, 0.1% 2-mercaptoethanol. Twenty percent SDS was added and the mixture was incubated at 65°C. Proteins were precipitated out by the addition of 5M potassium acetate. The supernatant was extracted with chloroform, and the DNA was precipitated twice with isopropanol.

Determination of YVMD susceptibility

YVMD susceptibility was determined by visual inspection in the field and by PCR amplification of the viral DNA. Susceptible plants showed yellowing of leaf veins and yellow spots on leaves. The disease was observable within two to four weeks after planting in susceptible Pichit 03. Plants were inspected for symptoms every two weeks in the field.

DNA fingerprinting

MFLP fingerprinting was conducted as described by Yang, *et al.* [4]. The sequences of microsatellite-anchored primers are shown in Table 1. Selective nucleotides of *Mse*I adaptor primer were CCG, CGT, CGG, CCT, CAA, CCA, CAG, CAC, CAT and CGA. MFLP products were separated on a 4.5% acrylamide, 7M urea gel [5] and stained with silver nitrate [6].

Table 1. Sequence of microsatellite-anchored primers for MFLP fingerprints.

Microsatellite-anchored primer	Sequence (5' to 3')
MF42	GTC TAA CAA CAA CAA CAA C
MF43	CCT CAA GAA GAA GAA GAA G
MF51	GGG AAC AACAAC AAC
MF78	GGC AAG AAG AAG AAG A
MF128	DVD TCT CTC TCT CTC TC*
MF201	CCC ATT GTT GTT GTT G

* D = A+G+T; V = A+G+C

Development of a PCR method for YVMV detection

Four PCR primers were designed based on synonymous nucleotides of the okra yellow mosaic viral AV1 coat protein gene from the Pakistani and Mexican isolates (GENBANK no. AJ002450 and DQ308546). The primer sequences were AV1F1 5'-CTC GTA ATT ATG TCG AAG

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CGA-3', AV1F2 5'-AAA CAG GCC TAT GAA CAG GAA A-3', AV1R1 5'-CTT AAG AGT AGC ATA CAC TGG-3' and AV1R2 5'-ACA AGG AAA AAC ATC ACC GAA T-3'. Thermal cycle conditions were 30 cycles of 30 sec at 95°C, 30 sec at 50°C and 1 min at 70°C. PCR products were analyzed on 1% agarose gels and stained with ethidium bromide.

Results

Genetics of YVMD resistance

F_1, F_2 and BC_1F_1 seeds were generated from the cross between B4610, a B-21-derived YVMD resistant line, and Pichit 03, a susceptible variety. Upon a natural inoculation with YVMV in the field, F_1 individuals exhibited prolonged resistance comparing to Pichit 03 but eventually developed YVMD symptoms. Therefore, the YVMD resistance mutation is semi-dominant. The segregation of resistance: susceptible individuals for F_2 and BC_1F_1 populations fit the expected ratios of 3:1 and 1:1, respectively, for a single dominant resistance gene (**Table 2**). Thus, the mutation could be treated as a dominant mutation when disease susceptibility was assessed at two to two-and-a-half months.

Table 2. Segregation of YVMD resistance among okra populations

Okra population	Total number of plants	Percentage of plants with YVMD‡	*p*-value*
Pichit 03	213	91.5	-
B4610	206	0	-
B4610 x Pichit 03; F_1	207	2.4	-
Pichit 03 x B4610; F_1	209	5.3	-
B4610 x Pichit 03; F_2	405	24.0	0.63
Pichit 03 x B4610; F_2	416	24.8	0.91
Pichit 03 x (B4610 x Pichit 03)	413	50.6	0.81
Pichit 03 x (Pichit 03 x B4610)	408	49.5	0.84

* Based on a single-locus mutation hypothesis.
‡ YVMD was assessed at 2.5 months.

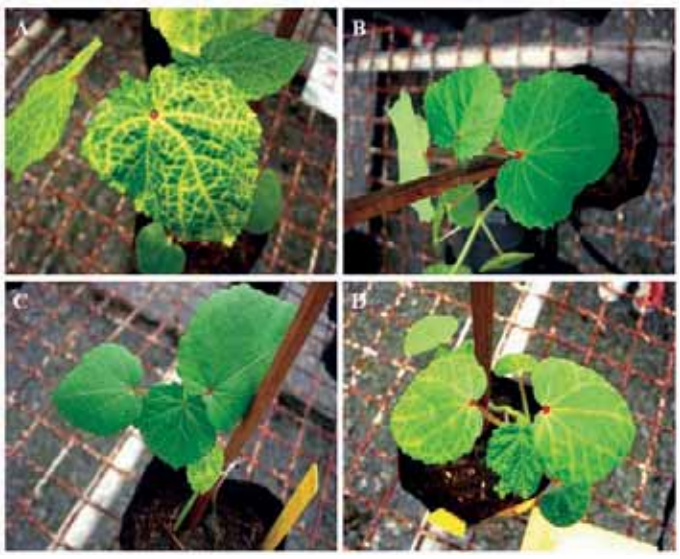

Figure 1 Okra seedlings individually inoculated with YVMV in the greenhouse. **(A)** Pichit 03. **(B)** B4610. **(C)** A resistant F_2 segregant. **(D)** A susceptible F_2 segregant.

In addition, 19 BC_1 individuals of the cross Okura x B4610 and 11 F_1 individuals of the cross Pichit 03 x B4610 were individually inoculated with the Pichit isolate of YVMV in the greenhouse in order to confirm the dominant nature of the mutation. All BC_1 and F_1 individuals appeared resistant at six weeks after inoculation, while all of the 22 Pichit 03 individuals tested had developed YVMD symptoms (data not shown). Forty-four F_2 individuals from the cross Pichit 03 x B4610 were also tested. Only 10 of them were found with the disease ($p = 0.67$ for a single locus dominant mutation) (**Fig. 1**). Thus, the data also supported the conclusion that the mutation appeared dominant and resulted from a single locus mutation.

Cosegregation analysis

MFLP fingerprints were performed on 52 YVMD resistant individuals and 52 susceptible individuals from the cross Pichit03 x (Pichit03 x B4610). Sixty primer combinations, consisting of six microsatellite-anchor primers and 10 selective *Mse*I-adaptor primers, were analyzed. A DNA fragment that showed potential linkage to YVMD resistance was identified with MF43 microsatellite-anchor primer and the *Mse*I-CAG selective primer (**Fig. 2**). The fragment exhibited the recombination frequency of 0.192 with YVMD resistance.

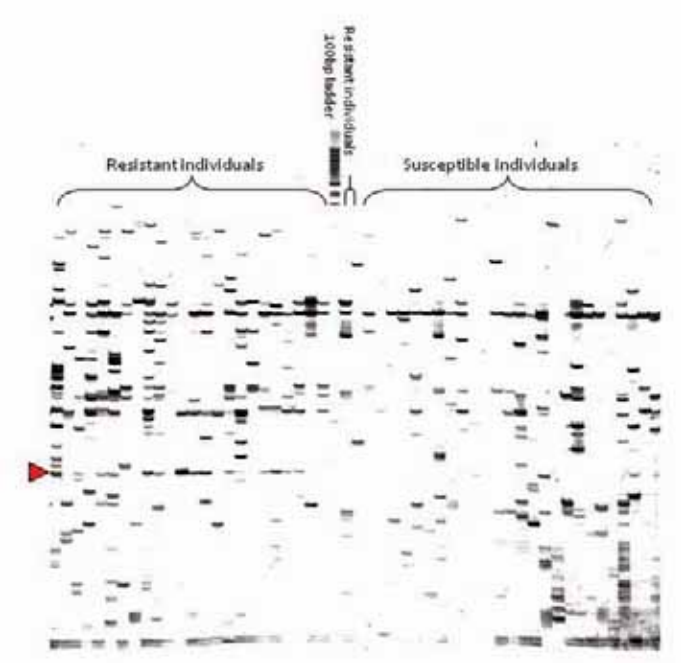

Figure 2 DNA fragment that showed linkage to YVMD resistance (red arrow), obtained from MFLP fingerprinting with primers MF43 (AAG repeats) and *Mse*I-CAG.

Development of a PCR method for YVMV detection

Four viral AV1-specific primers were tested against naturally inoculated susceptible Pichit 03 and resistant B4610 individuals grown at the Pichit Horticultural Research Center. Two primer combinations, AV1F1+AV1R1 and AV1F2+AV1R1, showed a single positive DNA band of the expected sizes from the inoculated Pichit 03 sample, while no PCR products were observed from the two resistant B4610 samples (**Fig. 3A**). Sequencing of the PCR product from the latter primer combination showed 88.5% sequence identity at the DNA level between the product itself and the YVMV AV1 sequence from the Madurai and the Pakistani viral isolates, indicating that the correct locus had been amplified (**Fig. 4**). Further tests showed that both primer combinations tested positive against YVMV isolates from Pichit province in the northern part of Thailand as well as from Kanchanaburi province in the western part of Thailand (data from one of the primer combinations is shown in **Fig. 3B**). Thus, they can be used to detect YVMV infection from samples grown in multiple regions of Thailand.

Discussion

The mechanism of YVMD resistance in okra is not yet understood. We showed that the YVMD resistance mutation, which was obtained through gamma-radiation induction, was semi-dominant. This is in contrast to most radiation-induced mutations, which are recessive in nature and result from loss of gene functions. However, in this study, we cannot yet conclude whether the YVMD mutation involves a loss or a gain of gene functions. Dosage effects might be involved as the heterozygotes showed prolonged resistance but eventually developed the disease.

In other crop plants, genes conferring resistance to mosaic viruses have started to be mapped. In soybean, at least three loci were shown to be involved in the resistance to the soybean mosaic virus [7]. AFLP technique was used to successfully identify linked markers, even in a population showing relatively low genetic polymorphisms. In this report, both AFLP and MFLP fingerprinting techniques were utilized because MFLP primers are anchored to microsattelite loci and therefore should reveal more polymorphisms than AFLP. A linked DNA fragment was successfully identified through MFLP fingerprinting. Nonetheless, additional linked markers are needed in order to identify the gene responsible for the resistance. Bulk segregation analysis and extensive AFLP and MFLP fingerprinting should prove to be an effective strategy for this purpose.

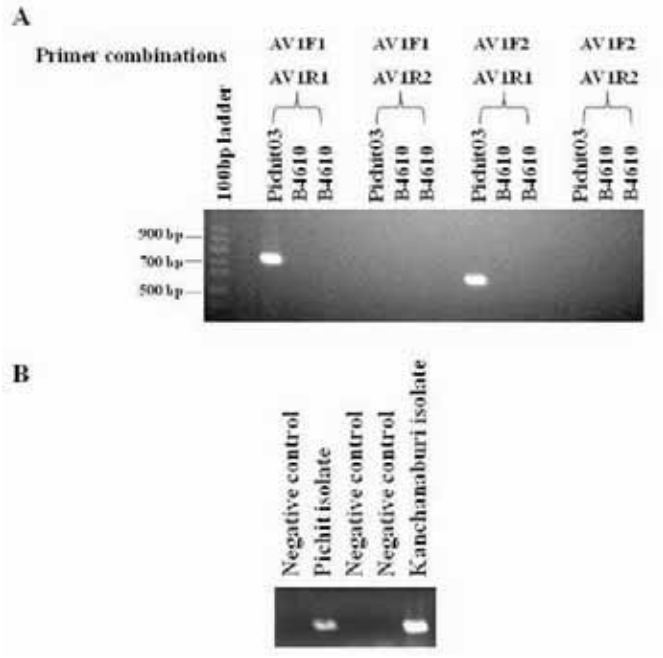

Figure 3 Detection of YVMV by a PCR method. **(A)** Two primer combinations, AV1F1+AV1R1 and AV1F2+AV1R1, successfully amplified the viral AV1 gene fragment. **(B)** Primer combination AV1F2+AV1R1 could detect Pichit and Kanchanaburi isolates of YVMV.

YVMD is caused by a Gemini virus. Identification of the disease is currently based on visual inspection and ELISA method, which is cumbersome. We have developed a PCR method for detection of YVMV in leaf tissues, based on the *AV1* gene sequence. The assay could identify the infection relatively easily and could detect at least two local isolates of the virus.

Figure 4 Sequence alignment of okra YVMV isolates from Madurai (India), Pakistan and Pichit province. Asterisks indicate sequence identity among the three isolates.

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Current Status of Mungbean and the Use of Mutation Breeding in Thailand

S Ngampongsai[1,*], A Watanasit[1], S Srisombun[2], P Srinives[3] & A Masari[1]

Abstract

Seeds of mungbean varieties Khampang Saen 1 (KPS1) and Chai Nat 36 (CN36) were irradiated with a dose of 500Gy Gamma-rays and treated with 1% ethyl methane sulphonate. The objectives of this experiment were seed yield improvement and powdery mildew resistance. A number of mutant lines were selected from M_2 onwards. Three promising mutants, M4-2, M5-1 and M5-5, gave 8-11% and 2-5% higher mean yield than those of KPS1 and CN36, but showed similar disease infection to their original parents tested during 1997-2006. The objective of the second experiment was to improve mungbean variety tolerance to beanfly, a key pest of mungbean. Seeds of var Khampang Saen 2 (KPS2) were irradiated with 600Gy Gamma-rays. A mutant line was selected and subsequently officially released as Chai Nat 72 (CN72) in 2000. It is the first mungbean variety released and developed through mutation techniques in Thailand. CN72 had lower beanfly infestation than a susceptible variety, CN36. The result of an addition trial conducted on calcareous soil showed that grain yield of mutant CN72 was superior to that of KPS2. The third experiment of the Mungbean Mutant Multilocation trials was conducted in two sites during 2003-2005. All mutants retained most traits of the original varieties, including yield. The highest yielding mutant across all five trials was CN72 which was similar to its progenitor (KPS2) and the local check, CN36. These three entries bore large seeds (70 g per 1,000 seeds), which is a desirable trait for Thai and international markets. An exotic entry, native variety showed least incidence of powdery mildew disease. It will be used as a source of disease resistance in the breeding programme.

Introduction

Mungbean (*Vigna radiata* (L.) Wilczek) is one of the most important grain legumes in Thailand, occupying an annual production area of over 300,000 ha. It can be grown during three seasons of the year, but the late rainy season, which starts between late August and September, is strongly recommended. Most of the annual mungbean production is used for bean sprouts. Therefore, germination and sprout quality are very important. Seven recommended mungbean varieties were cultivated from 1986 to 2000. They gave about 17-44% higher yield than local varieties. The popular varieties, Chai Nat 36, Khampang Saen 1 and Khampang Saen 2 are more resistant to major diseases, cercospora leaf spot (*Cercospora canescens*) and powdery mildew (*Oidium sp*.) (Thanomsub and Anat, 2005) [1]. Attempts have been made to develop new mungbean varieties by means of conventional breeding using mutation techniques and biotechnology.

Induced mutation using physical and chemical mutagens is one way to create genetic variation resulting in new varieties with better characteristics. The application of radiation and chemical mutation in mungbean breeding for various aspects were undertaken. The selection and development of mutants into recommended varieties for farmers have been successfully made in many countries (Chow and Loo, 1988; Lamsrijan, *et al*., 1988; Wongpiyasatid, *et al*., 1998 and 1999) [2,3,4,5]. Sandhu and Saxena (2003) [6] studied 34 mungbean mutant lines and found high variation in yield per plant and nutritional quality, especially contents of protein, methionine, tryptophan, sulphur, phenol and total sugars.

Current status of mungbean and the use of mutation breeding in Thailand

Induced Mutations in mungbean breeding in Thailand

In Thailand, induction of mutations is used to improve mungbean variety for higher yield and higher resistance to diseases than the previous recommended varieties. Seeds of varieties Khampang Saen 1 (KPS1) and Chai Nat 36 (CN36) were irradiated with a dose of 500Gy Gamma-rays and treated with 1% ethyl methane sulphonate. A number of mutant lines were selected from M_2 generation onwards. Yield trials were conducted in field crop research centers and farmers' fields from 1997 to 2006. Preliminary comparison of yield in 1997 revealed that most mungbean lines had yield and other agronomic characters that were not statistically different from the standard checks. Yet, some lines gave higher yields with less powdery mildew infection than the checks. Twelve lines were then selected and compared for regional yield trial.

Three mutant lines, M4-2, M5-1 and M5-5, gave 8-11% and 2-5% higher mean yield than those of varieties KPS1 and CN36, but they showed similar disease infection to the two original varieties (**Table 1**). These three elite lines are being tested for yield and adaptability in farmers' fields for the possibility to release.

Table 1. Grain yield (t/ha) and disease reaction of three mutant lines of mungbean compared with two check varieties, yield trials 1997-2006

Mutant line & variety	PYT	SYT	RYT	FT	Mean (%)	Cercospora leaf spot	Powdery mildew
M4-2	2.06	1.47	1.50	0.66	1.42(108)	MR	MR
M5-1	2.15	1.52	1.50	0.66	1.46(111)	MR	MR
M5-5	2.12	1.43	1.48	0.68	1.42(108)	MR	MR
CN36	2.07	1.42	1.42	0.68	1.40(106)	MR	MR
KPS2	1.95	1.33	1.36	0.67	1.32(100)	MR	MR

Source: Ngampongsai *et al.* (2006)[9]

PYT=Preliminary Yield Trial, SYT=Standard Yield Trial, RYT=Regional Yield Trial, FT=Farm Trial
MR = Moderately resistant CN36= Chai Nat 36, KPS2= Kampaeng Saen 2

The use of induced mutations for mungbean improvement

Beanfly (*Ophiomyia phaseoli* Tryon) is a key pest of mungbean in Thailand. Plants are infested at an early growth stage, often resulting in almost 100% plant death. In such circumstances, the plants that continue to grow remain stunted, and produce only a small yield. To

[1] Chai Nat Field Crops Research Center, Muang, Chai Nat 17000, Thailand
[2] OARD1, P.O.Box170, Chiang Mai University, Muang, Chiang Mai 50202, Thailand
[3] Department of Agronomy, Faculty of Agriculture, Kamphaeng Saen, Kasetsart University, Nakhon Pathom 73140, Thailand

* Corresponding author. E-mail: sumana56@hotmail.com

control beanfly, apart from insecticide spraying, induced mutation is used to improve the resistance of mungbean variety. One thousand seeds of variety Khampang Saen 2 (KPS2) were irradiated with 600Gy Gamma-rays. Progression from M_2 to M_4 generations was made by the single seed descent method. The M_3 selection was made under natural field infestations. In the M_5 generation, individual plants were selected for yield trials conducted sequentially, from preliminary trials through to, regional, farm and field trials.

The preliminary trial was conducted at Chiang Mai Field Crops Research Center in 1989. Observation of 10 plants of the insect tolerant selection Chai Nat 72 (CN 72) revealed only four larvae and pupae, compared to 8.5 larvae and pupae on the susceptible variety CN 36. A trial conducted in farmers' fields in 1995 showed a similar result. The number of larvae and pupae infesting CN 72 was 23.3 per 10 plants, compared to 28.3 per 10 plants for CN 36. According to the method reported by Talekar (1990)[7], CN 72 has moderate resistance to beanfly, whereas CN 36 is susceptible (**Table 2**).

CN 72 also produced a slightly higher yield than CN 36 in each evaluation trial (**Table 2**). An experiment conducted on a calcareous soil found that CN 72 produced as high a yield as CN 36 with similar yield components (**Table 3**) and was superior to its parent to KPS 2 in this respect. In addition, CN 72 also showed less yellow leaves compared to KPS 2 (**Table 3**). The resistance of CN 72 to beanfly may be due to low attraction or lack of palatability to the pest. The assertion is supported by Lin (1981)[8] who found that a beanfly-resistant line, LM 192, is a non-preference for beanfly due to its low attraction, lack of palatability and dense pubescence.

A mutant line was selected and subsequently officially released as Chai Nat 72 (CN72) in the year 2000. It is the first mungbean variety released and developed through mutation techniques in Thailand. CN72 had lower beanfly infestation than a susceptible variety, CN36. In addition, an experiment conducted on calcareous soil showed that CN 72 yield was superior to the original variety KPS2.

Mungbean mutant multi-location trials in Thailand

The IAEA/RCA project RAS/5/040 enhancement of genetic diversity in food, pulses, and oil crops and establishment of mutant germplasm network was executed during 2003-2005 under the joint support of the participating countries and IAEA. The project activities include (1) conducting mutant multi-location trials, and (2) mutation enhancement of crop genetic diversity. For mungbean multi-location trial, Thailand has conducted five yield trials during 2003-2005. The details of the trials are reported hereafter.

Five experiments of the mungbean mutant multi-location trials were conducted in two research sites in Thailand, viz. Chai Nat Field Crops Research Center (latitude 15 : 15 N, longitude 100 : 15 E, elevation 16 m above sea level) and Kasetsart University- Kamphaeng Saen Campus (latitude 14 : 01 N, longitude 99 : 58 E, elevation 5 m above sea level). Both locations have loamy clay soil representing the major mungbean production area of the Kingdom.

Each trial was conducted in a Randomized Complete Block Design (RCBD) with three replications. Each plot comprised six rows each of 6 m long, with the spacing of 50 cm between rows and 15 cm between hills within the same row with two plants/hill (total population of ~ 300,000 plants/ha). Data were recorded from the four middle rows, leaving the distance of 50cm at each end of the rows. Thus the plot area for yield determination was $10m^2$. Each trial was basally applied with 22.5, 45.0 and 22.5 kg per ha of N, P_2O_5 and K_2O prior to planting. The fields were irrigated as needed. In each trial, the data was deliberately collected from as many trial plots as possible.

The entries were nine pairs each of mungbean varieties and their corresponding mutants, plus a Thai local check (LM23-CN36). Thus, there were a total of 19 entries in the trials. The experiments were performed in the late rainy season of 2003, dry and late rainy seasons of 2004 and the dry season of 2005. All the mutants retained the same hypocotyls color, seed coat color and seed coat luster as their original cultivars, except for entry no. 10 (LM14-1560xNM92) which has darker purple hypocotyls than its progenitor (LM13-NM92) (**Table 4**).

Table 2. Number of larvae and pupae of beanfly, yield of the mutant variety Chai Nat 72 compared with check varieties

Variety	No. of larvae and pupae per 10 plants		Resistant level	Yield[1] (t/ha)
	CMFCRC	FF		
Chai Nat 72	4.0	23.3	MR	1.33
Chai Nat 36	8.5	28.3	S	1.27
Kampaeng Saen 2				

Source: Watanasit *et al.* (2001)[10]

CMFCRC= Chiang Mai Field Crops Research Center, FF= farmers' field in Chiang Mai
[1] yield trials conducted in research centers and farmers' fields from 1989-1995
MR = Moderately resistant, S = Susceptible

Table 3. Yield, yield components and yellow leaf score of three mungbean varieties sown on a calcareous soil at Nakorn Sawan Field Crops Research Center in 1993.

Variety	Yield (t/ha)	1,000 seed wt. (g)	No. of plant/pods	Yellow score[1]
Chai Nat 72	1.04a	60.6a	13.7a	2.0
Chai Nat 36	1.08a	60.5a	13.9a	1.0
Kampaeng Saen 2	0.66b	50.5b	7.5b	5.0

Source: Watanasit *et al.* (2001)[10]

[1] 1=normal leaf, 2=1-25% of leaf area turned yellow, 3=26-50% of leaf area turned yellow, 4=51-75% of leaf area turned yellow 5=76-100% of leaf area turned yellow due to Fe deficiency.

Table 4. Combined analysis of mungbean regional mutant multi-location trials from five locations conducted in Thailand during 2003-2005

	Entry Name	Hypocotyl color	Seed color	Seed coat luster
1	LM5-Camar	Green	Green	Shiny
2	LM6-PSj-B-II-17-6	Green	Green	Shiny
3	LM7-Gelatik	Green	Green	Shiny
4	LM8-Psj-S-31	Green	Green	Shiny
5	LM9-CV6601	Greenish purple	Green	Shiny
6	LM10-NM54	Greenish purple	Green	Shiny
7	LM11-NM20-21	Greenish purple	Green	Shiny
8	LM12-NM98	Greenish purple	Green	Shiny
9	LM13-NM92	Greenish purple	Green	Shiny
10	LM14-1560xNM92	Dark purple	Green	Shiny
11	LM15-NM51	Greenish purple	Green	Shiny
12	LM16-NM51xVC1973A	Greenish purple	Green	Shiny
13	LM17-2917A	Green	Green	Shiny
14	LM18-1-176	Green	Green	Shiny
15	LM19-Native Variety	Greenish purple	Yellow	Shiny
16	LM20-PACE3	Greenish purple	Yellow	Shiny
17	LM21-KPS2	Green	Green	Shiny
18	LM22-CN72	Green	Green	Shiny
19	LM23-Local Check CN36	Green	Green	Shiny

Source: Srinives *et al.* (2006)[11]
CN36 = Chai Nat 36, CN72 = Chai Nat 72, KPS2= Kampaeng Saen 2

All mutants retained most traits of the original varieties, including yield. The highest yielding mutant across five trials was CN72 (LM22) which was similar to its progenitor (KPS2) and the local check. These

three entries bore large seeds (70 g per 1,000 seeds), which is a desirable trait for Thai and international markets. The other large-seeded entries were LM6-Psj-B-II-17-6 and LM8-Psj-S-31, which gave lower yields than CN72. A number of desirable traits were observed in the entries that participated in the trial. LM5-Camar, LM9-CV6601 and LM11-NM20-21 had high number of seeds per pod. LM18-1-176 and LM19-Native varieties showed the least incidence of powdery mildew disease. These genotypes can be utilized in future mungbean breeding projects in the participating countries of the IAEA/RAS/5/040 Project (**Table 5**).

Table 5. Combined analysis of mungbean regional mutant multi-location trials from five locations conducted in Thailand during 2003-2005.

	Entry Name	No. of pods/plant	No. of seeds/pod	1,000 seeds wt. (g)	Seed yield (t/ha)	Powdery[1] mildew (1-4)
1	LM5-Camar	18.1a	10.2c-e	43.0g	1.32ab	2.00de
2	LM6-PSj-B-II-17-6	11.7e	9.2h	72.4a	1.21b	2.23a-d
3	LM7-Gelatik	14.5bc	9.9e-g	54.5e	1.19b	2.33a-d
4	LM8-Psj-S-31	12.0c-e	9.7f-h	71.7a	1.22b	1.80de
5	LM9-CV6601	17.5a	11.1a	38.9h	1.24b	1.67de
6	LM10-NM54	11.4e	10.3c-e	63.2bc	1.36ab	2.43a-d
7	LM11-NM20-21	17.9a	10.9 ab	40.3gh	1.32ab	1.57de
8	LM12-NM98	15.9ab	10.5 b-d	43.4g	1.21b	1.90c-e
9	LM13-NM92	12.7c-e	9.5 gh	60.1cd	1.12b	2.20a-d
10	LM14-1560xNM92	13.0c-e	10.7 a-d	54.8e	1.26b	2.33a-d
11	LM15-NM51	14.2b-d	10.8 a-c	49.1f	1.33ab	3.33ab
12	LM16-NM51xV-C1973A	10.7e	10.5b-d	59.9cd	1.34ab	2.57a-d
13	LM17-2917A	13.0c-e	10.2 d-f	56.5de	1.28b	2.13b-d
14	LM18-1-176	12.4c-e	10.4 f-h	53.7e	1.33ab	1.43de
15	LM19-Native Variety	15.7ab	10.5 b-e	43.2g	1.13b	1.00e
16	LM20-PACE3	12.9c-e	10.5 c-d	65.6b	1.33ab	3.13a-c
17	LM21-KPS2	11.9de	10.4 b-e	69.9a	1.3ab	1.77de
18	LM22-CN72	10.9e	10.5 b-d	72.7a	1.59a	3.43a
19	LM23-Local Check CN36	11.1e	10.5 b-e	72.5a	1.40ab	3.43a
	CV(%)	2.6	4.2	5.2	18.3	34.2

Source: Srinives *et al.* (2006)[11]

[1] Visual rating score: 1 = no infection, 5 = severe infection

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Intervarietal Differences in Response of Sunflower (*Helianthus annuus* L.) to Different Mutagenic Treatments

S Gvozdenovic[1,*], S Bado[2], R Afza[2], S Jocic[1] & C Mba[2]

Abstract

For much of the past century, mutagenesis has gained popularity in plant genetics research as a means of inducing novel genetic variation. Induced mutations have been applied for the past 40 years to produce mutant cultivars in sunflower by changing plant characteristics that significantly increase plant yield and quality. The present study was focused on generating baseline data to elucidate the role of genotypic differences in the response of sunflower to induced mutagenesis with the aim of expanding the applicability of the use of induced mutant stocks in the genetic improvement of the crop and in its functional genomics. The strategy adopted was to estimate the optimal treatment conditions (doses of mutagens) through relating the extent of damage in seedling progeny to the exposure levels of the initiating propagules to mutagens. Seeds of 15 elite sunflower genotypes commonly used as breeding stocks and grown on commercial scales were treated with a range of mutagens: Gamma-rays (γ rays); fast neutrons and with ethyle-methane-sulphonate (EMS) at different treatment doses. The three mutagenic agents affected seedling height, reducing it with increasing dosage. Based on the mutagen damage on seedling height, the 50% and 30% damage indices (D_{50} and $D_{30,}$ respectively) were estimated for the 15 sunflower genotypes for the three mutagens. The D_{50} (D_{30}) values for the sunflower lines ranged from 120 to 325Gy (5 to 207Gy) for gamma irradiation; 9 to 21Gy (0.1 to 10Gy) for fast neutrons and 0.69 to 1.55% (0.01 to 0.68%) concentration of EMS.

Introduction

Sunflower (*Helianthus annuus* L.) is one of the world's most important oil crops, used for human consumption and industrial processes. It is also used as a confectionery, ornamental plant and flower, and as bird feed. It is currently cultivated on over 21 million hectares world-wide annually. The largest sunflower producers in the world are Russia, the United States, Argentina, China, and France [1].

The main objective of sunflower breeding is to develop productive sunflower hybrid cultivars that are stable, high yielding, and resistant to biotic and abiotic stresses. Yield is a complex trait, is controlled by multiple gene effects. Seed yield is variously estimated as: number of plants per hectare (55,000-60,000), number of seeds per plant (>1,500), hectoliter mass of the seed (45-50 kg/ha), thousand seed mass (>80 g), low hull percentage (20-24%) and high seed oil content (>50) [2].

Induced mutations have been applied for the past 40 years to produce mutant cultivars in sunflower by changing plant characteristics for significant increase in plant productivity [3], [7]. Mutagenic treatments, usually on seed, have induced high-oleics, semi-dwarfs and dwarfs, male-sterile plants and other interesting variants such as earliness and seeds with thin hull [4], [5], [6].

[1] Institute of Field and Vegetable Crops (IFVC), Oil Crops Department, Novi Sad, Serbia
[2] Plant Breeding Unit Joint FAO/IAEA Agriculture and Biotechnology Laboratory, International Atomic Energy Agency Laboratories, Seibersdorf, Austria

* Corresponding author. E-mail: sandra@ifvcns.ns.ac.yu

In 1976, Soldatov produced a mutant of significant practical importance for sunflower breeding by treating the seed of the cultivar VNIIMK 8931 with a solution of 0.5% dimethyl-sulphate (DMS); M_3 lines possessing a high content of oleic acid in oil were obtained. After further breeding, the high-oleic cultivar Pervenetz was developed [4]. The high oleic content of this cultivar has proved to be very stable under varying temperatures and the trait can be easily transferred into other genotypes by normal breeding procedures.

The main objectives of this research were to increase genetic variation in sunflower inbred lines and to assess the efficiency of different mutagenic treatments, since basic information on this is lacking. The first step was to estimate optimal treatment conditions (doses). Germination of the M_1 seed provides a good test of the sensitivity of the material to the mutagenic treatment.

Materials and Methods

Fifteen genetically different sunflower inbred lines chosen for their importance in commercial hybrid production (**Table 1**) were used for this study. Seed of these genotypes varied morphologically. The Institute of Field Vegetable Crops, Novi Sad, Serbia, supplied the seeds.

For gamma irradiation, 50 seeds of each genotype were irradiated at 100, 200, 300, 400 and 500Gy using a Cobalt-60 gamma source at the IAEA Laboratories in Seibersdorf, Austria. Prior to mutagenic treatment, the seeds were kept in a desiccator over a 60 % glycerol/water mixture for seven days at room temperature for seed moisture equilibration.

For fast neutron treatment, 50 seeds were treated with five different doses: 10, 20, 30, 40 and 50Gy at the Atomic Energy Research Institute, Budapest, Hungary. The samples were bombarded inside a cadmium (Cd) capsule with wall thickness of 2mm. Exposure temperature was less than 30°C, at normal air pressure and humidity was less than 70%. The samples were rotated at 16 revolutions per minute. Ten days after the treatment, 25 seeds of each genotype were sown and germinated to assess radiosensitivity.

For chemical treatment, seeds were pre-soaked in distilled water for 24 hours. Twenty-five seeds of each genotype were treated with five concentrations of ethyle-methane-sulphonate (EMS) solution, 0.5, 1.0, 1.5, 2.0 and 2.5%, for 3.5 hours; treatment concentrations were based on studies of other species [8]. After EMS treatment, the seeds were washed and sown. The control, non-mutagenized seeds were treated similarly, except for exposure to the mutagen.

The treated seeds and the controls were sown in boxes in three replications using the flat method [9] in a glasshouse under controlled environmental conditions (22-35°C, lighting of 12-hour photoperiod). The parameter used to assess the dose response was the seedling height. The measurements were taken when cotyledons emerged above the soil and had split up (12 days after sowing).

The mean seedling height of the control was used as an index of the normal growth of each inbred line. The mean seedling height of each treatment was expressed as a percentage of the corresponding control value. Based on these values, regression equations were obtained.

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Radiobiological effects of mutagenesis were observed in the M_1, and calculated on the basis of the absorbed dose or EMS of the seedling height. According to [10] and [11] seedling height reduction of 30-50% is generally assumed to give high mutation yield. Seedling height is highly correlated to survival [12]. This is usually designated as D_{30} and D_{50}, respectively.

Table 1. List and characteristics of treated sunflower inbred lines

Inbred lines	Type of inbred line	Branching	Days to flowering	Plant height (cm)	Oil content (%)	Seed size ratio	Thousand seed mass (g)	Seed color	Seed coat type
HA-26	Standard female(B analogue)	no	62	126	44	0.39	46.15	black	thick
VL-A-8	Standard female(B analogue)	no	65	108	47	0.5	38.42	black	thick
HA-48	Standard female(B analogue)	no	72	150	48	0.49	44.30	black	thick
HA-19	Standard female(B analogue)	no	56	80	47	0.53	50.70	black	thick
OD-3369	Standard female(B analogue)	no	71	105	55	0.42	52.16	black	thick
V-8931-3-4-OL	High oleic	yes	73	95	54	0.47	47.47	black	thin
HA-26-OL	High oleic	no	65	119	47	0.40	51.96	black	thick
VK-66-tph$_1$	Altered tocopherol quality	yes	57	75	41	0.42	46.28	black	thick
VK-66-tph$_1$tph$_2$	Altered tocopherol quality	yes	58	64	37	0.47	52.46	black	thick
VK-66-OL-tph$_2$	High oleic and altered tocopherol quality	yes	60	68	28	0.44	50.96	black	thick
RUS-RF-168	Standard restorer	yes	74	134	40	0.49	38.31	black	medium
RHA-SELEUS	Standard restorer	yes	71	112	47	0.45	32.49	brown	medium
RHA-M-72	Standard restorer	yes	70	114	51	0.38	41.38	brown	thin
CMS-ANN-15	Standard restorer	yes	53	33	35	0.37	41.12	black	thin
RHA-S-OL-26	High oleic restorer	yes	69	88	55	0.38	28.43	cream	medium

Three mutagenic agents were used

Results and Discussion

All seeds, the control and the irradiated, germinated. The seedling height in all three treatments decreased with increasing dose. For gamma irradiation the D_{50} and D_{30} values for the 15 sunflower inbred line seeds ranged from 120Gy and 5Gy, respectively for inbred line HA-19 to 325Gy and 207Gy, respectively for genotype VK-66-tph$_1$. For fast neutron, the D_{50} and D_{30} for seeds of the 15 sunflower inbred lines seeds ranged from 9Gy and 0.1Gy, respectively (genotype HA-19) to 21Gy and 10Gy, respectively (genotype VK-66-tph$_1$tph$_2$). The trend was therefore similar to the responses to gamma irradiation by these genotypes. The D_{50} and D_{30} values for these 15 sunflower inbred line seeds treated with EMS ranged from 0.69% and 0.01%, respectively EMS concentration (genotype OD-3369) to 1.55% and 0.68%, respectively for the line HA-19 (**Table 2**).

The data indicated that all genotypes produced a wide range of responses. With respect to radiation damage by Gamma-rays, the genotype HA-19 showed the least radiation damage with VK-66-tph$_1$ displaying the highest damage. In the case of fast neutron, the genotype HA-19 was most affected while VK-66-tph$_1$ and VK-66-tph$_1$tph$_2$ had the least radiation damage. The study of EMS revealed OD-3369 to be least sensitive while VK-66-tph$_1$tph$_2$ again was highly susceptible. Reduction of seedling height was more pronounced in genotype HA-19 than any other genotype for both gamma and fast neutron irradiation and clearly demonstrated a genotypic response to mutagenic treatment. Interestingly, the same genotype showed the greatest resistance to high doses of EMS, inferring again a genotype - mutagen interaction. This line is very early maturing and it has round and large seed. Lines OD-3369 and V-8931-3-4-OL were generally more sensitive to all three mutagens than the others. These inbreds have very high oil contents in the seeds, normal sized seeds and high thousand seed mass. Inbred lines VK-66-tph$_1$, VK-66-tph$_1$tph$_2$ and VK-66-OL-tph$_2$ showed the greatest resistance to both physical and chemical mutagenic treatments. These genotypes are nearly isogenic lines, with different oil quality but low oil quantity. They have large, black seeds but a thick coat that is probably the reason for such high resistance to mutagenic treatments.

Table 2. D_{50} and D_{30} values for 15 inbreds for exposure to Gamma-rays, fast neutron bombardment and EMS solution

Genotypes	Gamma-rays (GY)			Fast neutrons (GY)			EMS (%)		
	D_{50}	D_{30}	S_e	D_{50}	D_{30}	S_e	D_{50}	D_{30}	S_e
HA-26	202	102	13.28	15	3.6	19.00	1.34	0.50	13.44
VL-A-8	218	100	12.54	12	0.6	22.95	1.41	0.55	12.03
HA-48	220	109	11.84	17	3.8	18.75	1.40	0.58	13.68
HA-19	120	5	22.76	9	0.1	25.67	1.55	0.68	9.82
OD-3369	151	18	20.34	11	0.08	24.56	0.69	0.01	22.39
V-8931-3-4-OL	155	44	15.96	13.5	1.5	21.21	0.82	0.07	22.95
HA-26-OL	181	76	13.39	12.5	1	22.27	1.16	0.43	14.16
VK-66-tph1	325	207	9.03	20	9	15.50	1.41	0.53	13.75
VK-66-tph1tph2	294	151	6.90	21	10	12.61	1.54	0.64	11.79
VK-66-OL-tph2	289	164	3.45	19	8	16.14	1.36	0.55	14.78
RUS-RF-168	201	101	14.33	20	7.3	20.86	1.09	0.30	14.88
RHA-SELEUS	206	95	13.43	15	2.6	21.80	1.15	0.39	12.40
RHA-M-72	188	93	19.03	13	1.7	22.34	1.46	0.62	16.91
CMS-ANN-15	237	146	14.89	13	0.4	20.52	0.94	0.25	13.51
RHA-S-OL-26	197	79	12.89	14.5	2	15.11	1.36	0.50	16.17

The three mutagenic agents affected seedling height, reducing it with increasing dosage. Based on the mutagen damage on seedling height, the D_{50} and D_{30} values for 15 sunflower genotypes were estimated for the three mutagens. Retardation of growth due to the mutagenic treatments has been used to determine the dose rate for mutation induction. It is

the most functional parameter to be used in radiobiological investigations because it is generally considered to be a result of primary injury due to nuclear DNA damage. Sensitivity in seedlings height had been demonstrated in earlier dose response studies of bean [13], soybean [14], and other crops.

In this experiment, we established relationships between the D_{50} values due to gamma and fast neutron irradiation and EMS to the thousand seed mass (TSM), seed size ratio, oil content in the seed, plant height and days to flowering (**Table 3**). A significant negative correlation was found between the treatment and seed oil content, indicating that genotypes with relatively high seed oil content were more sensitive to gamma irradiation, fast neutrons and EMS. Also, larger seeds were generally more resistant to EMS treatment than to gamma and fast neutron irradiation. There was a negative correlation between early flowering, short stature plants and gamma irradiation. Mutagenic damage depended on the biological traits of the variety.

Table 3. Correlations between biological traits and response to mutagenic treatments

Biological traits	Gamma-rays	Fast neutrons	EMS
TSM	0.15	0.00	0.14
Seed size ratio	-0.17	-0.18	0.38*
Oil content	-0.69**	-0.37*	-0.39*
Plant height	-0.39*	-0.20	0.11
Days to flowering	-0.41*	-0.14	-0.24
r(0.05)=0.349 r(0.01)=0.449			

The results obtained from this study indicated that the radiation damage due to mutagenic treatment was not similar amongst the genotypes. The same differential response to radiation among different genotypes in plant species was reported by many researchers. These intervarietal differences in radiation damage to seeds have been reported to be: a) under polygenic system in rice, tomato and barley [15], [16], [17], [18], [19], b) major gene control in einkorn wheat and soybean [20], [21], and c) influenced by heterozygosity in maize and peanut [22], [23], [24]. It is widely accepted that response to mutagens is species and genotype dependent, but the full explanation has not yet been provided.

The different D_{50} (D_{30}) values for sunflower inbreds were established: dose range of 120 to 325Gy (5 to 207Gy) for gamma irradiation, 9 to 21Gy (0.1 to 10Gy) for fast neutrons irradiation and 0.69 to 1.55% (0.01 to 0.68%) concentration of EMS. The radiation sensitivity studies indicated that all the genotypes treated exhibited a wide range of radiation damage to Gamma-rays and fast neutrons.

Based on the radiation damage, bulk irradiation with a dose giving rise to a 30% to 50% reduction in growth will be carried out and M_1 plants will be grown in the field. Different mutations will be observed in the field and promising mutants will be selected for further testing. Selection will be carried out in the M_2 generation for early flowering, short stature, deformations of leaves and heads, appearance of branches, head inclination, sterility and oil seed quantity and quality.

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A New Mutant for Yellow Mosaic Virus Resistance in Mungbean (*Vigna radiata* (L.) Wilczek) Variety SML-668 by Recurrent Gamma-ray Irradiation

K S Reddy

Abstract

The mungbean variety SML-668 is early, high-yielding and large-seeded but susceptible to yellow mosaic virus (YMV) disease. To develop YMV resistance in SML-668, a mutation breeding programme has been undertaken. Seeds of SML-668 were irradiated with 600Gy Gamma-rays and planted in the field. Three thousand plants in M_1 generation were harvested separately and planted in M_2. Ninety lines showed sterility and only 10 lines showed mutants for chlorophyll, small seed size, short pod length, dwarf plant type and profuse branching, but there was no YMV-resistant mutant. All the mutants along with normal plants of the segregating lines were harvested separately in M_2. In M_3 generation 2,500 normal lines were planted as single plant progenies and screened for YMV resistance and did not observe any YMV resistant mutant. Hence, the normal M_3 lines were made into two separate bulks and one bulk was irradiated with 500Gy as a recurrent irradiation and another was sown as it is. In M_3M_1 generation, a mutant showing very minor leaf symptoms for YMV, and without any pod symptoms was isolated. The mutant was purified by growing up to M_3M_6 generations. All the mutant plants showed very minor leaf symptoms but no symptoms in the pod. The pods and seeds were normal and also gave normal yield as compared to highly resistant check where two recessive genes controlling resistance is reported. The susceptible plants showed leaf and pod symptoms and showed severe yield losses. This mutant was used in crossing programme to study the genetics of YMV resistance.

Introduction

Mungbean (*Vigna radiata* L. Wilczek) is an important crop in India as well as in South East Asia. In India it is cultivated on 3.2 million hectares and the production is 0.95 million tons, with an average yield of 304 kg/ha [1]. The mungbean average yield fluctuates between 300 to 500 kg/ha for a decade in India. The yield losses (40-100%) reported due to biotic stresses is responsible for the fluctuation in the average yield. The biotic stresses like yellow mosaic virus (YMV), powdery mildew (PM) and *Cercospora* leaf spot (CLS) are major limiting factors for high yield. YMV disease has been reported to cause 32-78% reduction in grain yield under field conditions [2]. In mungbean, YMV disease resistance is governed by two recessive genes [3,4,5 and 6]. Thakur, *et al.* [7] showed that resistance was controlled by a single recessive gene. Two complimentary recessive genes controlling resistance to YMV have also been reported [8]. Considering the recessive nature of resistance, an induced mutation study was undertaken to develop YMV resistance in mungbean variety SML-668, which is early-maturing, high-yielding and suitable for summer cultivation in India, but is susceptible to YMV disease.

Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Center, Trombay, Mumbai 400 085, India

E-mail: ksreddy122@yahoo.co.in

Materials and Methods

Seeds of mungbean variety SML-668 were used for mutation studies. Seven thousand seeds were irradiated with 600Gy Gamma-rays. The M_1 was raised in the field and normal cultural practices of mungbean were followed. The germination percentage was recorded in M_1. Three thousand single plants from M_1 were harvested and planted as single plant progenies in M_2 and screened for YMV resistance under field infected conditions. Since no YMV resistant mutants were observed in M_2, about 2,500 lines, which did not show any mutants were advanced to M_3 and screened for YMV resistance in the field. No YMV resistant mutants were observed even in M_3. The entire population was harvested and pooled into two bulks and one of them was irradiated with 500Gy Gamma-rays as recurrent irradiation and the second bulk was planted as control to raise M_3M_1 and M_4 generations respectively. In the M_3M_1, a mutant with minor YMV symptom on leaf but no symptoms on pod was isolated and purified by raising up to M_3M_6.

The following crosses were made to study the minor leaf symptom YMV pod resistant mutant (MLYMVPR mutant) inheritance. The SML-668 x MLYMVPR mutant and MLYMVPR mutant x Kopergaon crosses were made and the F_1, F_2 and F_3 were screened for YMV disease.

Results

Mutation studies

After irradiation of 7,000 seeds of SML-668 with 600Gy Gamma-rays, only 4,060 (58%) seeds germinated in M_1. At the time of harvest 3,000 plants could be harvested individually as single plants and planted as plant to-row progenies in M_2. In M_2 90 lines showed sterility and only 10 lines showed the mutants for characters like chlorophyll, seed size, pod length, plant type and branching plant types (**Table 1**). Thus, the 600Gy Gamma-ray treatment was effective in inducing mutations in SML-668 variety. The remaining 2,900 lines did not show any mutations. Although high incidence of YMV disease prevailed in the field, no mutant for YMV resistance could be observed in M_2. From the 2,900 M_2 lines, about 2,500 plants were selected randomly and advanced to M_3. The M_3 population was screened for YMV disease under severe field infected conditions and no YMV resistant mutant could be isolated. Hence, the normal M_3 lines were pooled into two separate bulks, one of them was irradiated with 500Gy Gamma-rays as recurrent irradiation and the second one was advanced as it is. The treated bulk was planted as M_3M_1 and rest as M_4. In M_3M_1, a mutant, showing minor leaf symptoms for YMV and without any disease symptoms on the pod was isolated (**Fig. 1**). The mutant was purified by growing to M_3M_6 and screened for YMV pod resistant character. This character showed consistent performance in further generations. Although minor leaf symptoms were observed on leaves of mutant plants, no symptoms were observed on pods in any generation, even under high severity and incidence of YMV infection. The susceptible parent SML-668 and susceptible check Kopergaon were infected and expressed YMV symptoms on both leaves and pods reducing the yield considerably. The MLYMVPR mutant gave normal green pods with well-developed seeds compared to susceptible plants.

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Inheritance studies

The MLYMVPR mutant was used in hybridization with susceptible genotypes to study the inheritance. The SML-668 x MLYMVPR mutant and MLYMVPR mutant x Kopergaon crosses were screened for YMV reactions in F_1, F_2 and F_3. The F_1 plants were susceptible, showing symptoms on both leaves and pods. In F_2, the segregation was 3:1 for susceptible (leaf and pod infection) and leaf minor symptom YMV pod resistant characters respectively and segregation showed good fit to 3:1 ratio (**Table 2**). The F_3 segregation was one true breeding for susceptible (leaf and pod infection): two segregating for susceptible (leaf and pod infection) and minor leaf symptom YMV pod resistant reactions : one true breeding for minor leaf symptom YMV pod resistant characters and showed good fit for 1:2:1 ratio and confirmed the F_2 segregation (**Table 2**).

Table 1. Mutation frequency with respect to parent and mutant characteristics in M_2 of SML-668 mungbean variety

Mutation affecting characters	Parent characters	Mutant characters	No. of mutants isolated	Mutation frequency
Chlorophyll	Green	Chlorina	17	0.018
Chlorophyll	Green	Virescent	4	0.004
Growth habit	Tall (40-60cm)	Dwarf (20-35 cm)	18	0.020
Branching habit	2-4 branches per plant	4-6 branches per plant	5	0.005
Pod length	8-10 cm	4-6 cm	8	0.008
Seed size (100 seed weight)	Large seed size (5-6 g)	Small seed size (3-4 g)	14	0.015

Table 2. Inheritance studies of minor leaf YMV pod resistant mutant (MLYMVPRM) in F_2 and F_3

Crosses	Reaction scores of F_2 & F_3 and the number of plants			Total F_2 & F_3 plants	χ^2 value for 3:1	P–value
	S	S: M (seg.)	M			
SML-668 x MLYMVPR (F_2)	231		86	317	0.766	0.5-0.3
Kopergaon x MLYMVPR (F_2)	137		51	188	0.454	0.7-0.5
SML-668 x MLYMVPR (F_3)	73 (TB)	151	82	306	0.563	0.8-0.7
Kopergaon x MLYMVPR (F_3)	41 (TB)	87	46	174	0.287	0.9-0.8

TB= true breeding; S=susceptible; Seg=segregating; M= MLYMVPR mutant

Figure 1 (A) Minor leaf symptom YMV pod resistant (MLSYMVPR) mutant showing leaf symptoms and no pod symptoms. (B) Susceptible plant showing both leaf and pod symptoms.

Discussion

Mungbean yellow mosaic virus disease is a serious problem in India. SML-668 is a short duration (55-65 days) variety suitable for cultivation in the summer season under irrigated conditions which brings additional area under mungbean cultivation in India. But YMV disease is a major disease which can reduce the productivity to a great extent. Although SML-688 has many required characters for summer cultivation, it is highly susceptible (symptoms present on leaves and pods) to YMV disease. Induced mutation study was chosen to develop YMV disease resistance in SML-668 because the YMV resistance is recessive in nature and is controlled by two recessive genes and segregation for susceptibility and resistance was reported as 15:1 ratio [3,4,5 and 6]. It is assumed that the presence of any one dominant gene can result in susceptible reaction. These studies suggest that susceptibility is dominant over resistance. In another study conducted by Thakur, *et al* [7], the resistance was controlled by a single recessive gene. The recessive and two complimentary genes controlling resistance of YMV have also been reported [8]. The present study showed that the induction of mutation for YMV resistance in M_2 was not easy because getting simultaneous mutations in two dominant susceptible genes is extremely difficult in a single exposure of Gamma-rays. Hence, recurrent irradiation was chosen in this study. In this study a mutant isolated for minor leaf symptom YMV pod resistance (**Fig. 1**) is not comparable with the resistance controlled by two recessive genes where no symptoms were observed on both leaves and pods, but comparable in yield with respect to protected or resistant plants. The MLYMV pod resistant mutant when crossed with susceptible SML-668 (susceptible parent) and Kopergaon (susceptible check) the F_1 was susceptible and F_2 segregated in a 3:1 ratio for susceptible and mutant characters and 1:2:1 genetic ratio in the F_3 generation confirmed the F_2 segregation (**Table 2**). The present investigation showed that resistance in the minor leaf YMV pod resistant mutant is controlled by a single recessive gene. It is assumed that the two recessive genes involved in controlling complete resistance may have a major and a minor gene. The mutant obtained in this study is proposed as a major gene since it gives maximum protection from YMV disease. This hypothesis also supports the results obtained by Shukla and Pandya [8] where a single recessive and two complimentary recessive genes controlled YMV resistance. To confirm this hypothesis, a second recessive mutant gene has to be obtained. The resistant mutant obtained in this study is effective in reducing the yield losses by YMV disease compared to the SML-668 and is expected to give stable yield in summer cultivation. Further mutation studies will be aimed to target the second dominant susceptible gene for differentiation and characterization of two recessive genes controlling complete resistance.

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Isolation of Early Flowering Mutant in Cultivar C-306 Known for its Good Chapati-making Quality

B K Das* & S G Bhagwat

Abstract

About 85% of wheat grains produced in India are consumed in the form of *chapaties* or its variant form. Wheat varieties released in India have acceptable *Chapati*-making qualities, however, the variety C-306 is quoted for its excellent *Chapati*-making quality. Good *Chapati*-making quality requires medium strong dough and is influenced by protein content and protein quality. The variety C-306 is medium tall and late in flowering, and thus not suitable for large-scale cultivation. To reduce the duration of the variety, γ-ray induced mutagenesis was used. Mature seeds were irradiated with 200, 300, or 400Gy. About 400 plants in the M_1 generation were harvested individually and planted in the M_2 generation as plant to row progenies. In the M_2 generation, mutants that flowered early and showed reduction in height were observed. The mutants were carried forward in M_3 and M_4 generation as plant to row progenies. Although there were minor segregations in the lines, the early flowering and maturity behavior was consistent. The parent showed anthesis in about 75 days while the mutants showed anthesis from 50 to 63 days. Seven mutant lines were selected for quality analysis. These lines in the M_5 generation showed anthesis in 50 days, maturity in 90 days, and grain protein content ranging from 11.9 to 14.9% as compared to 13.1% in the parent. SDS-PAGE of total grain protein showed that the mutants had unaltered high-molecular-weight glutenin subunit pattern. Rheological properties were estimated using Brabender Farinograph. The mutants had comparable water absorption, dough development time, dough stability, degree of softening and quality number. The early mutants are being monitored for yield and quality parameters and are expected to retain good quality and possess improved agronomic characteristics.

Introduction

Wheat is the second most important crop in India in terms of area under cultivation and annual grain production. Nearly 85% of wheat grains are consumed in the form of *chapaties* or its variant form [1]. In recent years wheat varieties have been released which have acceptable *Chapati*-making qualities, however, the variety C-306 released in the year 1965 from HAU, Hissar is often quoted for its excellent *Chapati*-making properties [1, 2]. Good *Chapati*-making quality requires medium strong dough, and although it is not characterized in terms of biochemical components, *Chapati*-making quality is influenced to a considerable extent by the protein content and protein quality. The variety C-306 has agronomic characteristics that are not suitable for its large-scale commercial exploitation. The variety is medium tall and late in flowering [3]. Due to late flowering it often matures when the temperature starts rising at the end of winter season, and as a result, the grain filling is hampered. By reducing the duration of flowering and reducing the height, the suitability of the variety for large-scale cultivation can be improved. Direct mutants have been reported to be useful in cultivation [4]. This study reports isolation and quality assessment of Gamma-ray-induced early maturing mutants in the bread wheat variety C-306.

Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Center (BARC), Trombay, Mumbai-400 085, India

* Corresponding author. E-mail: bikram_das2001@yahoo.com

Materials and Methods

Mature seeds of C-306 were irradiated with 200, 300 or 400Gy dose of Gamma-rays and M_1 was raised in the experimental field at Trombay. The seeds of M_1 plants were harvested individually and planted in the M_2 generation as plant-to-row progenies.

The seeds of mutants isolated in the M_2 were harvested and subsequently grown as M_3, M_4 and M_5 as plant-to-row progenies. Days to anthesis and maturity were recorded in each generation.

The bulk harvests were used to estimate thousand kernel weight. Three replicates of 100 kernels were used to calculate 1,000-kernel weight.

Grain protein percentage was estimated by the Kjeldahl method using Kjeltec™ 2300 system (Foss Tecator). HMW-glutenin subunits were analyzed using SDS-PAGE according to Bhagwat and Bhatia (1993) [5]. Five grain bulks from each mutant line were used as sample to check the HMW-subunit patterns.

Rheological properties were estimated using Brabender Farinigraph E (Brabender, Germany).

Bulk harvest of each mutant line was milled using Tecator mill (0.8mm sieve). The whole meal was sieved through 40mesh sieve to obtain flour. Ten grams flour was used to determine water absorption percentage. Subsequently 10 gram samples were used to estimate dough development time, stability, degree of softening and quality number using the predetermined water absorption (Brabender /ICC method).

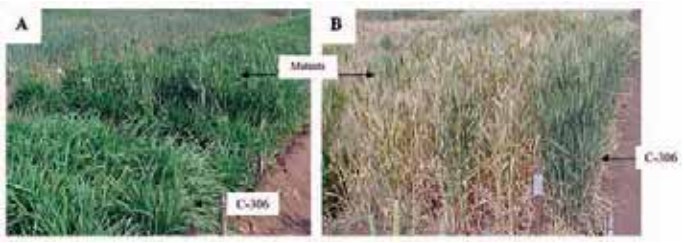

Figure 1 (A) Field view showing C-306 parent in the foreground and early flowering mutant (M_4) in background; (B) C-306 parent (extreme right) at grain filling stage and early mutant lines in M_5 generation nearing maturity.

Results

Raising the M_1 generation

In the M_1 generation the germination percentage was 29% (400Gy) to 88% (200Gy) compared to 91% in control.

Identification and evaluation of mutants

About 400 plants in M_1 generation were harvested individually and planted in the M_2 generation as plant-to-row progenies. In the M_2 generation that consisted of the 400 lines (~10,000 plants), mutants that flowered early and also showed reduction in height were observed. The

mutants were carried forward in the M_3 (35 lines) and M_4 (30 lines) (**Fig.1A**) generations as plant-to-row progenies. Although, there were minor segregations in these lines, the early flowering and maturity behavior was observed consistently. The parent variety showed anthesis in about 75 days from sowing while the different mutants showed anthesis from 55 days to 63 days.

M_5 generation

Twenty-three superior lines were carried forward to the M_5 generation. Each line was grown as three-meter row. These lines showed anthesis in 50 to 61 days. Seven lines (**Table 1**) that showed uniform height, anthesis time and good vegetative growth were selected for further analysis. The thousand kernel weight of five of the mutants were numerically higher than the parent (**Table 1**).

Table 1. Comparison of parent variety C-306 and mutants in M_5 generation for agronomic and quality traits

Sr. No	Parent/ Mutant	TKW (g)	HMWG subunits	Protein %	Water absorption (%)	Dough development (min)	Stability (min)	Degree of softening (FU)	Quality number
1	C-306	38.8	N, 20, 2+12	13.09	81.35	3.85	1.90	108	53.5
2	26-31	36.9	N, 20, 2+12	13.74	80.70	3.20	1.40	104	41.0
3	26-32	36.8	N, 20, 2+12	14.87	80.05	3.15	1.20	118	42.0
4	26-33	41.2	N, 20, 2+12	12.62	80.55	3.55	1.65	97	46.5
5	26-40	41.5	N, 20, 2+12	13.01	74.10	3.00	1.50	89	44.0
6	26-43	40.2	N, 20, 2+12	12.15	79.20	3.30	1.55	100.5	47.5
7	26-44	39.2	N, 20, 2+12	12.97	79.90	3.20	1.35	102	44.5
8	26-45	41.5	N, 20, 2+12	11.91	80.65	3.50	1.35	103.5	42.0

Values are means of two determinations on harvest of M_5 generation.

Quality analysis

The grain protein content of the parent was 13.1% while the range of protein content among the 23 mutant lines was 11.5 to 14.9%. Seven mutants selected for further analysis showed grain protein from 11.9-14.9%. The 23 mutants were analyzed using SDS-PAGE for their HMW-glutenin subunits. All the mutants except one showed HMWG subunit pattern which was similar to the parent (Null, 20, 2+12). Electropherogram of seven mutants and parent are shown in **Fig. 2**.

Rheological properties of dough

The seven mutant lines which were selected for studying dough properties showed that water absorption ranged from 74.1 to 80.7% as compared to 81.4% in case of C-306. The dough development time, dough stability, degree of softening and quality number were comparable to the parent (**Table 1**). Farinograph traces of the parent and two mutants are shown in **Fig. 3**.

Discussion

Mutation breeding has been applied to wheat for a variety of purposes [4, 6, 7]. Induced mutations have been shown to be useful in eliminating single defects in varieties which are otherwise useful. Obtaining a mutant that has all agronomic characteristics and having quality attributes of C-306 will be useful. In this experiment, the isolated early mutants showed anthesis in 50 to 61 days, which is as much as 25 days less than the parent variety C-306. The mutants matured in ~ 90 days compared ~113 days for the parent. The parent variety, due to its late-flowering often matures when the temperature starts rising at the end of winter season. The early flowering and maturing mutants are expected to escape the adverse weather conditions.

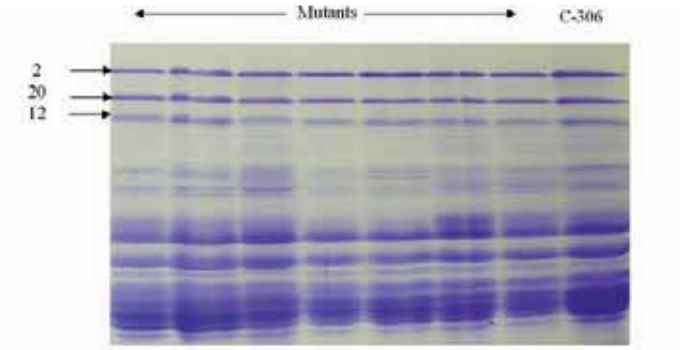

Figure 2 HMW-glutenin subunit patterns of parent and mutants as revealed by SDS-PAGE. L to R: lane 1-7: mutants, lane 8: C-306 (parent). The arrows indicate the presence of specific subunits.

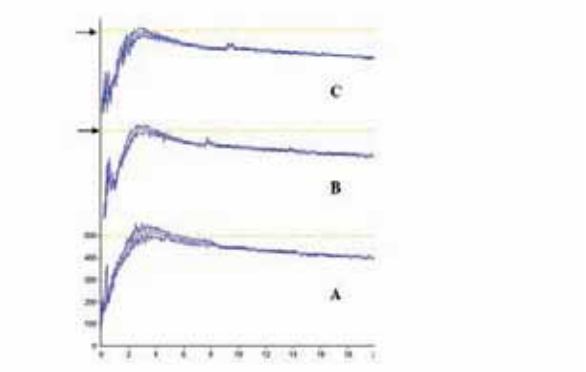

Figure 3 Farinograph traces of parent variety C-306 (A) and mutants 26-43 (B) and 26-44 (C). Arrow indicates 500 FU.

The mutants in the M_5 generation are expected to become more uniform in subsequent generations. The small differences in protein content and dough properties in the mutants and the parent could possibly be due to the difference in the conditions in which the grains developed and matured. The agronomic package for the mutants can be selected in such a way that its grain protein content and the dough properties would match the parent. Among the seven mutant lines, two mutants (26-43 and 26-44) were more comparable to the parent. These also were the highest in grain yield per line among the mutants, with 289.5 and 199.2g respectively. Their 1,000-kernel weights were numerically higher than the parent indicating increase in the grain size either due to genetics or to grain maturing in a favorable environment, or both.

The parent variety has hairy glumes; all the mutants used in the analysis showed plant morphology similar to the parent including hairy glumes. Some mutants with non-hairy glumes were observed. The early mutants isolated in this study are expected to retain good quality parameters and possess improved agronomic characteristics. The HMWG subunit '20' which is observed among the varieties with good *Chapati*-making quality, has not altered in the mutants. One mutant line was an exception and showed different subunit profile, the cause of which needs investigation. Grain protein quantity and quality influence the dough properties to a large extent and determine the quality of the end product. Since the mutants obtained in this study are comparable to parent variety C-306 in protein content and dough properties, they are expected to retain the *Chapati*-making quality.

The selected mutant lines will be evaluated in a replicated experiment to assess their yield potential.

ACKNOWLEDGEMENTS

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A Bentazon and Sulfonylurea-sensitive Mutant in Rice and its Application in Hybrid Rice

J Zhang[1,*], G Pan[1], X Wu[2] & J Tu[1]

Abstract

A rice bentazon-lethal mutant 8077S obtained by radiation, is being utilized in developing new hybrid rice systems. Genetic analysis revealed that the bentazon-lethal mutant was controlled by a single recessive gene, which is named *bel*. The mutant can be killed at the seedling stage by bentazon with a lethal dosage at 300 mg/l or above, while this dosage is safe for its F_1 hybrids and all other normal rice. This mutant is also sensitive to all the tested sulfonylurea herbicides and this sensitivity is also controlled by *bel*. Interestingly, another rice bentazon-lethal mutant Norin8m also obtained by radiation in Japan, was controlled by the allelic locus of *bel*, which is named as *bsl*. These two mutant genes were cloned by map-based cloning. Both mutant alleles had a single-base deletion respectively. There is a G deletion in the *bel.* and a C deletion in the *bsl.* The wild-type gene *bel.* encodes a novel cytochrome P450 monooxgenase, named CYP81A6. Otherwise, the use of photo-thermogenic male sterility (P/TGMS) system in two-line hybrid rice breeding is affected greatly by the sterility instability of P/TGMS lines caused by temperature fluctuation beyond their critical temperatures for fertility reversion. To prevent the hybrid seed contamination, we have developed three bentazon-lethal P/TGMS lines using 8077S by backcross and three new hybrid rice varieties using these P/TGMS lines had been registered. When these P/TGMS lines selfed by temperature fluctuation, the seedlings from the selfed seeds can be killed by spraying bentazon at seedling stage but the hybrid seedlings are safety. These new hybrid rice varieties have been cultivated in five provinces in China.

Key words: Mutant, radiation, bentazon, hybrid rice, cytochrome P450.

Introduction

Bentazon is a selected contact herbicide classified to benzothiadiazole, and is used to control most broadleaf weeds and sedges in most gramineous and large-seeded leguminous crops. Normal rice is resistant to bentazon and it is harmless to use up to 6000 mg/L of bentazon during the whole development stage. This herbicide disrupts photosynthesis of the target plant by blocking electron transfer in photosystem II (G. Retzlaff and R. Hamm. 1976). Bentazon-lethal mutants Norin8m were obtained from Co^{60}-γ-radiated conventional japonica cultivar Norin8 by Mori K. Previous studies showed that Norin8m mutants had recessive inheritance, governed by a single locus (Mori K., 1984). This single locus was named bsl. and mapped on the long arm of chromosome 3 (Liu and Lu, 2004).

On the other hand, selective responses of plants to a chemical products can be used to identify a specific type of plant from a mixture, and thus this selective chemical can be exploited as a marker. Rice bentazon sensitive lethality mutation maybe used as a selective marker. For this purpose, we induced the indica thermo-sensitive genic male sterility (TGMS) line W6154S by Co^{60}-γ-ray. A mutant 8077S which was sensitive to bentazon and sulfonylurea herbicides had been developed in this programme (Zhang J, *et al.*, 1999). Interestingly, these mutants also had recessive inheritance, governed by a single locus. This single locus was mapped on the long arm of chromosome 3 and named bel. (Zhang J, *et al.*, 2002). Then the bel. locus had been cloned by map-based cloning (G. Pan, *et al.* 2006).

In this paper, we summarized the agronomic characteristics and genetic pattern of the mutant 8077S and its applications in hybrid rice.

Obtaining of the bentazon-lethal mutant 8077S and its herbicides test

8077S was obtained through a mutation-breeding programme. Seeds from an indica TGMS line W6154S were treated with 350 Rad Co^{60}-γ-ray in 1995. The M_1 and M_2 plants were grown in the field with the temperature below the critical level required for fertility conversion of W6154S. M_3 plants were planted by families and some seeds from each M_2 plant were saved as seed sources since the sensitive mutant would be killed by herbicide. Six different herbicides including NC-311, bentazon, Londax, molinate, facet and Weinong, which are safe for the normal rice, were sprayed successively in the M_3 planting plot at one- to three-leaf stages. Recommended concentration rates for controlling weeds were used. Among 25,100 M_3 families tested, only one of them, numbered as 8077, was completely killed after spraying bentazon (**Fig. 1**), and this mutant was named 8077S.

Figure 1 The 8077S mutant from the M_3 families of radiated W6154S was planted in the field in 1997. The dead rice is 8077S.

To screen an herbicide to which M8077S is significantly sensitive, 29 herbicides belonging to 11 different chemical classes were tested using recommended rates for controlling weeds. The tested herbicides include bentazon and six sulfonylurea herbicides, i.e. Londax (bensulfuron), NC-311 (pyrazosulfuron), sulfometuron, metsulfuron, cinsulfuron, chlorsulfuron. The mutant 8077S and two controls including the original wild-type W6154S and a commercial cultivar Ce64-7, were used in this study. Among these herbicides, only bentazon selectively killed the seedlings of 8077S but was safe to W6154S (**Fig. 2**) and Ce64-7. 8077S was lethal to bentazon throughout the whole development stage. In addition, the mutant 8077S was also sensitive to all the tested sulfonylurea herbicides. The variety W6154S and the control variety had nearly normal

[1] College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, 310029, China
[2] Institute of Food Crops, Hubei Academy of Agricultural Sciences, Wuhan, 430064, China

* Corresponding author. E-mail: zhjwen@yahoo.com

growth and development with the applied herbicides. For all other herbicides, 8077S had a normal response without any visible injury. There was no significant difference in responses to other herbicides between the mutant and the control varieties. Therefore, the mutant M8077S is selectively sensitive to herbicides bentazon and sulfonylureas.

Figure 2 8077S died but the original donor W6154S is safe seven days after spraying with 1,250 mg/l bentazon.

For the concentration test, 8077S and the other two controls were planted in the field and sprayed with Bentazon at the three-leaf stage with eight different concentrations: 20, 39, 78, 156, 313, 625, 2,500, and 5,000 mg/L. The concentration test for Londax was planned in pot experiments. Each pot was applied with 5ml of Londax. Four different concentrations, 3, 15, 30, 300mg/L (equivalent to 3, 15, 30, 300g/h in the field respectively), were tested. The results for the concentration test with bentazon indicated that 8077S could be killed in two to seven days after spraying when the concentration was higher than 300mg/L. However, the controls were not affected significantly even when concentration was up to 5000mg/L. The oncentration test with Londax indicated that when the concentration increased to 15mg/L, the mutant was severely injured. Ten days later, the mutant was only about half as tall as that of the controls. When concentration increased to 300mg/L, plant growth of the mutant was severely inhibited and seedlings died eventually. However, this concentration was still safe to the controls. Control varieties could keep normal growth and tillering although they showed some injury on their leaves with a color slightly darker than normal. This phenomenon was also observed in the field test (**Fig. 3**).

Figure 3 Ten days after spraying with 15g/h Londax in the field, the bel-tagged P/TGMS line (left) was severely injured, but the restorer line (middle) and the F_1(right) are safe.

As a TGMS line, 8077S had an identical fertility conversion pattern and very similar agronomic traits as compared with its donor variety W6154S. When crossed with Ganhui 2, the mutant produced hybrids very similar to those produced with the original variety W6154S. This indicated that the *bel* gene did not have any significant effect on agronomic traits of the mutant and its hybrids except for bentazon sensitivity. 8077S also demonstrated other agronomic characteristics that the donor parent has including plant type, photo-thermo response, and combining ability.

Genetic patterns of the bentazon-lethal mutant 8077S

8077S was used as a female parent to cross with the original donor W6154S and five other indica varieties, Ganghui 2, Ce64-7, R1073, R1074 and R6175. At three-leaf stage, all F_1 plants were sprayed with 1,250mg/L of Bentazon. Plants were then scored as normal and dead based on their reaction to the herbicide seven days after spraying. The results showed that all the F_1 plants were resistant to bentazon with concentration even up to 5000 mg/L. This indicates that bentazon sensitivity of the mutant is genetically recessive.

When four F_2 populations derived from crosses 8077S/Ce64-7, 8077S/R6175, 8077S/R1073 and 8077S/R1074, were treated with 1,250mg/L bentazon at three-leaf stage, numbers of dead and normal plants, scored seven days after spraying, fit very well with the 1:3 ratio, as expected for single-locus segregation. The genetic pattern for single genes was also confirmed by two F_3 families where numbers of killed families, segregating families and normal families fitted the ratio 1:2:1. So the single bentazon lethality locus was named *bel*.

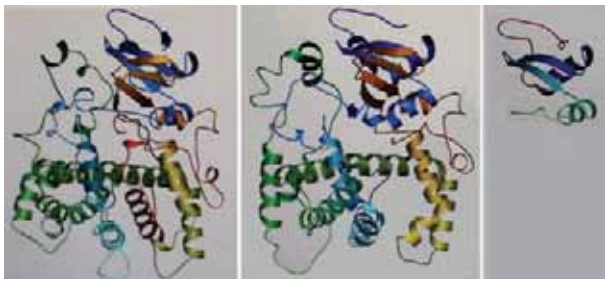

Figure 4 3-D structures of Bel. (left), bel. (middle), and bsl.(right) proteins predicted using Swiss-model.

Bentazon-sensitivity and allelism test of the two mutants 8077S and Norin8m

The plants of 8077S and Norin8m mutants and their wild-type controls were treated with the following concentrations of bentazon: 0, 50, 100, 200, 300, 600, 1,250 and 5,800 mg/l. Three plants (15–20 tillers per plant) were used for each treatment at maximum tillering stage and scored in seven days after the treatment. The data confirmed that both 8077S and Norin8m plants started to show symptoms to bentazon at 100 mg/l and the lethal concentration was about 300 mg/l. The threshold concentrations of the bentazon sensitivity for the mutant plants were about 60-fold lower than those for their wild-type controls. In addition, both 8077S and Norin8m plants were also sensitive to sulfonylurea herbicides.

Although *bel* for 8077S and *bsl* for Norin8m were shown to be located on chromosome 3, it was not clear whether they represented the same or different genes. To address this question, a cross was made between these two mutants. Thirteen F_1 and 800 F_2 progeny plants, along with their original parents, were treated with 1,250 mg/l bentazon at maximum tillering stage. The results showed that in contrast to the healthy plants prior to treatment, the parents, 13 F_1, and 800 F_2 progeny plants all died in seven days following the treatment, suggesting that *bel* and *bsl* were allelic.

Molecular mechanism of the bentazon-lethal mutants

Using primary gene mapping, 91 recessive susceptible individuals were selected from an F_2 mapping population developed from the cross between 8077S and P64S, showed that the *bel* was determined to be on the long arm of chromosome 3 and was linked to RM168 at a distance of 7.1cM. To gain information on the exact location of *bel*, 231 recessive susceptible individuals were selected from an F_2 mapping population developed from a cross between PA64Sm and 93-11 for fine mapping. The level of SSR polymorphism between them was highest among the parents tested. The detected polymorphic markers 3a, 7a, 8a, 14a in the genomic region from RM416 to RM3867 were used to survey these 231 recessive susceptible individuals. The data confirmed that the *bel* locus was located between the markers RM416-8a-3a and 7a-14a-RM3867.

The map distances between the *bel* and two closely linked markers of 3a and 7a were 0.1 and 0.4 cM, respectively. The markers 3a and 7a were found to be on the same BAC clone (AC084282) and the physical distance between the markers was 110 kb.

Further genomic analysis revealed that this 110kb sequence contains 25 putative genes, including a cluster of four tandem cytochrome P450 genes, namely CYP81A5, CYP81A6, CYP81A7 and CYP81A8 (GenBank Accession No: AAK63940.1, AAK63920.1, AAK63922.1 and AAK63925.1, individually). Considering that cytochrome P450s are ubiquitous heme proteins and known to play an important role in detoxification of natural and synthetic xenobiotics such as herbicides, we therefore treated those four cytochrome P450 genes as the primary candidates for the wild *Bel* gene.

To further address which gene among the four candidates corresponds to the *bel*/*bsl* locus present in 8077S and Norin8m, we comparably sequenced the PCR products of four genes amplified from 8077S and Norin8m and their wild type progenitors 8077S and W6154S. It was observed that among the four genes, CYP81A6 from 8077S was the only one that had a single-base deletion of G at 1,332th nuceitide of its coding sequence compared to W6154S. In order to verify this deletion mutation, we also amplified and sequenced the fragments flanking the mutation site from two *bel*-tagged *indica* lines 03B198 and 03B199 and two *indica* normal maintainer lines Minghui63 and 93-11, and identical results were obtained. For Norin8m, among the four genes only a single-base deletion of C was also detected in the coding region of CYP81A6 was observed in comparison to Norin8. These results suggested that the *CYP81A6* gene might be the *Bel* locus.

Based on the above results, we isolated the genomic fragment of the *CYP81A6* gene through PCR amplification. The total length of *Bel* is about 4 kb. The *Bel* gene contains a coding region of 1,542bp interrupted by an intron of 618bp.

The deleted base at *bel* is G that occurred at exon 2 and that at *bsl* is C that happened at exon 1. In genetics, any single base deletion can cause reading-frame shift on the subsequent sequence and creation of one or more termination codons before the native one, which leads to premature termination of translation. Both these alternations may have the effects on the mutation phenotype but which one is dominant depends on where the mutation site is located and where the effective termination codon is created. In case of *bel* protein, the premature termination caused the removal of 46 amino acid residues in the downstream part. This removal is exclusive of any important functional domain as presence in *Bel* and its mutation effect might thus be very limited. What the reading-frame shift altered, however, is the heme-binding motif (from FGMGRRRCPG to FGMGGGGAPA), in which a key amino acid residue of cysteine has been characterized as the fifth ligand to the heme iron and is strictly conserved nearly for all of the known P450 genes. Changing of this key amino acid is hence speculated to be the main reason for loss of function of *bel*. In case of *bsl* protein, only 225-aa residues are reserved, resulting in the removal of several conservative domains such as the E-R-R triad involved in the heme pocket locking, the I-helix responsible for oxygen activation, and the heme-binding domain. This longer polypeptide truncation is much likely the main reason for loss of function of *bsl*.

Furthermore, in the herbicide test, we observed that, the *bel* mutant was slightly more tolerant to bentazon than the *bsl* mutant. This result not only shows the existence of a correlation between the bentazon sensitivity and the truncated polypeptides length but also implies that the modified heme-binding domain with C to G substitution presence in the *bel* protein might still keep a partial function in the activity to bind to heme iron. **Figure 4** shows the 3-D structures of *Bel*, *bel*, and *bsl* proteins predicted using Swiss-model (Schwede T, *et al.* 2003). The comparison made between these 3-D structures provides the evidences that the truncated *bel* protein has the basic global structure as that of the wild *Bel* protein except two β-folder is removed from C-terminal and the modified heme-binding domain with C to G substitution is still toward the activation groove of the protein, a condition for this domain to play roles if available. However, more data is needed to confirm these deductions.

Application to secure purity in hybrid rice production

One of the most important applications of the *bel* gene in hybrid rice is to maintain hybrid purity. The two-line hybrid rice based the photo-thermo-sensitive genic male sterility (P/TGMS) have been broadly planted in China. However, most P/TGMS lines require a specific temperature (usually higher than a critical temperature) to maintain their sterility. Abnormal weather could bring the temperature down below the critical temperature that is required for conversion of P/TGMS lines from sterility to fertility, or simply called fertility conversion, which makes P/TGMS lines fertile or partially fertile in the location where they are supposed to be sterile in normal years. This results in a potential problem for seed production of two-line hybrid rice, i.e. a P/TGMS line producing seeds either from outcrossing as required for seed production or from selfing. The mixture of real hybrids with selfed seeds from the P/TGMS line cannot be used, resulting in a great loss to seed producers or to rice producers once false hybrid seeds are used in rice production. As insurance to the seed production, marking the P/TGMS lines using *bel*., named bentazon-lethal P/TGMS lines, could help remove the false hybrids from the mixture. If the hybrid rice seeds were contaminated with the selfed seeds of these bentazon-lethal P/TGMS lines, we sprayed bentazon at the seedling stage to selectively kill the seedlings from the selfed seeds of the P/TGMS lines. Then the hybrid rice purity in the field is insured.

Figure 5 After spraying with 1,250 mg/l bentazon, the bel-tagged P/TGMS line "Ben63S" (middle) died, but the restorer line "1175" (right) and the hybrid rice "Benliangyou639" F_1 (left) are safe.

The *Bel* gene can be also used for purity test in seed production. Hybrid rice seeds must be tested for purity before release to rice producers. Traditionally, seed samples are planted, and then a waiting period until they flower ensues, so they can be told from the false hybrid plants based on distinct agronomic traits. In order to obtain purity test results before the next planting season, seed samples are usually sent to a location where rice can be planted in the winter like Hainan, China. However, this is labor-intensive and also very expensive. Using bentazon sensitivity, false hybrids can be told by killing at two to three-leaf stages. A seedling tray in a greenhouse or in an incubator will be enough for a purity test required for any sample of hybrid seeds.

The technique for the use of the *bel* gene in seed purity management has been developed in our breeding programme. In the past decade, we have developed three bentazon-lethal P/TGMS lines (M8064S, B88S, B63S) using 8077S by backcross and three new hybrid rice varieties using these P/TGMS lines had been registered (Fengliangyou No 2, Benliangyou 639, and Benliangyou No 9) (**Fig. 5 and 6**). Now these hybrids have been broadly planted in Hubei, Guangxi, Jiangxi, Hunan and Anhui provinces in China.

Development of chemically induced male sterility in hybrid rice

Previous study showed that the expression of the *Bel*. gene was constitute expression, and W6154S transformants with the antisense displayed the bentazon- and sulfonylurea-sensitive phenotype similar to 8077S (**Fig. 7**). We may develop a novel herbicide-emasculation male sterile system by the antisense or RNAi of the Bel gene. In order to create the herbicide-emasculation male sterility line, the antisense or RNAi fragment of the *Bel* gene under the control of the tapetum-specific promoter such as the Osg6B has been constructed and introduced into the normal rice by *Agrobacterium*-madiated transformation, the tapetum of the transgenic rice was sensitive to the sulfonylurea and the other tissue of the plant resistance to the sulfonylurea, so that the transformants will be male sterile after spraying sulfonylurea herbicide (no published data).

Figure 6 The bel-tagged hybrid rice cultivar "Benliangyou No 9".

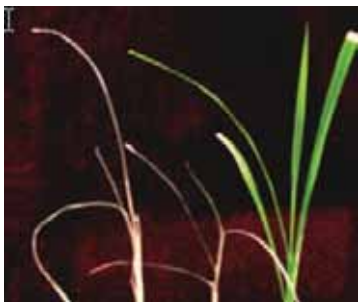

Figure 7 After spraying with 1,250 mg/l bentazon, the 8077S(left) and the W6154S transformants with the antisense of the Bel. gene (middle) died, but the original W6154S was safe.

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Dwarf Male-Sterile Wheat: A Revolutionary Breeding Approach to Wheat

L Yang*, B H Liu, H Q Zhai, S H Wang, H W Liu, Y Zhou, F H Meng, J P Yang, G Zhu, S L Chui, Q H Zhang & Y L Wei

Abstract

Dwarf male-sterile wheat is a new germplasm that linked the Taigu genic male-sterile gene *Ms2* with *Aibian1* dwarfing gene *Rht10* tightly on the same chromosome 4DS with 0.18 crossing-over unit. The progeny of dwarf male-sterile wheat always segregates into 1:1 for male-sterile plants with dwarfing gene *Rht10* and male-fertile plants without dwarfing gene *Rht10*. So the male-sterile plants are shorter than the male-fertile plants. It is very easy to identify male sterility plants based on plant height. Dwarf male-sterile wheat is favorable tool for wheat breeding in recurrent selection. A simple, effective and practical method in recurrent selection called the dwarf male-sterile wheat breeding system has been created. The new dwarf male-sterile wheat technical system consists of construction of a basic population, choice of male parent, selection of male-sterile plant and inter-crossing. Four new cultivars, i.e. RS981, RS987, RS518 and RS201 have been developed. Dwarf male-sterile wheat and its breeding technical system is an effective technology platform for wheat breeding in different ecological areas.

Introduction

Since Mendel's Law was re-discovered, wheat breeders, as well as breeders of other crops, started making use of genealogical selection via sexual hybridization to cultivate new wheat varieties. During the last hundred years, countless numbers of wheat-improved varieties have been produced that have helped solve difficult agricultural situations. The world wheat grain yields have been increasing substantially, especially in 1960s and 1970s, the semi-dwarfing high-yielding varieties and cultivation methods of the so-called "green revolution" were adopted worldwide [1].

But the genealogical selection has less and less effect on the progress of wheat genetic improvement since the 1980s. One reason is that genealogical selection limits the recombination and possibility of selection of favorable genes. Another reason is that this selection method just uses several varieties to do crosses and relates to the narrow genetic base of the developed varieties.

Recurrent selection (RS), firstly applied in cross-pollinating maize, is a population improvement method that increases the frequency of favorable alleles while maintaining genetic variation in the breeding population. The scale of manual crossing tampers with the effect of recurrent selection in self-pollinating wheat. One solution for this is to use male-sterile mutants instead of manual crossing in wheat recurrent selection. There are three important male-sterile genes that have been found in wheat. Driscoll (1978) described the production and use of a recessive male-sterility-inducing form of chromosome 4A which he designated "Cornerstone (*ms1*) in wheat breeding. One primary disadvantage of ms1 is that the amount of sterile plants is not always sufficient for recombination in wheat current selection [2]. *Ms3* is a dominant gene for male sterility found after EMS (ethyl methane sulfonate) treatment of the seeds of an alloplasmic common wheat with *Aegilops squarrosa* cytoplasm [3].

In 1972, a genic male-sterile form of common wheat was found at Taigu County, Shanxi Province, China, and it has been shown that the sterility controlled by a single dominant gene (designated *Ms2*). *Ms2* was located on the short arm of chromosome 4D with 31.16 centimorgan (cM) to the centromere [4,5,6]. During the past 30 years, *Ms2* has been introduced into a thousand backgrounds of Chinese varieties, even including octoploid tritical, and no self-fruitful seed has been found among them.

Chinese wheat breeders popularly adopt *Ms2* as a tool in wheat recurrent selection. However, two main shortcomings of *Ms2* have been found during its applications. One is that there is no difference of plant height between sterile plants and fertile ones. Therefore, many efforts must be made to identify plant sterility when flowering in the field. Another is that male-sterile plants easily get pollens from higher plants above. So the average height of recurrent population gets higher and higher with selection cycles.

Creation of dwarfing male sterile wheat

Chinese wheat breeders took into consideration the use of a trait to label *Ms2*. One of the most important short wheat germplasm resources, Ai-bian 1 (25.5 cm), was found in Shanxi Province, China. It is the shortest wheat in the world controlled by the dominant gene *Rht10*. It was also located on the short arm of chromosome 4D with almost 50 cM to the centromere [7]. The data showed that there were almost 20 cM between *Ms2* and *Rht10*. Could *Rht10* be used as a marker of *Ms2*? The re-test results showed the genetic distance of *Rht10* to the centromere was 31.17 cM instead of 50 cM [8]. It means that *Ms2* and *Rht10* should be linked closely.

In order to obtain the new recombination of *Ms2* linked with *Rht10*, the male-sterile Chinese Spring (about 108 cm) was pollinated with the dwarfing Ai-bian 1 (about 25.5 cm). All F1 hybrids were dwarfing phenotypes (about 48 cm), with one half of sterile plants and one half of fertile plants. The F1 dwarfing male-sterile plants were crossed back to double recessive, tall and fertile Chinese Spring. During 1984-1986, no recombination types were found among 321 recovered plants from the testcrosses. During 1986-1988, the population of the progeny was increased, of the 5,216 recovered plants from the testcrosses, 33 plants showed new phenotypes: one dwarfing sterile plant and 32 tall fertile plants, 2,632 and 2,551 plants showed tall sterile and dwarfing fertile, respectively. The material carrying *Ms2* for male sterility closely linked with *Rht10* for dwarfishness was named Dwarfing Male-Sterile Wheat [8].

During 1989-1990, to test the genetic distance between *Ms2* and *Rht10*, we crossed Dwarfing Male-Sterile Wheat (*Ms2Rht10/ms2rht10*) with the variety Fengkang 13 (*ms2rht10/ms2rht10*). The progeny of this cross had 1,874 dwarf sterile plants (*Ms2Rht10/ms2rht10*), 2,036 tall fertile plants (*ms2rht10/ms2rht10*), two dwarf fertile plants (*ms2Rht10/ms2rht10*), and five tall sterile plants (*Ms2rht10/ms2rht10*). Of all the

The National Key Facility for Crop Gene Resources and Genetic Improvement Institute of Crop Science, Chinese Academy of Agricultural Sciences Beijing 100081, China

* Corresponding author. E-mail: yangli@mail.caas.net.cn

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1,917 plants, seven of the recombination genotypes gave 0.18 cm of linkage value between *Ms2* and *Rht10*. It is an amazing thing that both perfect dominant genes link with each other so tightly.

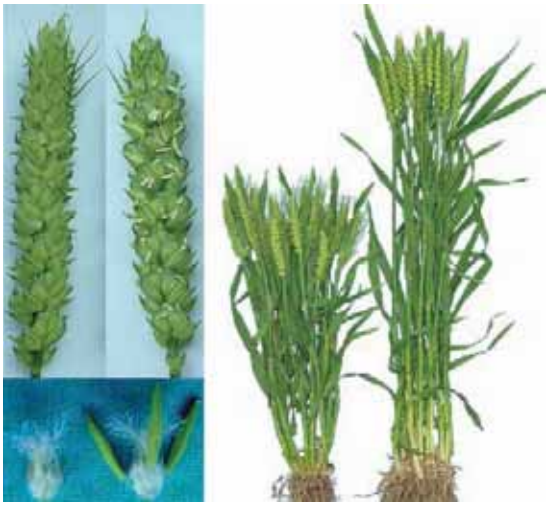

Figure 1 Dwarfing male sterility wheat and male fertility wheat.

Main characteristics of dwarfing male sterile wheat

Dwarfing male sterile wheat keeps all the advantages and abandons all the shortcomings of both of its parents, Taigu genic male sterile wheat and Ai-bian 1. (1) Dwarfing male sterile wheat has some characteristics of both the self-pollinating plant and the cross-pollinating one. It can be easily pollinated with any other wheat varieties or lines, and their offspring will segregate 50% dwarfing male-sterile plants and 50% normal fertile ones. Free cross-pollination of the dwarfing male-sterile plants leads to the interchange and recombination of favorable genes; and self-pollination of the normal fertile plants facilitates the homozygote and stability of favorable genes. (2) The plant male-sterility can be easily identified according to plant height in the early spring. Wheat breeders have enough time to design their plans to do crosses and to remove the inferior plants. (3) The plant height of the recurrent population can be easily controlled. The Dwarfing Male-Sterile Wheat can adequately and freely get the pollens from the fertile plants around. So the possibility of selecting favorable genes increases. (4) By means of dwarfing male sterile wheat, manual crossing is no longer a limit in wheat breeding. Many crosses can be used as breeding targets, so dwarfing male sterile wheat is a perfect tool for wheat breeding, especially for wheat recurrent selection.

During our effort over the past ten years, a new technical system of wheat recurrent selection and different wheat improved populations by means of dwarfing male sterile wheat were established in our institute. In the improved populations, yield potential, grain quality, lodging resistance and disease resistance have increased on a large scale. Many new varieties or lines with general good agronomic traits have been developed from these improved populations. Nobel laureate Dr. Norman Borlaug, father of Green Revolution, stated that the dwarfing male sterile wheat is "a revolution in wheat breeding."

Application effects of dwarfing male sterile wheat

Using dwarfing male-sterile wheat in recurrent selection, we have created a simple, effective and practical method and technology. The characteristics of the improved population, such as yield, quality, resistance and plant-type have increased more rapidly. We have selected four new cultivars, RS981 (Recurrent Selection), RS987, RS201 and RS209 and a series of new lines. Dwarfing male-sterile wheat and its breeding technology system are a technology terrace for wheat breeding in different ecological area. Recurrent selection by using dwarfing male-sterile wheat will lead to a revolution in the wheat breeding method.

Simple and effective recurrent selection technology in wheat breeding

Through several years practice in wheat breeding, we have developed a convenient, practical and relative quantitative recurrent selection technology system and method. Population of per cycle consists of seeds from dwarfing male sterility plants. According to our studies, dwarfing male sterility trait and pollen quality accepted by dwarfing male sterility plants are role important for recurrent selection. We have created a multi-selection technology on dwarfing male sterility plant selection, developed pollen resource selecting technology and controlled technology on the cross-pollinated.

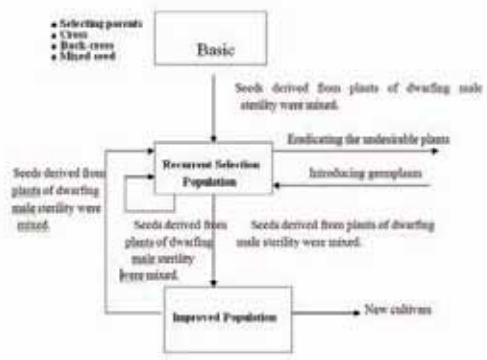

Figure 2 Procedure and method of recurrent selection breeding using dwarfing male sterility wheat.

Genetic diversity enhancement in the improved population

Through 10 years recurrent selection and intercross using dwarfing male-sterile wheat, the population of dwarfing male-sterile wheat has improved effectively. In improved population of dwarfing male-sterile wheat, characteristics such as yield potential, grain quality, lodging resistance, and disease resistance, have increased on a large scale. Many different kinds of male fertility plants with good agronomic traits were segregated in an improved population. Using dwarfing male-sterile wheat of Beijing of China as a basis population crossed with parents' material that adapt to different ecological regions, improved population of breeding goals will be established rapidly.

Achievements in new variety development

In the China National New Wheat Cultivars Demonstration Experiment in 2003, the new variety RS987, RS981, RS201, RS209 and RS981 that derived from dwarfing male-sterile wheat improved population were NO.1, NO.2, NO.3, NO.6 and NO.8 in order of yield increase respectively.

RS987 is a high-yielding wheat cultivar. In later years, the yield of RS987 was NO.1 in all yield trials such as the National Northern Winter Wheat Yield Trial, New Cultivars Demonstration Experiment and Production Registered Trial. The yield is significantly greater than that of check cultivar. RS987 was registered by China National Crops Cultivars Register Commission in 2003.

Conclusions

Dwarfing male sterile wheat and its technical system in recurrent selection has been popularized by more than 40 institutions of wheat breeding in China.

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Induced Mutations for Development of *B. Juncea* Canola Quality Varieties Suitable for Indian Agro-climatic Conditions

Y Y Barve[1], R K Gupta[1], S S Bhadauria[1], R P Thakre[2] & S E Pawar[2]

Abstract

Dry and presoaked canola quality 'Heera' seeds were treated with 0.01, 0.02 and 0.03% EMS with three and six-hour mutagenic treatments. Five mutants with early maturity (93-95 days) as opposed to 140 days of the parent were evaluated in trials. EH-1 was found superior in yield potential; however, the yield was lower than the control. Several selections with low glucosinolate, high erucic acid and canola quality were identified from the cross, mutant EH-1 and NU-6 (mutant derivative). Selection NUDH-YJ-6 with low glucosinolate, high erucic acid, 3.6g test weight and high oil content (46%) was at par in seed yield but 17% higher in oil yield in the multi-location trials of four years during 2003-04 to 2006-07 at 10 locations in zone III and zone IV of India. The advance selection derived from EH1 × NU6 was crossed with large seed mutant PB7. Several '00' selections were developed and studied for their agronomic characters. Two selections along with checks were evaluated for two years during 2005-06 and 2006-07. Both these selections were resistant to white rust disease and gave seed and oil yield comparable to national check Varuna. Another selection NUD-YJ- 5 with canola characters of maturity like Indian mustard varieties with small seed size has been registered with the National Bureau of Plant Genetic Resources (INGR NO-03034), ICAR, New Delhi.

Introduction

In India, nine annual oil seeds are cultivated on 30.25 million hectares with a production of 25.3 million tons and productivity of 1,067Kg/ha. Out of these, rapeseed mustard is second most important group of oil seed crops in India after groundnut and is being cultivated on 7.3 million hectare, with a production of 7.6 million tons. Per capita consumption of oil in India is increasing day by day and it is therefore imperative to have maximum productivity of oil crops. Mustard oil has characteristic pungency due to presence of glucosinolates in the seed meal and high erucic acid in oil, which renders mustard oil as less preferred over other cooking oils. None of the released varieties in India have the desired quality characters. Erucic acid content in the Indian cultivars is high averaging 49% compared to 25% in the European cultivars. The amount of glucosinolate varies from 150-240 µmole/g of defatted meal. Earlier Khalatkar, *et al* [1] had indicated high effectiveness of gamma radiations and EMS in the induction of mutations in genes controlling the synthesis of erucic acid and glucosinolates. Besides classical breeding, mutation breeding has demonstrated the plasticity of seed oil quality with significant alteration in fatty acid composition and no apparent detrimental effects on the crop agronomics [2] [3]. Larger seed size is one of the important yield-contributing traits in all of the crops. Improvement in seed size has been achieved through induced mutations in several crops. Pawar, *et al* [4] and Bhatia, *et al* [5] have demonstrated increase in seed size up to 25% in Pigeonpea and black gram by radiation induced mutation and recombinant breeding. The development of high value mustard oil having 30-40% oleic acid, less than 2% erucic acid, coupled with significant reduction in glucosinolate in the meal is the prime objective of this investigation. Both induced mutation and recombination breeding approaches were adopted in the present investigation for developing high yielding varieties of *B. juncea* with canola quality, yellow seed coat and white rust resistance.

[1] Dhara Vegetable Oil & Foods Co. Ltd. NDDB Campus, Anand 388001 India
[2] Department of Botany, RTM Nagpur University campus, Amravati Road, Nagpur - 440033 India

* Corresponding author. Email: yoganandbarve@gmail.com

Materials and Methods

The mutation breeding/hybridization programme was initiated during 1994-95 for developing canola quality early-maturing varieties with high yield potential. The canola quality 'Heera,' yellow seeded and white rust resistant *B. juncea* selection was developed at the Department of Botany, RTM Nagpur University, Nagpur, India by the late Dr. A. S. Khalatkar during 1992-93. It was late in maturity and not suitable for commercial cultivation in Indian conditions. It has been registered with the National Bureau of Plant Genetic Resources (INGR NO-03033), ICAR, New Delhi as a canola genotype developed in India. Heera seeds were water presoaked for 12 hours and treated with 0.01, 0.02 and 0.03% EMS with three and six-hour mutagenic treatments. Five mutants with early maturity (93-95 days) as opposed to 140 days of the parent were identified. They were dwarf, small-seeded with yellow seed coat.

NU-6 was selected in the segregating generation of EH-1 × Pusa Bold with high erucic acid and medium glucosinolate (30-40 µmole/g of seed) with medium seed size (4g test weight). In another experiment, the mutagenic treatment of 0.04% Sodium Azide was given to 12-hour water soaked seeds of advanced selection derived from cross EH1 × Pusa Bold. The mutant PB-7 with large seed size (5.5g test weight), zero erucic acid, medium glucosinolate (30-40 µmole/g of seed) was identified in the M2 generation.

An extensive hybridization programme using mutants EH-1 and PB7 and mutant derivative NU-6 was initiated for the development of agronomically suitable canola quality selections with high yield potential. Several selections with low glucosinolate, high erucic acid and canola quality were identified from the cross EH-1 × NU-6 and tested in multi-location trials. In another programme, mutant PB-7 was crossed with advanced derivatives of EH1 × NU6 having canola characteristics. In the segregating population, several '00' selections were developed and studied for their agronomic characters. Two selections along with checks were evaluated for two years during 2005-06 and 2006-07, and the yield performance is reported in the present communication.

Results and Discussion

Development of early mutants of Heera

Induction of early flowering / maturity is one of the most frequent characters modified in the mutation experiments in all the crops. In oil seed Brassica crops, several early flowering/maturity have been reported [6]. The mutation breeding programme was initiated for inducing early maturity in Heera. Five early flowering dwarf mutants (EH-1 to EH-5) were obtained in M_2. These mutants matured in 93 to 99 days as

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compared to 140 days that of control Heera (**Table 1**). They were dwarf, yellow seeded, and white rust resistant with canola characteristics. These were evaluated in multi-location trials but could not surpass the yields of non-quality variety Varuna. Among these, EH-1 was found to be superior in yield potential (**Table 2**).

Table 1 : Yield performance of EH selections at Nagpur

Mutant	Treatment	Days to maturity	Plant height (cm)	Yield (Kg/ha)
EH-1	12PSW + 3hrs EMS - 0.02%	93	175	1682
EH-2	12PSW + 6hrs EMS - 0.01%	95	177	1297
EH-3	12PSW + 3hrs EMS - 0.03%	97	165	1342
EH-4	12PSW + 3hrs EMS - 0.02%	93	169	1040
EH-5	12PSW + 3hrs EMS - 0.02%	99	166	1336
Heera	Control	140	202	1098

Table 2. Yield performance of EH selections in multi-location trial during 1997-98

Selection	Seed Yield (Kg/ha)					
	Allahabad	New Delhi	Ropar	Mehsana	Nagpur	Mean
EH-1	673.37	1416.67	411.38	895.83	244.40	728.33
EH-2	653.36	1576.39	282.09	867.42	208.11	717.47
Varuna (C)	1133.39	1000.00	634.70	1777.15	838.80	1076.81

Indian varieties are brown colored and have a higher fiber content, which is undesirable for animal nutrition. Genotypes with yellow seed color having low fiber content can also be used as genetic marker for '00' brassicas. Indian mustard high-yielding varieties are white rust susceptible and in traditional areas approximately 25% loss in the yields has been observed. Recombination breeding approaches have resulted in the development of 667 new crop varieties out of 2,252 mutant varieties worldwide [7]. In the present investigation emphasis has been given to developing yellow seeded, white rust resistant, large seed sized, canola quality genotypes using recombination breeding approaches.

Seed size improvement of early mutants

High-yielding mutant EH-1 was crossed with popular non-canola quality variety Pusa Bold with large seed size (5.5g /1000 seed). In the segregating population NU-6 was identified with high erucic acid and medium glucosinolate (30-40 µmole/g defatted meal). The seed size of NU-6 was 4g/1000 seed.

In another programme, advance selection in the F_4 generation from the cross EH-1 × Pusa Bold was treated with 0.04% sodium azide and in the M_2 generation, 47 plants were selected having zero erucic acid and medium glucosinolate. In the M_3 generation, 12 plants were selected on the basis of zero erucic acid, medium glucosinolate and good plant type. In the M_4 generation, one plant with same quality character and extra large seed size (5.5g/1000 seed) was isolated. The mutant PB-7 was studied for its breeding behavior and was stabilized in the M_6 generation. This mutant was extensively used in further crossing programme for the development of bold seeded, double low, high-yielding selections.

Development of high-yielding, high-oil containing selections

Extensive hybridization was carried out with EH-1 and NU-6 as parents, and several single and double low selections were identified. Out of them, one selection NUDH-YJ-6, with high erucic acid and low glucosinolate quality, high oil content (46%) and 3.6g/1000 seeds was identified. Due to its high oil content, it was evaluated first in station trial and then in multi-location trial in zone III and IV for three consecutive years from 2003-04 to 2006-7. Though the seed yield was at par with the national check over the period and locations during the trial, the selection NUDH-YJ-6 gave 16% higher oil yield than the national check (**Table 3**).

Table 3. Mean yield performance and quality of '0' selection in multi-location trial during 2003-04 to 2006-07 in zones III and IV of India (10 locations)

Variety	Erucic Acid (%)	Glucosinolate (µmole/g seed)	TW (g)	Oil content (%)	Mean seed yield (Kg/ha)	% increase	Mean oil yield (Kg/ha)	% increase
NUDH-YJ-6	49.5	7.0	3.6	46	1950	4.3	897	17.0
Varuna (C)	46	110.8	4.2	41	1869	0.0	766	0.0

Mustard oil is not preferred in central and southern India due to its pungency. Low glucosinolate selection NUDH-YJ-6 having 16% higher oil yield over the national checks certainly will help to increase the area under the non-traditional ecosystem thus making the alternate oil available for people of the region. The seed meal having low glucosinolate content will be ideal as cattle and bird feed.

Development of canola quality genotype

Selection NUDH-YJ-5, derived from the cross EH-1 × NU-6 with canola quality, yellow seed coat, small seed size (3g/1000 seed) and maturity like Indian mustard varieties was developed. It was also resistant to white rust disease. NUDH-YJ-5 has been registered with National Bureau of Plant Genetic Resources (INGR NO-03034), ICAR, New Delhi as canola genotype suitable for Indian agroclimatic conditions. This selection also could not surpass the yield of national checks in the trials.

In order to increase the seed size of canola quality selections, mutant PB-7 was crossed with the derivative of (EH1 × NU6). In F6, two selections viz. 1A/5-5-1-5 and 1A/5-23-29-18 were identified as having true breeding behavior in quality characters, disease resistance and genetic purity. Both these selections had shown promising yield potential and thus were evaluated in multi-location trial for two years in zone III and IV of Indian agroclimatic conditions (**Table 4**).

Table 4. Performance of '00' selections in multi-location trial during 2005-06 to 2006-07 in zones III and IV (six locations)

Variety	Erucic Acid (%)	Glucosinolate (µmole/g seed)	TW (g)	Oil content (%)	Mean seed yield (Kg/ha)	% increase	Mean oil yield (Kg/ha)	% increase
1A/5-5-1-5	0	17.0	3.3	42	2053	1.6	862.26	4.1
1A/5-23-29-18	0	10.0	3.6	41	2000	-1.0	820.00	-1.0
Varuna (C)	46	110.8	4.2	41	1869	0.0	828.2	0.0

Conclusion

The yield potentials of NUDH-YJ-6 having low glucosinolate, high erucic acid and significantly higher oil content (46%) was comparable with Indian popular varieties, however, it gave 17% higher oil yield. Canola quality selections 1A/5-5-1-5 and 1A/5-23-29-18 were comparable in yield with national checks. Seed size improvement could not be achieved in this programme. Research efforts are being made to improve the seed size of these selections.

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Development of Improved Varieties of Rapeseed and Mustard Through *In Vivo* Mutagenesis and Hybridization in Pakistan

A S Syed* **& K Rahman**

Abstract

Rapeseed and mustard are the second most important source of vegetable oil in Pakistan. However, due to low productivity, these crops have been pushed to marginal lands, which resulted in a narrow genetic base. Mutation breeding research in conjunction with classical breeding techniques were therefore initiated at NIFA in 1989 to induce useful genetic variability in characters of economic importance in Oilseed Brassicas. The research efforts have resulted in the development of three varieties, namely Abasin-95 in 1996, NIFA-Raya in 2003 and Durr-e-NIFA in 2005. These varieties were approved by Seed Council of North West Frontier Province (NWFP) for commercial cultivation in the irrigated and rainfed areas of the province. Abasin-95 and NIFA-Raya are the first ever mutant varieties respectively of rapeseed and mustard in Pakistan. Durr-e-NIFA was developed from a hybridized population of a cross between 'Dunkeld' and 'Abasin-95'. All the three varieties possess high yield potential, medium-to-high oil content, early maturity and broader adaptability to rainfed and irrigated environments in comparison with the local check varieties and respective parents. These varieties are being cultivated by growers on appreciable areas.

This paper reports the developmental history and performance of these varieties.

Introduction

Pakistan has been facing edible oil shortage for the many years despite modest progress made in the development of agricultural sector. The total domestic requirement of edible oil during 2006-07 was 3.107 million tons, out of which 0.857 million tons was locally produced and rest (1.787 million tons) was imported to meet the short fall, which cost the national exchequer a huge sum of more than 1.3 billion US dollars during 2006-07 [1, 2]. The huge bill for imported edible oil can be reduced considerably by increasing domestic oilseed production. Rapeseed (*Brassica napus* L. and *Brassica campestris* L.) and mustard (*Brassica juncea* L.) are important oilseed crops in Pakistan and their share in total area and production of all oilseed crops grown in the country is over 31% and 28% respectively [1]. The area and production of canola (*Brassica napus* L.) has been increased by over 300% during 2000-01 to 2006-07 [2]. Brassica oilseeds are well entrenched in the cropping system of Pakistani growers, and hold promise in narrowing the gap between production and consumption if made competitive with other field crops [3]. The oilseed Brassica improvement programme at NIFA is therefore directed towards evolving high-yielding canola quality (low in erucic acid and glucosinolates) varieties of rapeseed and mustard, with high oil content and tolerance to biotic and abiotic stresses. This paper reports the development and release of two mutant varieties i.e. Abasin-95 of rapeseed and NIFA-Raya of mustard, and one hybrid variety, Durr-e-NIFA, of rapeseed for commercial cultivation in NWFP.

Nuclear Institute for Food and Agriculture (NIFA), P. O. Box-446, Peshawar-25000, Pakistan

E-mail: sanwarshah@nifa.org.pk

Materials and Methods

The procedure outlined in [4] was followed for induction of mutations and raising M_1 to M_3 generations. About 10,000-15,000 uniform seeds of canola cv. Tower and Canadian Canola type mustard line, DLJ-3, with about 8-10% moisture were irradiated at 1,000, 1,200, and 1,400 *Gy* gamma rays (^{60}Co gamma source) in 1988 and 1994-95 respectively. The treated seeds in both cases were planted in the field in isolation as M_1 generation. Selection for desirable mutants was carried out in M_2/M_3. RM-152-2 was selected in M_3 while MM-1266 was selected in M_2 from rapeseed and mustard mutagenized populations respectively. In case of hybridization, an Australian Canola type variety "Dunkeld" was crossed with mutant variety "Abasin-95" during 1995-96. A recombinant line "NH-97-1/5-1" was selected in F_2. After confirming genetic stability of these mutants and recombinant line "NH-97-1/5-1," they were thoroughly assessed for yield in different replicated trials at NIFA, Multi-location Adaptation Yield Trial (MLAYT) in NWFP and National Uniform Rapeseed/Mustard Yield Trials (NURYT, NUMYT) through out Pakistan, which were laid out according to Randomized Complete Block Design (RCBD) with four replications, having six rows, 5m long and 30 cm apart. The yield trials data were analyzed using computer software [5]. The seed quality of different genotypes for total oil content, fatty acid profile and total seed glucosinolates were analyzed by Near Infrared Reflectance Spectroscopy (NIRS) system at NIFA Oilseed Analytical Lab.

Results

Abasin-95

The M_5 mutants of cv. Tower were tested in Preliminary Yield Trial (PYT) during 1990-91 at NIFA and mutant RM-152-2 significantly out-yielded the parent (Tower) and a commercial cultivar (PR-7) by producing 1994 kg/ha grain yield. This mutant was tested in Advanced Yield Trials (AYT) during 1991-92. RM-152-2 again significantly out yielded the control cvs. and produced 2308 kg/ha grain yield against 1,632 kg/ha yield of control cvs. [**Table 1**]. RM-152-2 was assessed simultaneously in a MLAYT in NWFP and in a 30-entry NURYT for two consecutive years, i.e. 1992-93 and 1993-94.

In MLAYT, RM-152-2 significantly out-yielded the check variety in all locations in both the years, producing 2049 kg/ha grain yield (**Table 1**). The summary of yield and agronomic data of NURYT are presented in **Table 2**. This mutant line produced third highest grain yield of 1,605 kg/ha amongst 30 entries (candidate varieties) for two consecutive years in NURYT (average of 15 locations). RM-152-2 outclassed Pak cheen by 13.3% & DGL by 5.2% (two non-canola controls), and Shiralee and Westar (two canola controls) by 10.2% and 18.4% respectively. R M-152-2 matured significantly earlier than control cvs. ranging from six to 18 days, at different irrigated and rainfed sites in the national trials. These results clearly indicated genetic stability of RM-152-2 over years and locations. Results regarding oil content, erucic acid and glucosinolates [**Table 3**] indicated that RM-152-2 possessed 46% oil (range 43-47% at different sites) against the 42% of Tower (parent) and 43% of Pakcheen

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(control). It contains less than 3% erucic acid ($c_{22.1}$) and 25 micromoles total glucosinolates per gram of oil free meal, hence RM-152-2 falls in to the canola standard of Pakistan [6], which requires less than 5% erucic acid in oil and less than 40 micromoles of total glucosinolates per gram of oil-free meal. Based on its high yield potential, early maturity and its broader adaptability to diversified climates, RM-152-2 was approved by the NWFP Provincial Seed Council in December 1996 for irrigated and rainfed areas of NWFP under the name of Abasin-95 [7]. Abasin-95 was also approved and registered by the National Seed Council in 1997 as an improved mutant variety of rapeseed in Pakistan.

Table 1. Summary of yield and other characteristics of Abasin-95 and commercial cultivars in PYT, AYT AND MLAYT from 1991 to 1994

Cultivars	Maturity (days)	Plant height (cm)	Yield /ha (kg)
PYT			
RM-152-2 (Abasin-95)	175.5	179.3	1994
PR-7 (Control)	177.0	175.4	1523
Tower (Parent)	171.0	180.8	1516
AYT			
RM-152-2 (Abasin-95)	170.0	168.8	2308
PR-7 (Control)	179.5	169.9	1632
Tower (Parent)	173.4	189.7	1598
MLAYT, NWFP (average of 6 locations)			
RM-152-2 (Abasin-95)	-	-	2049
PR-7 (Control)	-	-	1542
Tower (Parent)			1600

Table 2. Summary of yield and other characteristics of Abasin-95 and commercial cvs. in NURYT, 1992-93 and 1993-94 (mean of 15 sites)

Cultivars	Maturity (days)*	Plant height (cm)*	1000 seed wt. (gm)*	Yield /ha (kg)	Yield increase of RM-152-2 over controls (%)
RM-152-2 (Abasin-95)	172.6 (164-184)	162.4 (150-180)	4.4 (3.7-5.1)	1605.1 (Potential 3.3 t)	
Pakcheen (Control)	175.9 (160-184.5)	156.3 (133-175.7)	3.8 (2.8-5.0)	1416.3 (Potential 2t)	13.3
DGL (Control)	177.0 (166-186)	151.6 (124.1-175.1)	4.1 (3.1-5.7)	1526.3 (Potential 2.50)	5.2
Shiralee (Control)	179.3 (168-188)	159.6 (134-197)	3.4 (2.9- 4.2)	1456.9 (Potential 2.4)	10.2
Westar (Control)	178.7 (158-187)	156.9 131-185)	3.6 (2.9-4.9)	1355.2 (Potential 2.2)	18.4

*Data recorded at different locations is given within parenthesis.

Table 3. Oil content, erucic acid and total glucosinolate of RM-152-2 (Abasin-95), Pakcheen and Tower (control)

Entry name	Oil content (%)*	Erucic acid (%)	Glucosinolates (µ mole/g)
RM-152-2 (Abasin-95)	46.0 (43-47)	02.98	25.0
Pak cheen (Control)	43.0 (41-44)	33.52	68.8
Tower (Parent)	42.3 (41-43)	10.31	41.9

*Ranges are given within parenthesis.

NIFA- Raya

The stable mutants of DLJ-3 were tested for yield and other agronomic traits in different yield trials. The summarized results of these trials are presented in **Table 4**. MM-1266 produced highest yield (1908 kg/ha) and significantly out-yielded the parent by a margin of 100% in PYT. The following year, MM-1266 was tested in AYT at NIFA. The line exhibited sustainable yield performance (2042 kg/ha) and produced 84% more yield than the control (parent). Based on yield performance of MM-1266 in PYT and AYT under irrigated conditions, it was tested in a 16-entry MLAYT in NWFP during 1999-2000. The pooled data over site indicated that MM-1266 produced over 1,458 kg/ha grain yield and significantly out-yielded the parent by a margin of 63%, which was used as check (in three irrigated and two rainfed environments). MM-1266 was assessed for adaptability in diversified environments through out Pakistan in NUMYT for two consecutive years. MM-1266 out yielded the non-canola check (BM-1) in six out of 14 sites in NUMYT, during 2000-01 and 2001-02. MM-1266 also out yielded Pb-10, the low erucic acid candidate variety, by a wide margin in all locations in these trials.

Initially seed of MM-1266 contained high oil content (range 44-47%) with 1.30% erucic acid and 22 micro moles of total glucosinolates per gram oil-free air dried solid [**Table 5**]. Thus MM-1266 is well within the canola standards of Pakistan [6] and its oil is fit for human use and meal for animal feed. MM-1266 is uniform, stable and morphologically distinct from the parent cultivar. The NWFP Provincial Seed Council approved MM-1266 on October 8, 2003 for sowing in irrigated and rainfed areas of NWFP under the name of NIFA-Raya [8].

Table 4. Summary of yield and other characteristics of MM-1266 (NIFA-Raya) and commercial cvs. in different yield trials, 1997-2002

Cultivars	Maturity (days)	Plant height (cm)	Yield /ha (kg)
PYT			
MM-1266 (NIFA-Raya)	101	201.2	1908
BM-1 (Control)	75	205	1280
DLJ-3 (Parent)	147	273.7	950
AYT			
MM-1266 (NIFA-Raya)	96.2	211.5	2041.8
BM-1 (Control)	-	-	-
DLJ-3 (Parent)	140.7	289.7	1104.5
MLAYT, NWFP (average of 5 locations)			
MM-1266 (NIFA-Raya)	-	-	1458.6
DLJ-3 (Parent/check)	-	-	892.3
NUMYT (2 Yrs. Average of 14 locations)			
MM-1266 (NIFA-Raya)	-	-	1518
BM-1 (Control)	-	-	1610
Pb-10 (canola line)	-	-	1238

Table 5. Oil content, erucic acid and total glucosinolate of MM-1266 (NIFA-Raya), BM-1 (control) and BP-10 (candidate variety)

Entry name	Oil content (%)	Erucic acid (%)	Glucosinolates (µ mole/g)
MM-1266 (NIFA-Raya)	44-47	1.3	22.0
BM-1 (Control)	40.8-42.0	49.7	104
Pb -10	42-44	0.9	41

Durr-e-NIFA

The stable recombinant line 'NH-97-1/5-1' was tested for yield and other agronomic traits in PYT during 1999-2000, AYT during 2000-01, MLAYT during 2001-02 and NURYT during 2002-03 and 2003-04 trials. The summarized results of these trials are presented in **Table 6**. NH-97-1/5-1 produced the highest yield (2168 kg/ha) and significantly out-yielded the parent/control in PYT. The following year, NH-97-1/5-1 repeated the excellent performance in AYT at NIFA and exhibited sustainable yield performance (2630 kg/ha) and produced over 25% more yield than the parent and 29% than check. Based on yield performance of

NH-97-1/5-1 in PYT and AYT under irrigated conditions, it was tested in a 16-entry MLAYT in NWFP during 2001-02. The pooled data over sites indicated that NH-97-1/5-1 produced 2,083 kg/ha yield and significantly out-yielded the parent and check varieties. The line was assessed for adaptability in diversified environments throughout Pakistan in NURYT for two consecutive years, 2002-03 and 2003-04. NH-97-1/5-1 produced 1,855 kg/ha grain yield and out yielded the check varieties (Shiralee and Dunkeld) on the basis of an average of 20 locations in both the years by a margin of 12.5% and 21.5% respectively. NH-97-1/5-1 contained oil content ranging from 39.6 to 42.9% with 2.4% erucic acid and 19 micromoles of total glusinolates per gram oil-free air-dried solid [**Table 7**]. Thus NH-97-1/5-1 is well within the canola standards of Pakistan [6]. Based on high yield potential and its wider adaptability to diversified climates, NWFP Provincial Seed Council on September 18, 2005 approved NH-97-1/5-1 as new improved variety under the name of Durr-e-NIFA for commercial cultivation in irrigated and rainfed areas of NWFP [9].

Table 6. Summary of yield and other characteristics of Durr-e-NIFA and commercial cvs. in different yield trials, 1999-2004.

Cultivars	50% flowering (days)	Plant height (cm)	Yield/ ha (kg)	Aphid[1] Attack	Alternaria blight[2]
PYT					
NH-97-1/5-1 (Durr-e-NIFA)	116.5	190.7	2168	2.0	MR
Abasin-95 (Control/Parent)	109.5	198.9	2067	3.0	MR
AYT					
NH-97-1/5-1 (Durr-e-NIFA)	99.0	182.5	2630	2.1	MR
BM-1 (Control)	80.8	210.0	2020	2.3	R-MR
Abasin-95 (Parent)	101.3	201.0	2100	2.9	MR
MLAYT, NWFP (average of 6 locations)					
NH-97-1/5-1 (Durr-e-NIFA)	-	-	2083	-	-
Abasin-95 (Parent)	-	-	1641	-	-
BM- I (Control)	-	-	1147	-	-
NURYT (2 Yrs. average of 20 locations)					
NH-97-1/5-1 (Durr-e-NIFA)	-	-	1855	-	-
Shiralee (Control)	-	-	1649	-	-
Dunkeld (Parent)	-	-	1527	-	-

[1] Aphid attack was recorded on the basis of aphid colony size (cm) on the main branch.
[2] R-Resistant, MR: Moderately Resistant

Table 7. Oil content, erucic acid and total glucosinolate contents of Durr-e-NIFA and control

Entry name	Oil content (%)	Erucic acid (%)	Glucosinolates (μ mole/g)
NH-97-1/5-1 (Durr-e-NIFA)	39.6 – 42.9	2.4	19.0
Shiralee (Control)	36.1 – 41.8	4.0	60.0

Discussion

Gamma-ray induced mutations have been instrumental in creating useful genetic variability in characters of economic importance in rapeseed and mustard cultivars, which led to the development of improved mutant varieties. The improvement made in some polygenically inherited characters through induced mutations, such as grain yield and seed oil content, might be due to genetic changes induced in certain other related but simply inherited characters like plant architecture, photoperiod response and seed coat, which could have positive effects on seed yield and oil content [10, 11, 12,]. The Canadian canola germplasm is photoperiod sensitive under Pakistani conditions as such germplasm is bred and adapted to a long photoperiod (usually about 17 hours daylight) and hence not adapted to the short day length of winter season (about nine hours daylight) in Pakistan. The Canadian canola cultivars therefore, have more vegetative growth period and very short reproductive phase due to which they grow very tall and produce less grain yield. The success of development of mutant variety 'Abasin-95' and 'NIFA-Raya' from mutagenized population of respectively Canadian variety 'Tower' and 'DLJ-3' through gamma irradiation was possible only by breaking the photoperiod sensitivity and a clear manifestation of Gamma-ray induced useful mutations. Other researchers [13, 14, 15, 16, 17] reported results that are in full agreement with our findings.

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M 127- A Promising Tomato Variety Developed Through Induced Mutation Technique

R Peiris*, T K Wickramasinghe & S P Indrasena

Abstract

Bacterial wilt (BW) is the most serious constraints for tomato cultivation in Sri Lanka. At present, the producers and consumers are more interested in yield and quality of produce. The objective of this study was to develop genotypes having BW resistance, high yield potential (>20 t/ha) with desirable fruit qualities. Application of induced mutations was practiced on the Manik variety. It is a well-adapted variety with BW resistance, large fruit size with low fruit weight (76 g) due to large empty locular cavities. Several beneficial mutants better than the parent variety were identified in the M_2 generation and confirmed in the M_4 generation. The five most promising mutants were evaluated for BW resistance, fruit quality and yield. During dry and wet seasons, the yield evaluation studies were conducted in research and farmers' fields. The mutant M 127 gave significantly higher yields (32.2 t/ha) than the check variety T 245 (21.7 t/ha) during the both seasons. Bacterial wilt screening in the field and laboratory demonstrated that M 127 was moderately resistant. The National Coordinated Varietal Trials confirmed that it was a promising mutant under different agro-ecological zones in both dry and wet seasons. Farm trials indicated that farmer acceptability was higher for the mutant than the check variety. The mutant M 127 possesses high fruit weight (158.6 g), red, slightly flattened firm fruits. It is highly acceptable for table purposes. In the near future the mutant M 127 will be officially released to farmers and at present, it is utilized as a donor parent in the development of new HF3 hybrid under the heterosis breeding programme.

Introduction

Tomato (*Lycopersicon esculentum* Mill) is one of the most widely cultivated vegetables in Sri Lanka. Bacterial wilt (BW) is the most devastating soil borne disease affecting the yield of the crop. Therefore, in the past much attention has been paid to the development of varieties that have BW resistance. However, at present the producers and consumers are more interested in yield and quality of the produce. Therefore, this study was initiated at Horticultural Crops Research and Development Institute from 1996-2005 with the objective of developing genotypes that have high yield potential (>20 t/ha) with desirable fruit quality characters. Application of induced mutations was practiced on the Manik variety, a well adapted variety, which has BW resistance and poor fruit quality characters such as irregular shape, low fruit weight (76 g) due to large empty locular cavities.

Materials and Methods

About 5,000 seeds of the Manik variety were irradiated with 320Gy Gamma-rays and seeds were sown in upland nursery beds to establish the M_1 population [1, 2]. The 14-day-old seedlings were transplanted in the field with the spacing of 80 cm between rows and 50 cm between plants. Recommended fertilizer levels of 50 kg N/ha, 150 kg P_2O_5/ha and 80 kg K_2O/ha were applied at appropriate time. Stacking of plants was done 20 days after transplanting. Weeding and irrigation were carried out as required. Three rows of control (untreated) seedlings were planted at both ends of each plot. Various preliminary observations from seedling to maturity were recorded on 100 randomly tagged plants of treated and untreated material. At maturity, fruits from first raceme and second raceme were harvested separately from each surviving M_1 plants to form the M_2 population. Fruit progenies of the M_2 generation consisted of 10,000-15,000 plants. Each fruit progeny had 21 plants. Three rows of original variety (control) were planted at both ends of each plot for screening. The M_2 populations were thoroughly screened for mutations. Mutants were selected on the basis of BW resistance, earliness yield/plant, fruit color, size and shape. Several beneficial mutants better than the parent variety Manik were identified. For the confirmation of mutants, the generations were advanced up to M_4. The 10 most promising mutants were evaluated in preliminary observational plots and five were selected for yield evaluation studies. The yield of the mutants was tested during dry and wet seasons in Randomized Complete Block Design with three replications with the check variety T 245.

The plot size was 2.4 cm×3.5 cm with 3 rows/plot and 21 plants/row. The field establishment method was similar to raising of the M_1 generation. Data on days to 50 % flowering, plant height at first harvest, plant type, reaction to BW disease, marketable fruit yield/plot and fruit quality characters such as fruit weight, fruit size, shape, color, brix, number of locules and % acidity as citric acid were recorded.

Bacterial wilt screening was conducted in the field and laboratory. In the laboratory the disease was recorded at three-day intervals from day after inoculation with an isolation of *Ralstonia solanacearum* using the following scale:

0	No symptom
1	One leaf wilt
2	Two or three wilted
3	All leaves wilted except top two or three
4	All leaves wilted
5	Plant death

The type of plant reaction was based on disease percentage as follows:

Highly resistant (0)	0%
Resistant (1)	1-25%
Moderately resistant (2)	26-50%
Moderately susceptible (3)	51-75%
Susceptible (4)	76-99%
Highly susceptible (5)	100 %

Horticultural Crops Research and Development Institute, Gannoruwa, Peradeniya, Sri Lanka

* Corresponding author. E-mail: peirisranjani@yahoo.com

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The mutant M 127 was tested in National Coordinated Varietal Trials (NCVT) in different agro-ecological zones. The standard yield evaluation procedure was carried out in NCVT. Finally it was tested in farmer fields during wet season (10 sites) and dry season (five sites) using T 245 as the check variety.

Results and Discussion

In the M_1 generation, seedling emergence, seedling survival at 14 days and at maturity decreased in treated population. Days of flowering delayed in treated population (**Table 1**).

Table 1. The performance of the M_1 generation population of tomato variety Manik

Parameter	Untreated	Treated
Emergence at 7 days	98.5	86.5
Seedlings survival at 14 days	86.0	62.0
Days of 50 % flowering	42.0	49.0
Plant survival at harvest	82.0	52.7

In the M_2 generation, several beneficial mutants better than the parent variety were observed. In the M_4 generation, 10 promising mutants were identified and five were selected for further evaluation. The BW screening data of the selected five mutants is given in **Table 2**.

Table 2. BW reaction of the selected mutants.

Variety / Mutant	Reaction 1	Reaction 2
M 110	MR	MR
M 121	MR	R
M 127	MR	R
M 120	MR	MR
M 65	R	MR
Manik	R	R
Marglobe*	S	S
KWR**	HR	HR

Reaction 1- In the laboratory
Reaction 2- In the field
* Susceptible check
** Resistant check

HR - Highly resistant
R - Resistant
MR - Moderately resistant
S - Susceptible

Yield evaluation of mutants carried out during the dry and wet seasons in the research fields clearly revealed that there were significant differences in yield among the mutants or varieties (**Table 3**). Out of the tested mutants, M 127 was found to be most promising giving an average yield of 32.2 t/ha.

Table 3. Yield performance of mutants and check variety T 245 in research trials

Mutant/Variety	Dry season	Wet season
M 110	26.2c	22.3c
M 120	27.8bc	21.5c
M 121	28.2b	26.9b
M 127	32.2a	28.2a
M 65	18.2 e	12.4 f
T 245	20.8 d	20.7 d
Manik	18.0 e	18.1 e
CV %	10.0	10.2

Means followed by the same letter are not significantly different at 5% level based on DMRT

Fruit quality characters of M 127. Manik and T 245 are presented in **Table 4**.

Table 4. Fruit quality characters of M 127, Manik and T 245.

Variety	Weight (g)	Color	Shape	Locules Number	Citric Brix Acid %	Size	Locule cavity	Mutant
M 127	158.0R	SF	5	1.1	4.5	B	F	
T 245 (check)	80.5	OR	SF	6	0.9	4.1	M	F
Manik (Parent)	50.2	ORYS	BL	7	4.0	0.4	B	E

R - Red
SF - slightly flattened
B - BigF - Filled
OR - Orange
BL - Blocky
M - Medium
E - Empty
ORYS - Orange red yellow shoulder

M 127 had slightly flattened, red, heavy fruits (158.0 g), whereas the parent variety, Manik had blocky shape, orange-red fruits with yellow shoulder and low fruit weight (50.2 g).

In the NCVT trials conducted for more than one season in different agro-ecological zones, clearly revealed that M 127 has a better yield potential than the check variety T 245 (**Table 5**).

Table 5. Yield (t/ha) of M 127 and T 245 in National Coordinated Varietal Trials

Agro-ecological zone				
Variety/ Mutant	Wet zone	Intermediate zone		Dry zone
	Mid country (a)	Low country (b)	Up country (c)	Low country (d)
M 127	32.8	25.8	30.3	12.7
T 245 (check)	20.8	19.8	20.1	10.2

(a) Gannoruwa — Average of 06 seasons
(b) Makandura — Average of 3 seasons
(c) Bandarawela — Average of 3 seasons
(d) Mahailukpallama — Average of 3 seasons

In all agro-ecological zones, the mutant gave higher yields than the check variety T 245 (**Table 5**). On-farm trials conducted in 15 sites indicated that farmer acceptance of M 127 mutant variety was higher than the check variety T 245. The farmers also reported fewer incidences of pest and diseases and good keeping quality in M 127 mutant variety.

Conclusion

M 127 is a promising mutant that has moderate resistance to BW, high yield potential (32.2 t/ha) and acceptable fruit quality characters such as high fruit weight (158.6 g), red, slightly flattened firm fruits with a long shelf life (14 days). In near future, M 127 will be officially released to farmers and at present it is utilized as a donor parent in a heterosis breeding programme.

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Induction of Dormancy in Spanish Groundnut Seeds (*Arachis hypogaea* L) Using Cobalt-60 Gamma Irradiation

N Benslimani[1,*] & L Khelifi[2]

Abstract

Irradiation has been used in several countries to create genetic variability in groundnut (*Arachis hypogaea* L). Several mutated lines were isolated. Our research aims are to induce genetic variability to make a selection of an improved local population of groundnut especially with regard to the dormancy characteristic of the seeds. In this context, dried seeds (14% moisture content) of four Spanish-type local groundnut populations were treated with the dose range of 50 to 450Gy in order to study their radiosensitivity at the laboratory level. The optimal irradiation doses were determined for two groundnut populations Berrihane (P1) and Tonga oust (P3). The measurement of field agronomic characters allowed us to choose a single population that was investigated during two generations. The obtained results have shown a significant effect of irradiation through statistical analyses. Concerning the seed dormancy tested on every M_2 plant, the obtained results demonstrated the existence of such a feature. However, one has to wait for the next generations in order to evaluate the evolution of the dormancy characteristics with respect to time.

Introduction

Radiomutagenesis is known to significantly improve the appearance frequency of induced changes. It can thus be used to widen the genetic variability to be integrated in many varietals selection and creation diagrams [1]. It enabled the improvement of many characters for different species: lupin, chickpea and groundnut [2]. Fundamental work regarding groundnut was carried out. It focused on various mutagen agent effects: X-rays [3]; [4] fast neutrons [5]; Gamma-rays [6]; [7] and chemical agents [8]. That work dealt with improvable characters (ramifications, morphology of the young shoots, maturity, seeds and pods morphology). Thirty-three cultivars were obtained worldwide. Twenty of them were developed in China. Nineteen varieties were created directly by a selection of mutant plants resulting from mutagen-treated seeds. The remaining 14 were obtained after a selection from crossings making use of mutant lines [2].

Two types of groundnut features are generally aimed at:

1. Characters related to the adaptation to the ecological and agro-technical conditions.
2. Characters related to the use of the obtained types (size, form, aspect, contents of oil and proteins, cellulose, organoleptic quality, resistance to seeds handling) [9].

The Virginia groundnut type exhibits a seed dormancy ranging from one to three months after harvest. This character is a significant agronomic advantage as it prevents premature field germination [10]. However, the Spanish type does not exhibit such dormancy. They constitute the targeted population of the present work which focuses on the Spanish type groundnut dormancy character. It consists of inducing gamma radiation changes on four Spanish type Algerian groundnut populations in order to produce mutant lines with dormant seeds [11]. These would enable farmers to obtain an adapted variety and to thus avoid considerable production losses.

Materials and Methods

The material is composed of four local non-dormant groundnut seeds (P_1: Berrihane, P_2: Boumalek, P_3: Tonga Ouest and P_4: El Frine), from the Algerian region of El-Kala (14% moisture content). They have been irradiated in a Co-60 unit at different doses namely: D_1 (50Gy), D_2 (100Gy), D_3 (150Gy), D_4 (200Gy), D_5 (300Gy) and D_6 (450Gy).

For the sake of studying radiosensitivity, a number of 25 seeds per dose and per population including the control are germinated in Petri dishes containing filter paper. They were afterwards soaked in water then placed in a controlled atmosphere room (temp: 22°C, humidity: 51 %, photoperiod: 16 h/day). The biological effects of the mutagen treatments were studied by surveying the percentage of stem seed germination and root's length.

Two populations and three gamma doses were retained for the field-work. Two hundred and twenty five seeds per dose and population were irradiated and sown at the National Institute for Agriculture experimental station. Each piece of land corresponded to a treatment (irradiation dose) per population. The elementary pieces were laid out in complete random blocks with five replications by population; a total of 10 blocks. Each block comprised the different doses. The seedlings of M_1 were surveyed during their whole development cycle. Every mature M_1 plant was collected together with the control. A sampling of five seedlings per dose and per population was carried out in order to investigate the main stem, root and secondary ramification lengths, ramifications' number, seedlings pods number, and weight of 100 pods. Ten mature pods were taken from each plant to survey the M_2 plants. The remainder were collected and mixed according to both irradiation dose and population to study the germination process dynamics.

The M_2 generation study was made on the Tonga Ouest M_1 plants population. For each block, the plant material was as follows: 169 M_1D_0, 161 M_1D_1, 120 M_1D_2, 110 M_1D_3 plants. Two pods for each M_1 plant including control seeds were selected and respectively numbered. The experimental device was a random block with two replications each of consisting of four basic plots for four different doses. Each dose contained various numbered plants from each seed collected for the M_1 plant. The methodology focused on the measurement of the branches, leaves, flowers, pods, seeds, roots, plant height and the germination test in every M_2 plant (single-seed descent).

Discussion

Seed radiosensitivity

Variance analysis revealed a significant population effect for the number of germinated seeds. The comparison between two populations made it possible to distinguish two homogeneous groups. Group A represents the average number of germinated seeds which is the most significant

[1] Algiers Nuclear Research Center, PO Box 399, Algiers, 16000 Algeria
[2] National Institute for Agriculture, El-Harrach, Algiers, Algeria

* Corresponding author. E-mail: benslimaninabila@yahoo.fr

with 24.1 and 23.4 as compared to group B which average germination number is 22.66 and 21.80 (knowing that the number of germinated seeds is 25 per population). The number of germinated seeds was neither affected by the irradiation dose nor by population-irradiation dose interaction. Consequently, the former is not a significant criterion for the useful irradiation doses choice. Hence, it becomes of interest to investigate other parameters (**Fig. 1**).

Average Stem Length Variance analysis does not show significant effect for this parameter. However, for all the studied populations the increase in the doses involves a significant stem length reduction, except for D_4 dose (200Gy). The two by two comparison of the average figures gives four homogeneous groups (**Figs. 2, 4, 5**).

Average Root Length: Variance analysis indicates a significant population effect for this parameter. This allows one to distinguish two homogeneous groups. Group A with the P_1 population represents the most raised root length and group B being the remainder of P_2, P_3 and P_4 populations. Similarly, the root length is inversely proportional to the irradiation doses. The dose effect is highly significant. The two by two comparison of the mean values makes it possible to distinguish five homogeneous groups (**Figs. 2, 3, 5**).

Irradiation dose effect on stem and root growth The slowing of stem and root growth due to increased irradiation doses was observed in different species. The criterion used by Guhardja to determine useful doses is the one inducing approximately a 30% reduction in the stems' length as compared to the control [12]. Konzak and Mikaelsen recommend a growth reduction ranging from 30% to 50% and advised the use of three irradiation doses (100, 200 and 300Gy) [13]. In the present case, a clear root length decrease is noted, which reaches respectively 46.5% and 59% for 450Gy. For the continuation field experiment 100, 200 and 300Gy doses are retained. This would involve about 30% of stems and roots length decrease (**Fig. 6**).

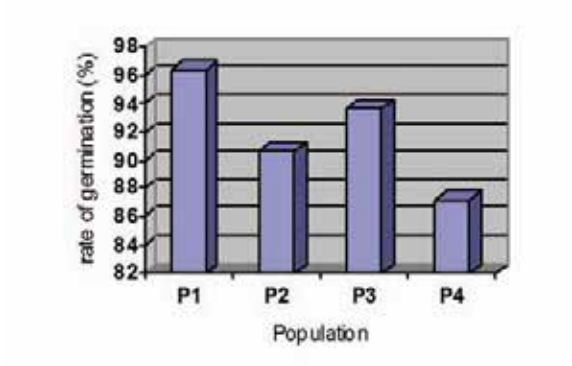

Figure 1 Population effect on seed germination (any dose)

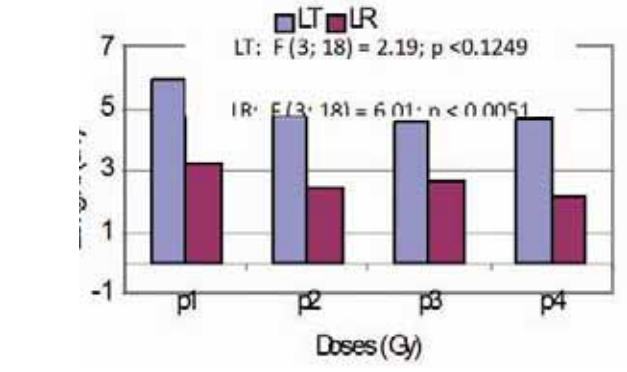

Figure 2 Population effect on the stem and root mean length (any dose)

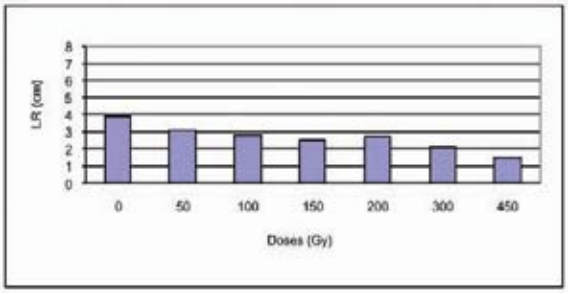

Figure 3 Irradiation doses effect on root mean length (mean of four populations).

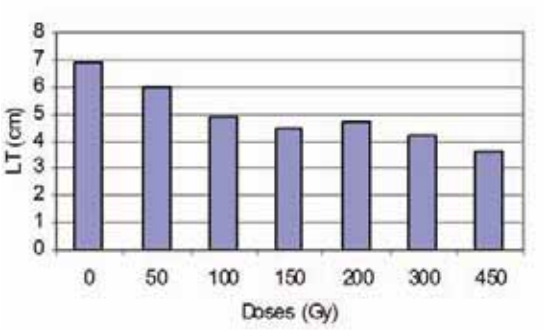

Figure 4 Irradiation effect on stem means length (mean of four populations)

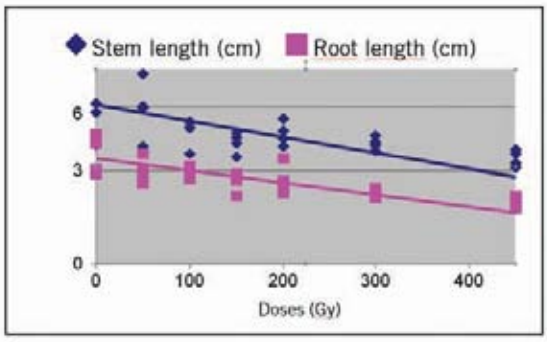

Figure 5 Irradiation effect on root and stem mean length (mean regression of four populations).

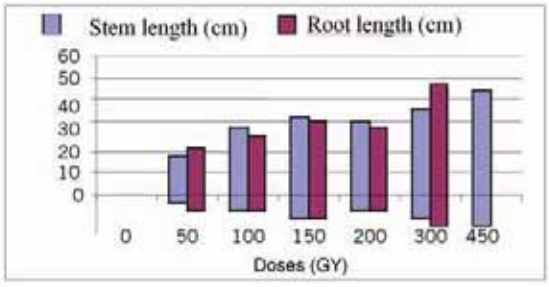

Figure 6 Doses effect on root and stem length reduction (mean of four populations).

M_1 and M_2 generations field survey

Statistical analysis for the M_1 characters (main stem length, primary branching number, main branching length, total pods weight, pod number**,** 15 pods weight, 15 pods seeds weight, 15 pods shell weight, shelling percentage) shows no significant effect from the doses. Therefore, the M_1 plants behave in the same way (**Table 1**). For M_2 characters as mentioned in the **Table 1**, variance analysis shows that the dose effect is significant to highly significant.

Table 1. Statistical analysis of the M_1 and M_2 characters.

Characters	M1		M2	
	F	Dose effect	F	Dose effect
Main steam length	0.55	NS	47.98	***
Primary branching number	1.03	NS	16.64	***
Main branching length	0.98	NS	117.38	***
Number of pods	0.95	NS	15.48	***
Total pods weight	0.90	NS	24.50	***
15 pods weight	1.56	NS	41.12	***
15 pods seeds weight	0.24	NS	49.97	***
15 pods shell weight	2.028	NS	7.26	***
Shelling percentage	0.22	NS	35.03	***
Biomass diameter	/	/	19.83	***
Two main branching diameter	/	/	3.53	**
Plant dry mater weight	/	/	10.77	***
Roots dry mater weight	/	/	2.64	***
Leaf surface	/	/	27.81	***
Total number of flowers	/	/	38.60	***
Total pegs number	/	/	17.12	***
Reproductive efficiency	/	/	22.13	***
Percentage of pegs fructification	/	/	3.22	*

NS: Non Significant, *: Significant, **: Highly significant , *** : Extremely significatn significant

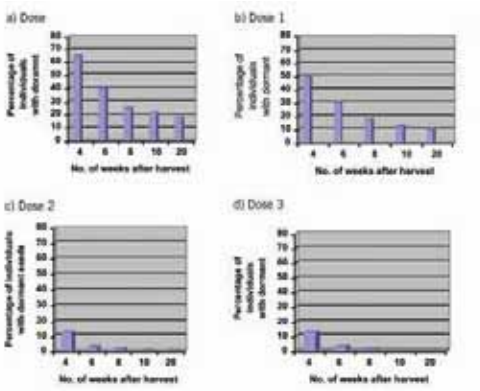

Figure 7 Percentage of plants with dormant seeds.

Dormancy study

The germination study of the M_1 plants control, D_1 and D_2 seeds shows that all seeds have germinated after 48 hours. For M_2 seeds germination testing (all M_2 plants) the main observation made by doses is as follows:

Dose 0: Four weeks after harvest about 65 % of individuals are dormant seeds. This rate falls after five months to reach 17%, which represents 22 individuals over 167 plants (**Fig. 7a**).

Dose 1: The same comments apply for the control at this dose, as 50% of individuals are dormant seeds four weeks after harvest. This rate rises to 15% after five months; which represents about 14 individuals with dormant seeds from 160 plants (**Fig. 7b**).

Dose 2: The decrease in the dormant seeds plants rate is rather drastic from 14% after four weeks to 0.5% after five months which corresponds to one dormant seed over 111 plants (**Fig. 7c**)

Dose 3: The same trend as for D2 has been noticed except that after 2.5 months no dormant seeds could be observed (**Fig. 7d**).

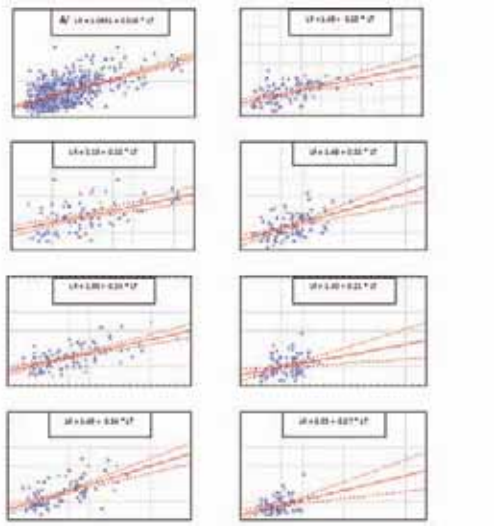

Figure 8 Stem length - root length relationship: A (all doses and populations), D_0 to D_6 (mean of the four populations per dose).

Discussion of results

Population P_1 (Berrihane) shows a root length slightly more significant compared to the remainder of the populations for all the irradiation doses. Berrihane and Tonga Ouest populations present the most significant germinated seeds average number (laboratory test) as compared to Boumalek and El Frine.

A highly significant positive correlation between stem and root length (**Fig. 8**) is noticed for the four populations and the seven doses. This type of correlation persists for the doses D_0, D_1, D_2, D_3 and D_4. Beyond the D_4 dose, there is loss in stem-root correlation (**Fig. 8A**). This was checked on the level of the stem and root growth reduction (spherical cloud near the origin (**Fig. 8-D6**). This reduction was already reported for other species [14]. It can be attributed to mitotic division's reduction in the meristematic apical cells [15]. In our case, the retained doses for field study seeds irradiation are: 100, 200 and 300Gy. This corresponds to the useful doses (200 – 300Gy) as suggested by Brunner to induce changes for same species [16]. This choice was also made on the basis of other studies which recommend a stem and root length reduction ranging from 30% to 50 %. For the first and second generation studies, the fundamental analysis of variance shows no significant effect of radiation doses at the M_1 level. The plants behave nearly in the same way for all doses. However, the M_2 variance analysis has helped identify a very high significant dose effect for each character study. This suggests the possibility of characters segregation for this generation. This enables one to say that the applied irradiation effects are real at the genetic level. The study of the seeds dynamic germination for the M_1 revealed an effective germination for all the seeds after 48 hours. Regarding the M_2 seeds, the obtained results show that D_2 and D_3 doses indicate significant amount of seeds germination during the first 30 days after harvest. After three months of conservation, the seeds almost do not show dormancy (appreciatively 100 % germination). Twenty-two lines of the D_0 and 14 lines of the D_1 were found to exhibit dormant seeds even five months after harvest. Consequently, the hypothesis of the presence of dormancy in local populations has to be considered. On the other hand, the effect of radiation doses should not be overlooked, since it's also the doses 200 and 300Gy which showed a complete seed germination. For the D_1, 14 lines remain dormant even five months after harvest which suggests that low radiation doses may be the cause of dormancy that is observed in

these lines. However, one must not lose sight of the fact that the lines are segregated and therefore must await future generations to draw the appropriate conclusions.

Conclusion

Regarding groundnut (*Arachis hypogaea. L*), very little progress was made through the conventional improvement methods because of a lack in the germplasm genetic variability of the species. The selection after radiation induced change offers a considerable source of genetic variation. This work enabled us to study seed radiosensitivity of four local Algerian groundnut populations of the non-dormant Spanish type. This experimentation has shown that the four groundnut seed populations are totally sensitive to the applied mutagen. For the M_1 seed dormancy, there is no variation on control and gamma irradiation seeds. In the M_2, the variation is important particularly for D_0 and D_1. Ultimately, all results require confirmation because only two generations were analyzed. Starting from the M_3, the most interesting plant characters will be identified and selected. The main agronomic traits will be evaluated on the selected plants in the fourth and fifth generations. The most interesting character will be confirmed in the sixth generation to obtain stable genotypes.

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Mutation Breeding for Rice Improvement in Tanzania

A Luzi-Kihupi[1,*], J A Zakayo[2], H Tusekelege[2], M Mkuya[3], N J M Kibanda[3], K J Khatib[4] & A Maerere[1]

Abstract

The mutation breeding programme based at Sokoine University of Agriculture (SUA), Morogoro, Tanzania aims at reducing plant height and maturation period of popular indigenous cultivars while maintaining some of the good qualities of the parents. Dry seeds of the indigenous popular cultivars were irradiated with 170, 210, 240 and 250Gy Gamma-rays from ^{60}Co at IAEA Seibersdorf Laboratories in Vienna in 1987, 1994 and 2001. The irradiated seeds and controls were sown at SUA. M_1 panicles were harvested, and planted as M_2 panicle-to-row progenies. M_2 plants were selected and advanced to M_3 and subsequent generations using pedigree selection method using plant height, early maturity and grain type as selection criteria. In another procedure, Single Seed Descent (SSD) method was used, whereby one seed was randomly selected from each M_2 plant to raise the M_3 generation. Apart from this, some improved mutants have been used in the cross breeding programme. The selected variants with improved plant type have been evaluated in multi-locational trials and on farmers' fields. Mutants that were selected using single seed descent were found to be very early in maturity and were resistant to rice yellow mottle virus (RYMV). After several years of multilocation and on-farm trials, SSD 35 was released in 2005 as a new variety under the name of Mwangaza. On the other hand, the improved mutants originating from cultivar 'Salama' also combined high yield potential and resistance to RYMV. Semi-dwarf Supa mutant, M-100 was backcrossed to 'Supa' variety and one high-yielding line selected from this cross has been recommended for cultivation in Zanzibar. Other lines originating from crosses between mutants and other varieties have been found to be resistant to rice yellow mottle virus and also combine high yield potential and acceptable grain quality.

Introduction

The importance of rice in Tanzania is increasing. Currently, it is a food crop in the diet of 60 percent of the people in Tanzania whose population is growing at a rapid rate (2.8%) resulting in a continuous increase in demand for rice and pressure to increase production [1]. Current average yield in the country estimated at 1.7 tons per hectare, however this is low compared to that of other countries like Korea and Japan where yields are above six tons per hectare [2]. One of the reasons for the low yield is that the farmers grow a number of traditional varieties that are tall and prone to lodging. Moreover, these varieties have long maturation period and are not suitable for areas with marginal rainfall pattern. The occurrence of rice blast and rice yellow mottle virus has also contributed to the declining yield of rice. In order to address the above constraints, rice breeders have been employing both conventional and non-conventional breeding methods. The first programme of mutation breeding was initiated in 1972 [3] with financial assistance from the International Atomic Energy Agency (IAEA). Faya Theresa and Kihogo Red varieties were used in the FAO/IAEA "Coordinated Mutation Breeding Programme for the Improvement of Grain Protein Content and Quality." Some mutants selected from this project combined high grain yield and high protein content [4].

The current mutation breeding based at Sokoine University of Agriculture (SUA) aims at reducing plant height and maturation period of the popular indigenous cultivars while maintaining some of the good qualities of the parents. This project was funded by the International Atomic energy Agency in the 1990s. Prior to 1999, the project was under coordinated project titled "Improvement of Basic Food Crops in Africa through Plant Breeding Including the use of Induced Mutation." The activities were later expanded under the Technical cooperation project titled "Improvement of Basic Food Crops of Tanzania Using Nuclear Techniques" [5,6].

Materials and Methods

The materials used in these studies were the local popular varieties. Afaa Mwanza 1/159, Supa India, Salama, Kaling'anaula, Ringa Nyeupe, Kihogo Red, Usiniguse and SSD35, have so far been subjected to irradiation at the Seibersdorf laboratories in Vienna. This paper presents the improvement of Supa India and Salama cultivars.

Supa and Salama varieties (rainfed lowland and upland varieties respectively) are widely grown cultivars in Tanzania. Supa has excellent cooking and eating qualities but its yield potential is low. It is too tall, photoperiod sensitive, has a long maturation period and is also susceptible to diseases such as rice blast and rice yellow mottle virus (RYMV). Salama, which was recommended for cultivation since the late 1970s, is high yielding but too tall and susceptible to rice blast. Dry seeds of Supa and Salama cultivars were sent to Vienna for irradiation in May of 1994. The materials were irradiated using 170, 210 and 240Gy Gamma-rays from ^{60}Co at IAEA Laborotory, Seibersdorf, in Vienna.

The seeds of the irradiated and non-irradiated control were sown at SUA farm immediately upon arrival. The M_1 panicles were harvested, and planted as M_2 panicle-to-row progenies. M_2 plants were selected using plant height, early maturity and grain type as selection criteria and harvested individually. About 80 plants were selected per treatment and advanced to M_3 and subsequent generations using pedigree selection method. In another procedure using the same M_2 plants, Single Seed Descent (SSD) method was used, whereby one seed was randomly selected from each M_2 plant to raise the M_3 generation. The M_4 plants were selected individually and M_4 progenies were planted as progeny rows. The selected variants with improved plant type were selected and evaluated at SUA, Dakawa and KATRIN in Morogoro region, Tanzania [5].

Use of Mutants in Cross Breeding

A number of mutant lines, viz. Afaa Mwanza mutants 4, 6, 12, Salama mutant M55 and Supa mutants M-100, SSD 1, SSD35 were hybridized

[1] Sokoine University of Agriculture, Department of Crop Science and Production, P.O. Box 3005, Morogoro, Tanzania,
[2] Dakawa Agro-Scientific Research Center, P.O. Box 1892, Morogoro,
[3] Agricultural Research Institute, KATRIN, Private Bag, Ifakara, Tanzania,
[4] Kizimbani Agric. Research Institute, P.O.Box 159, Zanzibar

* Corresponding author. E-mail: aluzikih@yahoo.com

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with different varieties such as Supa, Jefferson, PSBRC 50, IR 8 and Kihogo Red for further improvement.

Results and Discussion

The five mutants selected from irradiated 'Supa' variety using single seed descent were found to be very early maturing and resistant to rice yellow mottle virus. From the pooled data of the two sites and two seasons, all the characters except panicle weight and grain weight showed significant differences (**Table 1**). Rice breeders worldwide made good use of induced mutations to generate variation and produce some good varieties [7,8,9,10]. The mutants flowered earlier as compared to the parent. Short duration varieties (105-115 days) are excellent in marginal areas because they grow rapidly during the vegetative phase and are thus more competitive with weeds. They reduce weed control costs and utilize less water [11]. All the mutants except mutant SSD 35, significantly outyielded the parent. Mutant SSD 3, which yielded the highest, had a mean of 5296 kg/ha in the two locations. Mutants SSD1, SSD3, SSD5, SSD7 and SSD35 were also resistant to rice blast and rice yellow mottle virus. These improved mutant lines were also photoperiod insensitive. After several years of multi-location and on-farm trials, SSD 35 was released in 2005 as a new variety under the name of Mwangaza. From the results presented, it is clear that mutagenesis reduced the maturity period of the original cultivar up to 24 days. This is significant improvement on this trait. In China, Yua feng Zao variety, which matures 45 days earlier than the original variety IR 8, was developed through gamma irradiation. The new variety still has high yield potential [12].

The results of evaluation of other mutants originating from Supa and Salama varieties are shown in Table 2. The Supa mutants, though high yielding, are susceptible to blast and RYMV. However, the mutants originating from Salama cultivar combined high yield potential with resistance to RYMV (**Table 2**). These mutants are Salama M-19, Salama M-38, Salama M-55 and Salama M-57.

Mutants in cross breeding

In a cross breeding programme, a number of variants have been selected which combined high grain yield, good grain quality and resistance to rice yellow mottle virus (Table 3). Supa BC is a line, which was selected from a cross of a dwarf mutant of Supa (M-100) and the original Supa variety (Table 2). The line was accepted as a new variety in Zanzibar. It is still maintained on the Tanzanian mainland and shows good prospect to be released as a new variety in areas where RYMV is not a problem.

In the present study, gamma radiation was used to induce useful mutations resulting in the release of two new varieties. The improved mutants selected from the irradiated materials and the lines obtained

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

Genotype	Treatment	Days to 50% flowering	Plant Height (cm)	No. of Panicle/m2	Panicle length (cm)	Panicle weight (g)	1000 grain wt.(g.)	% filled grain/ panicle	Grain yield kg/ha	RYMV Score
SSD1	170Gy	71bc	118.3a	160.7	21.9c	4.3	32.9	82.2ab	4816b	1
SSD3	170Gy	72bc	127.0ab	167.2	23.9ab	4.6	32.5	81.2ab	5296a	3
SSD5	170Gy	72bc	120.0b	163.7	23.9ab	4.4	31.9	79.7b	4655b	3
SSD7	170Gy	72bc	121.1ab	168.3	23.7a	4.7	31.9	83.4ab	3799b	1
SSD35	170Gy	70c	120.0b	146.5	24.9bc	4.7	34.7	84.5a	2956c	1
Supa	Control	94a	122.1a	184.3	25.5	4.1	32.1	71.2c	2935d	7
Mean		75	120.6	165.1	23.5	4.4	32.7	80.4	4556	
Sx (+/-)		0.53	12.77	9.97	0.54	0.17	0.4	1.23	150	
CV(%)		1.73	5.63	14.8	5.62	3.1	3.1	3.76	8.46	

Table 2. Performance of *Supa & Salama* mutants grown at SUA, Morogoro (2002)

Plot no.	Description	Days to 50% FI	Plant height (cm)	Panicle length (cm)	Panicle/m²	1000 grain wt (g)	Panicle wt (g)	Yield (kg/ha)	Resistance to RYMV
1	Supa M-6-11	94 a	128 a	22.2 d	139 cd	36.9 ab	1.5 a	2,660 ab	Susceptible
2	Supa M-14-17	95 a	126 b	21.9 d	145 d	32.6.1 ab	2.1 f	2,700 ab	Susceptible
3	Supa M-22-17	92 ab	128 b	22.1 d	136 cd	35.5 ab	1.7 def	2,951 a	Susceptible
4	Supa M-70-10	90 abc	126 b	22.9 d	156 bc	33.7 ab	2.6 cd	3.152 a	Susceptible
5	Supa M-101-22	93 a	119 b	22.5 d	187 ab	36.0 ab	2.0 de	2,925a	Susceptible
6	Supa-M-26-19	93 ab	129 b	23.1 d	165 bc	34.7 ab	2.2 bc	3,082 a	Susceptible
7	Supa M-106	94 a	127 b	23.1 cd	172 bc	36.4 ab	2.0 cd	3,258 a	Susceptible
8	Supa BC	85 bc	90 c	24.7 b	187 ab	26.5 ab	1.0 cd	2,676 ab	Susceptible
9	Salama M-55	87 abc	125 b	22.5 d	194 ab	32.4 ab	2.6 cd	3,161 a	Resistant
10	Salama M-19	85 bc	144 a	26.4 a	160 bc	29.2 abc	3.2 ef	2,240 bc	Resistant
11	Salama M-30	87 abc	122 b	19.9 e	211 a	31.6 bcd	2.8 bc	1,845 c	Resistant
12	Salama M-57	83 c	132 ab	24.5 bc	110 de	35.8 de	2.7 b	2,156 bc	Resistant
13	Salama (control)	83 c	145 a	23.3 bcd	92 e	27.2 de	3.6 bc	1,965 c	Resistant
14	Supa (control)	94 a	125 b	23.0 cd	161 bc	36.9 e	2.1 bc	3,150 a	Susceptible
Mean		**89.6**	**126**	**23.6**	**158.3**	**33.5**	**2.3**	**2,709**	
Sx (+/-)			**12.64**	**1.52**	**34.6**	**4.01**	**0.83**	**530**	
CV		**4.71**	**5.90**	**3.94**	**13.02**	**7.14**	**21.25**	**14.58**	

Table 3. Grain quality characteristics of some RYMV resistant rice genotypes

S/n	Cross	Grain length (mm)	Size category	Length:bredth	Grain shape	Gelatiniza-tion Temp.	Amylose content (%)	Type
1	Mutant 12 /M-100/ Supa	6.825	Long	2.382	Medium	Low	24.87	Intermediate
3	Mutant 6 /Salama M-55	7.295	Long	2.906	Medium	Low	25.85	high
8	Mutant 12/PSBRC 50	6.4	Long	2.169	Medium	Low	26.12	high
9	Mutant 12/PSBRC 50	7.3	Long	2.684	Medium	High	22.38	Intermediate
10	Jefferson/SSD1	7.075	Long	2.67	Medium	Intermediate	23.27	Intermediate
11	Jefferson/SSD1	7.485	Long	2.752	Medium	High/Intermediate	22.65	Intermediate
23	Jefferson/SSD 35	7.665	Extra long	2.898	Medium	High/Intermediate	20.78	low
25	Jefferson/ SSD 35	7.605	Extra long	2.848	Medium	High/Intermediate	22.65	Intermediate
32	Jefferson/SSD 35	7.805	Extra long	2.864	Medium	Intermediate	22.3	Intermediate
49	IR 8/M-100/Supa	7.97	Extra long	2.903	Medium	High	24.43	Intermediate
52	IR 8/M-100/Supa	7.63	Extra long	2.852	Medium	High	21.76	Intermediate
59	IR 8/M-100/Supa	6.955	Long	2.322	Medium	High	22.47	Intermediate
68	M-100/Kihogo Red	6.705	Long	2.883	Medium	Intermediate	20.7	low
69	M-100/Kihogo Red	6.74	Long	2.774	Medium	Intermediate	18.01	low
70	Supa (Control)	7.585	Extra long	2.69	Medium	Intermediate	15.45	low
71	M100 (Control)	6.36	Medium	2.634	Medium	Low	11.45	low
72	Kihogo Red (Control)	6.545	Long	2.503	Medium	Low	20.96	low

from cross breeding will be a suitable source of germplasm in breeding and genetic studies.

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Use of Induced Mutations to Adopt Aromatic Rice to Low Country Conditions of Sri Lanka

R Pathirana[1,3], T Vitiyala[1] & N S Gunaratne[2]

Abstract

Two aromatic rice accessions, Au 27789 and IR Basmati were used in mutation breeding by subjecting 12,000 seeds of each variety to γ-ray doses of 200 or 300Gy from a ^{60}Co source. Based on agronomic characteristics, 635 M_2 plants were selected and grown as M_3 progenies. Sixty plants were selected from non-irradiated parental varieties using the same criteria, and tested along with mutant plant progenies. Both doses of γ-rays were effective in creating genetic variability for agronomic characteristics, with high heritability values when M_2 parent to M_3 progeny regression-based heritability was compared with selection in non-irradiated control varieties. Three mutant lines with compact plant type, erect and larger flag leaf, compact panicles and acceptable quality recording the highest yield were tested in five locations over four seasons using two recommended cultivars as controls. The mutant line 22/3 with a medium level of aroma recorded more than 2.5 t/ha, higher than the average yield of rice (1.5 -2 t/ha) in low-country wet zone. It has a compact panicle and narrow leaf angle allowing denser planting, which may help further increase the yield. The mutant lines maintained superior kernel length, linear elongation ratio and expansion index, all of which are important characteristics of aromatic long grain rice.

Introduction

Rice occupies 34% (870,000 ha) of the total area of cultivated land in Sri Lanka with 1.8 million families engaged in its cultivation. Sri Lanka currently produces 2.7 million tons of rough rice annually, which satisfies around 95% of the domestic requirement. Rice provides 45% total calorie and 40% total protein requirement of an average Sri Lankan (IRRI 2008). Despite the introduction of semi-dwarf, high-yielding cultivars, rice production in Sri Lanka has stagnated since the mid-1990s. Overproduction in some years has resulted in lower prices and a subsequent decrease in production. The introduction of high quality rice varieties with good export potential was seen as one way of stabilizing market prices by exporting the excess during years of overproduction. Here we report attempts to develop quality aromatic rice suitable for export, with the aims of increasing farmer income and making rice cultivation more profitable.

Materials and Methods

Two accessions, AU 27789 and IR Basmati, selected for good agronomic and quality traits from a collection of aromatic rice germplasm introduced from IRRI, were used in mutation breeding. Seeds (12,000) of each accession were treated with γ-ray doses of 200 or 300Gy from a ^{60}Co source. Based on agronomic characteristics, 635 M_2 plants were selected and grown as M_3 progenies. Sixty plants each were selected from non-irradiated parental varieties using the same criteria. Five random plants from the middle part of each row were used for recording agronomic characters. To compare variation generated by irradiation with natural variation in parent varieties, M_2 parent to M_3 progeny regression-based heritability values were computed. Six of the best mutant lines (28 ING and 39/1 -selected from Au 27789 irradiated with 200Gy Gamma-rays, 22/3, 3/51 and 3/48 – selected from IR Basmati irradiated with 200Gy Gamma-rays and 4/104 a mutant of IR Basmati irradiated with 300Gy Gamma-rays) were selected in the M_5 and tested in a field trial in a randomized complete block design over two seasons. Following these tests, three mutant lines were selected for multi-location testing at five experimental sites in the Matara District: Mapalana, Kotapola, Thihagoda, Komangoda and Gombaddala. Field experiments were conducted using a randomized complete block design with a minimum of three replications using recommended non-aromatic variety BG 379/2 and a new high-yielding non-aromatic cultivar RU 102 as controls. Agronomic and quality traits (Juliano and Perez 1984) including aroma (Bijral and Gupta 1998) of grains were assessed. Means from three samples per plot were used in the analysis of variance, performed separately and for combined seasons, allowing assessment of genotype x environment interaction.

Results and Discussion

Performance of aromatic rice accessions in the Matara District, Sri Lanka

Aromatic rice cultivars introduced from IRRI recorded very low yields. They were tall and susceptible to lodging. Most accessions did not have the agronomic characteristics of modern rice varieties, such as semi-dwarfism, an erect stem with narrow leaf angle, large and erect flag leaf, and compact and dense panicles (**Table 1**). Many accessions were susceptible to rice blast and did not produce any grain. Two accessions, IR Basmati and AU 27789, recorded acceptable yield and good quality and were therefore selected for the mutation-breeding programme (**Table 1**).

Heritability in mutant populations

Mutation breeding was carried out on two selected aromatic accessions, IR Basmati and AU 27789, to improve their adaptability and agronomic performance. Both doses of γ-rays used, 200Gy and 300Gy, created useful genetic variability. This resulted in higher heritability when M_2 parent to M_3 progeny regression-based heritability values were compared with those for selection in non-irradiated (0Gy) control varieties (**Figure 1**). In AU 27789, lower γ-ray dose seemed to induce more useful variation (**Figure 1**).

Field performance and adaptability of mutant lines

The three mutant lines with the highest yield in preliminary experiments (28 ING, 22/3 and 39/1; **Table 2**) were included in multi-location testing. They were more compact than the parent lines and had a larger and more erect flag leaf, compact panicles and acceptable quality (**Table 2**).

Genotype x season interaction for yield in the analysis of variance of data from multi-location testing was not significant. Therefore, the

[1] Department of Agricultural Biology
[2] Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Kamburupitiya, Sri Lanka
[3] New Zealand Institute for Plant & Food Research Limited, Private Bag 11600, Palmerston North, New Zealand

* Corresponding author. E-mail: PathiranaR@crop.cri.nz

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Table 1. Performance of aromatic rice accessions introduced from IRRI in Sri Lanka (mean of two seasons of testing at Mapalana Research Farm, Matara District)

Rice material	Leaf angle	Flag leaf angle	Leaf area* (cm^2)	Flag leaf area (cm^2)	Plant height (cm)	Panicle number	Panicle length (cm)	Grain Yield (kg ha^{-1})
Aromatic accessions								
Average (range)	43.5 (24-56)	34.6 (15-136)	17.6 (8-21)	15.6 (6-16)	112.5 (96-138)	10.3 (0-21)	21.3 (13-32)	763.7 (0-1323)
IR Bastmati	42.4	38.9	18.6	13.8	114.7	13.0	24.7	1187.4
AU27789	44.3	35.1	17.2	16.1	112.0	9.4	26.4	1213.9
Non-aromatic rice								
BG 379/2	15.2	8.3	24.8	18.4	77.3	6.3	27.2	2875
RU 102	13.8	8.2	27.5	22.3	100.4	4.4	29.0	3541

*Leaf immediately below the flag leaf

Table 2. Yield and growth characteristics of mutants in the preliminary trials (mean of two seasons testing)

Variety/Mutant line	Leaf angle	Flag leaf angle	Leaf area* (cm^2)	Flag leaf area (cm^2)	Plant height (cm)	Panicle number	Panicle length (cm)	Yield (kg/ha)	Aroma
28 ING	40.1^{a}	28.1^{b}	18.1^{c}	17.2^{b}	99.5^{ab}	8.9^{abc}	23.5^{cde}	1661.3^{de}	High
39/1	26.1^{b}	20.0^{c}	19.9^{bc}	18.4^{b}	82.8^{cd}	7.6^{bcd}	24.0^{cd}	1878.4^{d}	Low
22/3	18.8^{c}	14.0^{d}	19.5^{bc}	18.2^{b}	91.6^{bc}	7.3^{cd}	24.6^{bc}	2351.3^{c}	Medium
3/51	41.5^{a}	35.6^{a}	$18.4.^{c}$	17.6^{b}	88.0^{bc}	10.7^{a}	21.4^{e}	1497.3^{ef}	Low
3/48	44.0^{a}	30.8^{b}	19.4^{bc}	15.8^{b}	90.2^{bc}	10.0^{a}	22.2^{de}	1310.8^{f}	Low
4/104	41.9^{a}	27.9^{b}	17.1^{c}	17.5^{b}	96.9^{ab}	9.7^{ab}	24.0^{cd}	1538.0^{ef}	Low
IR Bastmati	42.3^{a}	39.1^{a}	$18.4.^{c}$	14.3^{c}	109.5^{a}	10.4^{a}	23.1^{cde}	1216.4^{g}	Medium
AU27789	45.2^{a}	34.6^{a}	$17.1.^{c}$	17.2^{b}	105.2^{a}	10.0^{a}	24.5^{bc}	1311.7^{fg}	Low
BG379/2	15.8^{cd}	8.56^{e}	22.5^{b}	17.6^{b}	75.9^{d}	5.6^{de}	26.5^{ab}	2928.7^{b}	None
RU102	13.2^{d}	7.9^{e}	28.7^{a}	22.0^{a}	105.3^{a}	4.7^{e}	28.5^{a}	3481.5^{a}	None
CV (%)	9.3	9.5	8.2	10.9	6.7	14.4	4.7	6.5	

Means with the same letter in a column are not significantly different at 0.05 level according to Duncan's New Multiple Range Test.
*Leaf immediately below the flag leaf

Table 3. Average performance of aromatic rice mutants at five locations in the Matara District, Sri Lanka (mean of four seasons)

Variety/ Mutant line	Leaf angle	Flag leaf angle	Leaf area* (cm^2)	Flag leaf area (cm^2)	Plant Height (cm)	Panicle number	Panicle Length (cm)	Yield (kg ha^{-1})	Aroma
28 ING	41.3^{a}	28.8^{a}	18.8^{c}	17.4^{b}	99.9^{a}	8.4^{a}	23.4^{d}	1915.0^{e}	High
39/1	25.7^{b}	20.2^{b}	19.0^{c}	18.1^{b}	83.0^{c}	7.6^{b}	23.7^{cd}	2121.9^{d}	Low
22/3	19.2^{c}	14.0^{c}	20.3^{c}	18.5^{b}	91.1^{b}	6.7^{c}	24.5^{c}	2575.8^{c}	Medium
BG379/2	15.6^{d}	8.7^{d}	22.6^{b}	18.1^{b}	75.9^{d}	5.3^{d}	26.3^{b}	3187.6^{b}	None
RU102	13.2^{e}	7.7^{d}	28.9^{a}	21.7^{a}	104.0^{a}	4.5^{d}	28.2^{a}	3687.6^{a}	None
CV %	10.7	9.5	10.6	9.6	6.3	15.1	5.0	7.1	

Means with the same letter are not significantly different at 0.05 level according to Duncan's New Multiple Range Test.
*Leaf immediately below the flag leaf
Quality of aromatic rice mutant lines

Table 4. Kernel characteristics of aromatic lines compared with standard varieties.

Variety	Kernel Length (mm)	Kernel Breadth (mm)	L/B Ratio	Length after cooking (mm)	Breadth after cooking (mm)	Linear Elongation Ratio	Breadth-wise Expansion Ratio	Expansion index
28 ING	7.93^{a}	1.77^{c}	4.49^{a}	10.73^{a}	2.00^{c}	1.39^{b}	1.12^{b}	1.2^{a}
39/1	8.12^{a}	1.98^{b}	4.10^{b}	10.94^{a}	2.21^{b}	1.35^{b}	1.12^{b}	1.21^{a}
22/3	7.01^{b}	1.72^{c}	4.08^{b}	10.04^{b}	1.97^{c}	1.48^{a}	1.13^{b}	1.25^{a}
BG 379/2	5.57^{c}	2.40^{a}	2.32^{c}	6.21^{c}	2.83^{a}	1.08^{c}	1.16^{a}	0.95^{b}
RU 102	4.16^{d}	2.47^{a}	1.69^{d}	4.50^{d}	2.89^{a}	1.06^{c}	1.18^{a}	0.93^{b}
CV%	4.70	4.18	5.89	4.39	4.06	3.03	1.79	6.28

Means with the same letter in a column are not significantly different at 0.05 level according to Duncan's New Multiple Range Test.

data of four seasons was combined for statistical analysis and the results are presented in **Figure 2** and **Table 3**. Separate and combined analysis of data from the five locations gave similar results and trends for all characters showing that these characters are expressed consistently over seasons and are highly genotype dependent.

Although the yields of mutant lines were significantly lower than the standard cultivars, the line 22/3 with a medium level of aroma recorded mean grain yield of more than 2.5 t ha^{-1} (**Table 3**), which is much higher than the average yield of rice in the Matara District, which fluctuates between 1.5 -2 t ha^{-1} (Weerasinghe and Lexa 1988; Pathirana and Chandrasiri 1991). It has narrow flag leaf and leaf angle allowing denser planting, which may help further increase the yield. Panicle is more compact than in the parent accession and in the other two mutant lines.

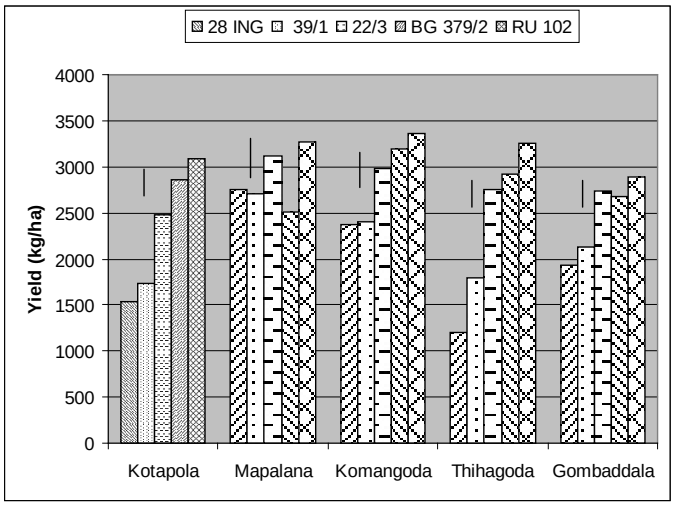

Figure 1 Heritability of agronomic characters based on M_2 parent to M_3 progeny regression compared with selection in non-irradiated plants in two aromatic accessions.

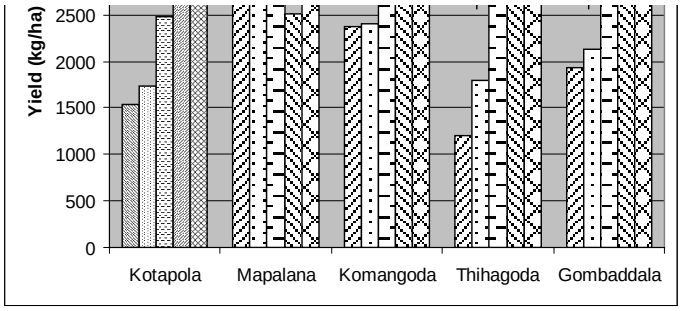

Figure 2 Yield performance of three mutant and standard varieties at five locations in the Matara District, Sri Lanka (mean of four seasons, bars represent LSD).

The mutant lines maintained the quality of parent lines, recording highest kernel length, linear elongation ratio and expansion index (Table 4), all of which are important characteristics of aromatic long grain rice (Sharma 2002). High milling returns and good cooking quality are often associated with aromatic or scented rice. Grain elongation at cooking is a special characteristic of several high grain quality varieties such as Basmati 370 and Nga Kywe. Such grades fetch approximately two times the price of average grade rice in international markets (FAO 2002). Mutant line 22/3 recorded a linear elongation ratio similar to Basmati rice from Pakistan (Sakila, *et al.*1999). Our surveys in Colombo, Matara and Galle revealed that high quality aromatic Basmati rice is almost three times the price of white rice produced from widely cultivated Sri Lankan varieties. Thus cultivation of these lines, 22/3 in particular, will be more profitable. Investigation and implementation of agronomic practices to optimize the yield and quality of new mutant lines will help increase the profitability of cultivation. Their further improvement may be possible through hybridization among mutants.

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Characterization of Pre-breeding Genetic Stocks of Urdbean (*Vigna mungo* L. Hepper) Induced Through Mutagenesis

V Kumar[1,*], A K Sharma[1], V P Singh[2] & M Kumar[1]

Abstract

Pre-breeding genetic stocks using different doses of EMS, Gamma-rays and combination of both (EMS and Gamma-rays) were induced in two urdbean cultivars viz., PU-19 (Pant Urd-19) and PU-30 (Pant Urd-30). Out of a total 14 of macro mutations selected from the different treatments of the mutagens in PU-19, narrow leaf mutant exhibited significantly a higher yield/plant as compared to the parent and some other mutants viz., non-hairy, tall, and tendriller showed at par grain yield. All the seed and pod color double mutations selected from the PU-30 showed significantly higher yield. Such breeding stocks can be used for the further genetic enhancement of this crop.

Introduction

Black gram (*Vigna mungo* L. Hepper), popularly known as urdbean, urid or mash is an important self-pollinating diploid grain legume and belongs to the family Leguminosae and subfamily Pepilionaceae. It is an important food legume crop of the Indian subcontinent. Genetic enhancement for yield, synchronization, and tolerance to major biotic and abiotic stresses is a major concern due to comparatively less genetic diversity in this crop. Since genetic variability is a prerequisite for any successful breeding programme, and the creation and management of such induced variability becomes a central base for the improvement of any crop species. Creation of genetic variability followed by screening and selection of the best genotype is a major target for this crop. Mutation is considered as one of the easy, rapid and effective tools of crop improvement. Spontaneous mutation cannot be expected to serve the cause of crop improvement effectively due to its very low i.e., 10-7-10-9. Induced mutations may be induced using treatment with certain physical (Gamma-rays) or chemical mutagens. Selection of macro mutation for different contrasting traits can be used as a variety or as a parent for the bringing of desirable traits into the otherwise well-adapted cultivars. Induction of the useful macro mutations for increasing genetic diversity and utilization of such trait-specific genetic stock for further crop improvement would certainly be useful.

Description of scenario selection procedures

Independent initiating event methodology

Four hundred healthy, pure, uniform and dry (9.5% moisture) seeds of two cultivars viz., PU-19 and PU-30 of urdbean were used for each treatment of Gamma-rays, EMS and a combination of both. Seeds were treated with ^{60}Co Gamma-rays (10, 20, 30 and 40 kR) at I.A.R.I., New Delhi. Pre-soaked seeds were treated with (0.2, 0.4, 0.6 and 0.8 %) of freshly prepared aqueous solution of EMS in phosphate buffer (pH 7.5) for 8 hours. For combination treatments, four hundred seeds for each treatment were treated with Gamma-rays first and then with 0.2% EMS solution. Treated seeds with EMS were thoroughly washed in running water for four hours to remove the residual effect of the chemical and then dried on blotting paper. Three hundred and fifty seeds of each treatment were sown in the M_1 generation at the experimental plot of Agricultural Research Farm, Banaras Hindu University, Varanasi during the summer of 2001. The M_2 generation was raised during *Kharif*, 2001 following plant to progeny row method. The M_1 seeds were space planted (35x15 cm) in the randomized block design with three replications to raise the M_2 generation during *Kharif*, 2001. The population was screened for macro-mutations.

The macro-mutants selected in the M_2 generation were advanced to the M_3 generation following plant to progeny during the summer of 2002, in a randomized block design with three replications to study their breeding behavior and performance nearly homozygous promising mutant lines of for yield and yield traits. The observations made on the normal looking plants, selected in the M_2 generation for micro-mutation were advanced to the M_3 generation. The experiment was conducted in randomized block design with three replications. The data on yield and yield traits was recorded as mentioned in the M_2 generation from 15 randomly selected plants from each replication. Soil of the experimental site was sandy loam to clay loam types with pH-7.9, 0.6 percent organic carbon of the field. The data obtained for different yield and yield attributing traits under study were subjected to statistical analysis as suggested by [2].

Confinement in the near field

The broad spectrum of macro or viable mutations was (plant with altered phenotype) identified in M_2 generations in both the cultivars. Out of the all the all the mutations with altered phenotypes 31 types of macro mutations were identified in PU-19 whereas, 35 types of macro mutations were identified in PU-30. The macro mutations isolated in the M_2 generation grew in the M_3 generation following the plant to progeny row for their characterization. Many true breeding mutants having distinct morphological feature (s) were isolated in the M_3 generation. Out of the total number of viable macro mutations identified in the M_2 generation, 13 true breeding mutant lines from each cultivar were selected and characterized in the M_3 generation. The main features and the treatment of mutagen against the mutants isolated from PU-19 and PU-30 are as follows and the mean performance of all mutant lines are presented in **Tables 1 and 2**, respectively.

True breeding mutant lines of cv. Pant Urd-19

Non-hairy: This mutant line was isolated from the combination treatment of EMS and Gamma-rays 30kR+0.2%, pods are non-hairy, high-yielding, moderately susceptible to the MYMV, but resistant to CLS.

Tall: Tall mutant is 10 cm taller (44.40 cm) in comparison to the parent, and was isolated from the 0.6% EMS treatment, has a higher number of pods and branches per plant, resistant to MYMV and CLS.

Tendriller: It was isolated from the combination treatment 20kR+0.2%, tall, high-yielding, higher pod length, resistant to the MYMV and CLS.

[1] Directorate of Research, Sardar Vallabh Bhai Patel University of Ag. & Tech. Meerut, India
[2] Department of Genetics and Plant Breeding, Institute of Agricultural Sciences,Banaras Hindu University, Varanasi-2210 05 (U.P.), India.

* Corresponding author. E-mail: vipinch1@yahoo.co.in

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Short pod: Short pod mutant was isolated from the 10kR treatment of Gamma-rays, medium height, small pod (3.68 cm), low-yielding and resistant to the MYMV and CLS.

Late: Late maturity as well as late flowering, isolated from 40kR treatment of Gamma-rays, fewer pods per plant, more branches per plant, low-yielding, higher 100-seed weight, moderately susceptible to the MYMV but resistant to CLS.

Oval leaf: Leaves were oval shaped, isolated from 20kR dose of Gamma-rays, dwarf, less number of pods and branches per plant, comparatively early in maturity (87 days), resistant to the MYMV and CLS.

Narrow leaf: Leaves were narrow and comparatively long, high number of pods and number of seeds per pod, identified from the 20kR+0.2% combination treatment, resistant to the MYMV and CLS.

Bunchy pod: The pods formed a bunch, isolated from the 0.4% treatment of EMS, larger number of pods per plant, dwarf, high 100-seed weight, less infestation of pod borer, resistant to the MYMV and CLS.

Small seeded: Seeds are small in size, low 100-seed weight, isolated from the 30kR treatment of Gamma-rays, low-yielding, comparatively more infestation of pod borer, resistant to the MYMV and CLS.

Flat seeded: Shape of the seed was flat, isolated from the 20kR+0.2% combination treatment, medium height, medium yielding, comparatively more infestation of pod borer, resistant to the MYMV and CLS.

Early: Matured in 81.45 days, in isolated form with 0.8% dose of EMS, low yielding, comparatively more infestation of pod borer, resistant to the MYMV and CLS.

Pigmented stem: Stem was violet to red in color before maturity and turned to black at maturity, isolated from the 30kR treatment of Gamma-rays, more number of seeds per plant, medium-yielding, comparatively less infestation of pod borer, resistant to the MYMV and CLS.

Long petiole: Length of petiole was more as compared to the parent, isolated from the 10kR+0.2% combination treatment, medium yielding, comparatively more infestation of pod borer, resistant to the MYMV and CLS.

True breeding mutant lines of cv. Pant Urd-30

Seed mutnat-1: This mutant was isolated from Gamma-rays treatment 30kR, early maturing as compared to the control, golden (shining) color seed with brown pod, high yielding, less infestation of pod borer resistant to CLS and MYMV.

Seed mutnat-2: Yellow (without shining) seed with brown pod, isolated from the 20kR+0.2% combination treatment, more number of pods per plant, high yielding, higher seed weight, resistant to CLS and MYMV.

Seed mutnat-3: Isolated from the 30kR+0.2% combination treatment, seeds were shining black color with brown pod, high yielding, resistant to CLS and MYMV, high 100 seed weight and more number of pods per plant.

Seed mutnat-4: Seeds were shining spotted (yellow with black) with brown pod, isolated from the 30kR+0.2% combination treatment, high yielding, resistant to CLS and MYMV.

Seed mutnat-5: Brown pods, black rough seeds, tall and medium yielding, resistant to CLS and MYMV, isolated from the combination treatment 20kR+0.2%.

Seed mutant-6: Black pods, golden (shining) seeds, dwarf and low yielding, resistant to CLS and MYMV, isolated from the Gamma-rays treatment 30kR.

Seed mutant-7: This mutant was isolated from the 6% treatment of EMS, black pods with spotted shining seeds, tall, and medium yielding, resistant to CLS and MYMV.

Crinkled leaf: Leaves were small and crinkled, isolated from the 0.6% treatment of EMS, medium yielding, comparatively more infestation of pod borer, resistant to the MYMV and CLS.

Bushy: Dwarf with higher number of branches per plant, isolated from the 40kR treatment of Gamma-rays, low yielding, more infestation of pod borer, resistant to the MYMV and CLS.

Bold seeded: Isolated from the combination treatment 20kR+0.2%, bold seed, higher seed weight (5.0g) and grain yield per plant, medium yielding, medium height, less infestation of pod borer, resistant to the MYMV and CLS.

Tall: Plant height was 6-7 cm more than the parent, isolated from the 30kR treatment of Gamma-rays, yield per plant was almost equal to the parent, less number of the pods per plant, less infestation of pod borer, resistant to the MYMV and CLS.

Early: This mutant line matured 16 days early in comparison to the parent, it was isolated from the 0.6% treatment of EMS, low yielding, medium height, less number of pods per plant, less number of the pods per plant, comparatively more infestation of pod borer, resistant to the MYMV and CLS.

Dwarf: Plant height was 14 cm less as compared to the parent, isolated from the 0.8% treatment of EMS, high yielding, high seed weight, less number of the pods per plant, less infestation of pod borer, resistant to the MYMV and CLS.

Data perusal from the **Table 1** revealed that out of the total 14 macro mutations selected from the different treatments of the mutagens in PU-19, narrow leaf mutant exhibited significantly higher grain yield per plant (6.24g), compared to the parent and some other mutants viz., non-hairy, tall, and tendriller showed at par grain yield. The late maturing mutant exhibited maximum plant height (48.70 cm) and maximum number of branches per plant (4.82). Almost all the mutants showed resistance to CLS and MYMV, except non-hairy and late mutants, which were moderately susceptible against MYMV. Although the late maturing mutant exhibited a much smaller number of pods per plant as compared to the parent (PU-19), this mutant can be used for forage and green manuring purposes. Early maturing mutants take only 81 days to mature, which is significantly less than the parent PU-19. Several workers have reported tall, dwarf, bushy and tendriller types of mutants (13, 11, 6). Narrow, oval, broad and crinkled types were also reported in different pulse crop following mutagenic treatments [5, 7]. Leaf abnormalities were attributed to the chromosomal breakage, disturbed auxin synthesis, disruption of mineral metabolism and accumulation of free amino acids [4].

Data presented in the **Table 2** revealed that macro mutations identified in PU-30, bunchy pod mutant exhibited less infestation of MYMV and pod borer compared to the parent. All seed and pod color mutations showed a significantly higher yield. All seed and pod color mutations have been submitted to the National Gene Bank, NBPGR, New Delhi for their registration and out of these Brown pod with Yellow Rough Seeded mutant has been registered [9]. The maximum number of pods per plant (35.32), was exhibited by the Seed mutant -4 followed by seed Mutant-3 (33.06). Such high-yielding with shining seed color mutations can be released as a variety [14]. Male sterility results in malformation of male reproductive organs caused by gene transformation of stamens into carpel-like organs. Chemical mutagens probably induce sterility due to increased sensitization of the embryo and seeds as a result of presoaking and decreased intrasomatic selection. Male sterility may be attributed to gene mutation or deletion [16]. The anthocynine pigmented mutants were isolated in the M_3 generation which had pinkish color stems and branches, but it turned black at maturity. Such a type of mutant has been reported by [10, 11, 15]. The macro mutations of seed and pod color were induced in the M_3 generation. Such seed mutations for different seed color were reported in pulse crops, for example, buff and black in arhar [1], yellow in soybean [16] and dull green to shining green [3] and golden-yellow [12] in mungbean. [12] suggested that golden yellow testa mutant was likely to involve a single gene but the simultaneous variations for yield and other morphological characters indicate a gross change or perhaps a very closely linked group of genes [8].

Figure 1 Normal pods with short and non-hairy pod mutants.

Figure 2 Normal with brown color and non-hairy bunchy pod mutants.

Table 1: Mean value for yield and traits of some induced mutant lines of Pant U 19 in the M_3 generation

Mutant line	Days to Flowering	Days to maturity	Plant height (cm)	No. Of branches plant^{-1}	No. pods plant^{-1}	Pod length (cm)	No. of seed pod^{-1}	100 seed weight	Grain yield plant^{-1}
Non-hairy	37.50	92.52	38.93	4.12	26.12	4.66	5.02	4.48*	5.54*
Tall	40.00	96.66	44.60*	4.70*	26.23	5.16*	5.32*	4.41	5.50
Tendriller	41.20	94.43	45.90*	4.52	25.30	5.43*	5.29*	4.24	5.42
Short Podded	37.24	90.97	36.73	3.27*	18.87*	3.68*	4.25	4.25	4.64
Late Maturing	48.56*	116.23*	48.70*	4.82	19.66*	4.41	5.04	4.40	5.27
Oval Leaf	37.75	87.50	28.10	3.43	19.66*	4.55	5.32*	4.23	5.35
Narrow Leaf	36.88	93.60	30.23	3.61	26.51	4.60	5.56*	4.79*	6.24*
Bunchy Podded	40.77	95.43	26.70*	3.45	30.75*	4.54	4.46	4.52*	5.36
Small Seeded	38.60	88.81	32.55	4.33	24.41	4.57	4.73	3.73	5.28
Flat Seeded	40.23	92.54	30.67	3.95	20.56	4.41	4.56	3.88	5.33
Early Maturating	34.56*	81.45	25.34	3.86	22.45	4.08	4.50	3.76	4.95
Pigmented Stem	37.22	90.37	30.23	3.67*	24.70	4.51	5.06	4.18	5.40
Long Petiole	41.45	91.33	28.45	4.30	20.54	3.76	4.11	3.79*	5.29
Parent (Pant U 19)	39.17	88.31	33.18	3.94	28.03	4.53	5.38	4.13	5.50
SEm±	1.04	1.45	1.11	0.12	0.67	0.065	0.340	0.078	0.231
LSD (5%)	2.19	3.05	2.33	0.26	1.41	0.137	0.714	0.164	0.485

*Indicates lowest and highest values

Table 2. Mean value for yield and traits of some induced mutant lines of Pant U 30 in the M3 generation.

Mutant line	Days to Flowering	Days to maturity	Plant height (cm)	No. Of branches plant^{-1}	No. pods plant^{-1}	Pod length (cm)	No. of seed pod^{-1}	100 seed weight	Grain yield plant^{-1}
Seed mutant-1	45.81*	88.67	36.74	3.45	35.31*	5.11*	5.13*	4.79	7.26*
Seed mutant-2	43.10	94.66	34.25	3.20	30.34*	4.50	5.00*	5.03*	6.958
Seed mutant-3	41.48	86.34	41.06*	4.00	33.06*	4.88*	5.20*	4.90*	7.45*
Seed mutant-4	40.23	85.33*	35.13*	3.44	35.32*	4.80	4.75	4.88	7.23*
Seed mutant-5	37.80	96.66	40.48	3.37	28.06	4.26	4.61	4.65	5.74
Seed mutant-6	36.55*	88.17	34.44	4.04*	26.90	3.70	4.87	3.76*	4.50
Seed mutant-7	43.43	90.30	34.35	3.74	27.97	3.70	4.34	4.63	5.32
Crinkled Leaf	40.57	93.43	35.658	3.46	20.32	3.57	4.41	3.80	4.58
Bushy	43.70	99.65	33.53	4.54*	23.65	3.86	4.36	3.86	4.41
Bold Seeded	44.85	95.77	35.41	3.28	18.76*	3.58	4.25	5.00*	4.95
Tall	40.33	97.67	44.54*	3.43	20.43	4.12	4.42	4.25	4.81
Dwarf	37.09	87.79	24.52*	4.00	25.32*	4.34	4.78	4.50	6.44*
Early Maturating	35.69	83.80*	30.43	3.56	22.87	4.00	4.33	4.13	4.23
Parent (Pant U 30)	42.79	92.07	37.49	4.15	26.05	3.68	4.74	3.87	4.88
SEm+	1.04	0.89	0.99	0.23	0.65	0.32	0.09	0.13	0.19
LSD (5%)	2.19	1.87	2.08	0.48	1.37	0.68	0.18	0.27	0.40

*Indicates lowest and highest values

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Development of High Yielding, Late Maturing Kenaf (*Hibiscus cannabinus*) Using Gamma Irradiation

M O Balogun*, S R Akande, J A Raji, B A Ogunbodede, G O Agbaje & O O Adeyeye

Abstract

Two mutants of Kenaf (*Hibiscus cannabinus*) derived from gamma irradiation of Cuba 108 (Mutant 1) and Tainung-1 (Mutant 2), their parents and two landraces were evaluated for maturity period and fiber yield traits in the forest, derived and southern Guinea savannah agro-ecologies of southwest Nigeria. Mutant 2 was consistently late maturing across locations with an average of 80 days compared to 71 in the parent. Fiber yield and maturity period were highest in forest and lowest in Southern Guinea savannah agro-ecologies due to photoperiod and rainfall differences. Mutant 2 was most stable, and had the highest mean value of 26,158kg/ha for fiber yield followed by 17,611kg/ha in Mutant 1. Mutant 2 is suspected to be photo-insensitive and recommended for equatorial climates.

Introduction

Kenaf is an important fiber plant that alleviates global warming by absorbing carbon dioxide gases due to its rapid growth rate. However, Africa produces only 2.91% of the global production [1]. This is due in part, to photosensitivity of most of the varieties.

Photosensitive varieties of Kenaf initiate flowering when day length reduces to 12.5 hours, and are suited for countries above the tropics. In contrast, these cultivars flower very early at latitude 0^0 to 10^0 N or S, where day length is more uniform from June to September, causing a reduction in vegetative growth and low fiber yields. Photo-insensitive cultivars are therefore preferred since they flower late, or when they flower early their vegetative growth is not significantly reduced [2]. To develop varieties that are adapted to Nigerian agro-ecologies, induced mutagenesis was used to create genetic variability for maturity period and fiber yield in Kenaf.

Methods

Dry seeds of two varieties of Kenaf were exposed to $Cobalt^{60}$ source of Gamma-ray at doses of 200 and 400Gy and the M_2 population was screened for mutants in terms of maturity period and fiber yield. Cuba 108 was irradiated with 200Gy (Mutant 1) and Tainung-1 irradiated with 400Gy (Mutant 2) were mostly high yielding and late maturing, respectively, and therefore selected. The selections were planted up to the M_5 generation when they became stable. To formulate recommendations for areas of optimal cultivar adaptation [3, 4], the selections were planted in multi-locational trials alongside the parents and two local varieties at Ikenne, Ilora and Ballah corresponding to forest, derived and southern Guinea savannah agro-ecologies respectively in southwest Nigeria.

Results and Discussion

Mutant 2 was consistently late maturing in the three locations (**Fig. 1**) compared to other genotypes, except for Mutant 1 at Ikenne, which matured two days later. Maturity periods varied with location in other genotypes. The average maturity period for Mutant 1 was 80 days, compared to 71 in the parent.

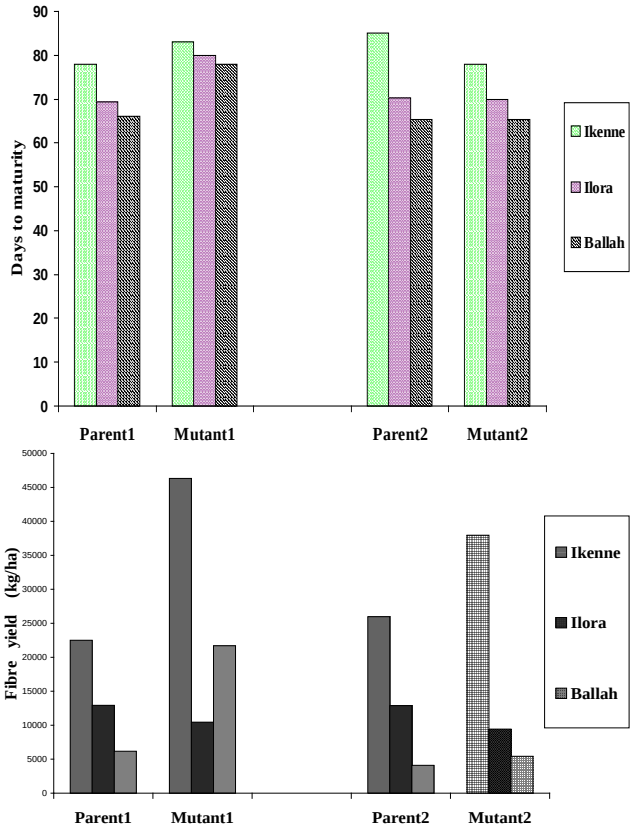

Figure 1 Maturity period and fiber yield in two mutants of Kenaf and their parents in three Nigerian agro-ecologies.

Although at flowering, Mutant 2 plants were taller than other genotypes in Ikenne (**Table 1**), they were shorter than other genotypes in Ilora and Ballah despite longer days to maturity at these locations. Mutant 2 had the highest percentage gain in height after flowering, 293.08% compared to 90.13% in the parent line (**Table 1**).

The highest fiber yield was recorded in Ikenne across genotypes (**Table 1**). At Ikenne, both mutants had higher fiber yields than other genotypes. Mutant 2 had the highest value of 46,321kg/ha (Figure 1), followed by Mutant 1 with 37,966.5kg/ha. These were in comparison with 22,498.7kg/ha in Tainiung-1 (Mutant 2 parent) and 25,978.3kg/ha in Cuba 108 (Mutant 1 parent). One of the landraces (Local 35) had the lowest fiber yield of 17,427.67kg/ha.

Obafemi Awolowo University, Institute of Agricultural Research and Training, Moor Plantation, P.M.B. 5029, Ibadan, Nigeria

* Corresponding author. E-mail: kemtoy2003@yahoo.com.

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Table 1. Mean vegetative growth traits and fiber yield of Kenaf genotypes grown at forest, derived from southern Guinea savannah agro-ecologies of Nigeria

Genotypes/Trait	Agro-ecologies			Mean (Genotypes)
	Ikenne	Ilora	Ballah	
Height at flowering (cm)				
Mutant 1	182.92b	152.67a	77.00a	137.53A
8B	168.17c	148.00a	69.17ab	128.44BC
Tainung-1 (Parent 2)	145.67d	154.67a	73.00a	124.44CD
Cuba 108 (Parent 1)	147.92d	142.00a	72.00ab	120.64DE
Local35	146.83d	143.67a	59.27b	116.59E
Mutant 2	239.92a	123.33b	40.33c	134.53AB
Mean (Agro-ecologies)	171.90A	144.06B	65.13C	
Error mean square	63.77			
Gain in Height after flowering (%)				
Mutant 1	9.55a	27.46a	74.54b	37.18B
8B	10.12a	24.29a	69.92b	34.77B
Tainung-1 (Parent 2)	13.63a	13.97a	90.13b	39.24B
Cuba 108 (Parent 1)	12.69a	37.51a	62.63b	37.61B
Local35	19.47a	14.92a	103.42b	45.94B
Mutant 2	4.82a	33.12a	293.08a	110.34A
Mean (Agro-ecologies)	11.71C	25.21B	115.62A	
Error mean square 779.56				

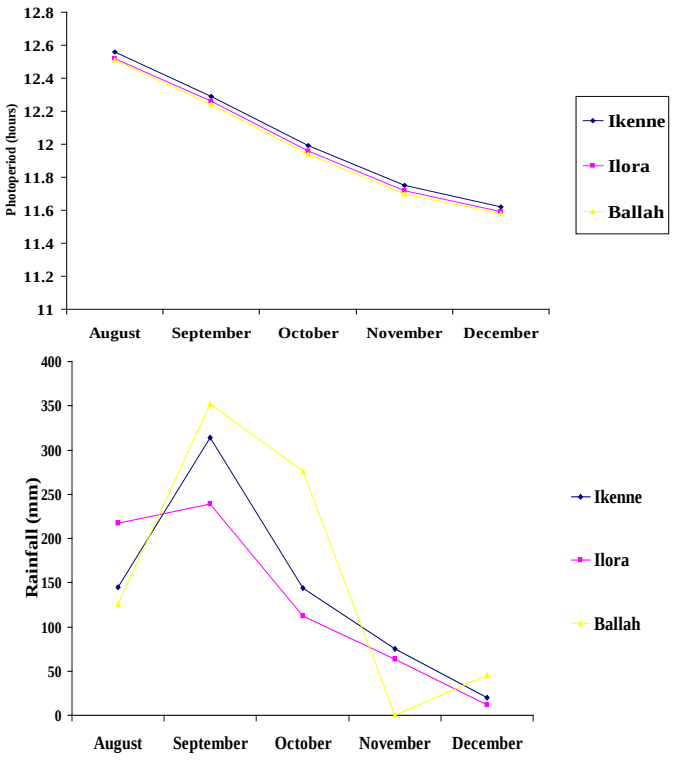

Figure 2 Rainfall distribution and rainfall patterns at the three agro-ecologies during the growing season.

Mean in each column followed by the same lower case letters are not significantly different at p=0.05. Genotype and location means followed by the same upper case letters are not significantly different at p=0.05.

Highest fiber yields were recorded in Ikenne for the Kenaf genotypes evaluated in this study. Kenaf fiber yields have been reported to be highest in regions with long growing seasons and abundant moisture [5], which are characteristic of Ikenne.

Also, Kenaf is a short-day annual that remains vegetative until the number of daylight hours fall below 12.5 hours, when flowering occurs [2, 6]. Ballah, Ilora and Ikenne are located on 13, 7 and 6 degrees latitude respectively, and this is associated with an increasingly wetter climate and later shortening of day length (**Fig. 2**). The growing season is longest at Ikenne and shortest at Ballah.

Low fiber yield was recorded in Mutant 2 at Ballah and Ilora as it was yet to reach its maximum vegetative growth when it flowered. This was as a result of water stress due to no rainfall in November at Ballah (**Fig. 2**). Vegetative growth was therefore retarded, but resumed with sparse rain in December, causing its high percentage gain in height after flowering at this location. Also, day length decreased soon after planting at Ballah and Ilora, making the plants (especially the relatively photosensitive genotypes) flower earlier to produce seeds before the dry season. The initiation of flowering is associated with reduced vegetative growth, and in turn, low fiber yield. Planting should therefore be done earliest in Ballah, followed by Ilora and Ikenne to allow for ample vegetative growth and high fiber yields before cessation of rains.

Conclusion

The consistently late maturing, high yielding Mutant 2 is recommended for the three locations, while Mutant 1 will also yield well at Ikenne. Fiber yield will be optimum when planted early in the growing season. Induced mutagenesis was successfully used to develop high-yielding genotypes of Kenaf adapted for specific climates (**Table 2**). This could be a very useful tool for creating genetic diversity that will cope with global climate change in a sustainable way.

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