



Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf

# Mutation Breeding Newsletter & Reviews

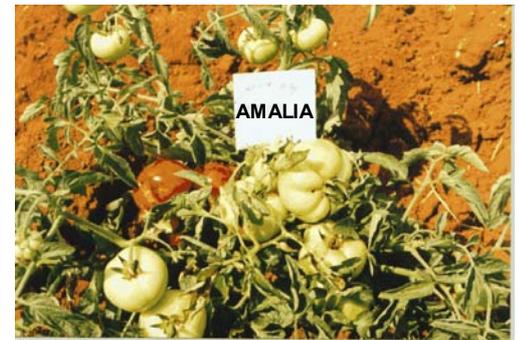
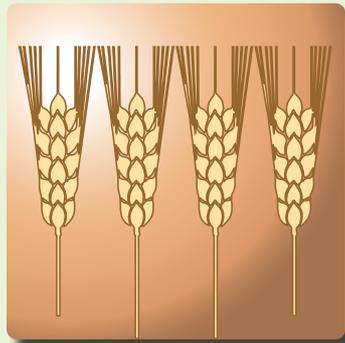
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*A mutant line R15 (left) developed using  $\gamma$  rays from variety AMA (right) showed enhanced drought tolerance (Instituto Nacional de Ciencias Agrícolas, La Habana, Cuba) – Details on page 6*

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## To Our Readers

Since the first issue of Mutation Breeding Newsletter (MBNL) was born in May 1972, and her sister Mutation Breeding Review (MBR) ten years later, there were 46 issues of MBNL and 13 MBR published, both MBNL and MBR, being the only specialised publications on mutation breeding worldwide. Our contributors and readers have enthusiastically supported them. During the past half century, mutation induction has matured from a focal research area to sophisticated technologies in modern plant improvement. Doubtlessly, these two publications played unique and important roles in fostering the development and application of mutation techniques in plant research, germplasm innovation, and new variety development. We are indebted to our predecessors at Plant Breeding and Genetics Section, and especially to Dr Alexander Micke, to have born and raised these publications and fostered their spread.

In 1998, a new newsletter, Plant Breeding and Genetic Newsletter (PBGN), became a regular bulletin, covering all activities in our Section. Therefore, a major function of the MBNL was largely replaced by PBGN. On the other hand, the FAO/IAEA Mutant Variety Database (<http://www-mvd.iaea.org/MVD/default.htm>) has also taken over some functions of these two publications. Because of this and other reasons, we are facing a dwindling number of suitable submissions from outside for these two periodicals, and MBNL and MBR have become irregular publications since 2001. Even though, we, as well as many of you, contributors and readers, still believe that these two publications have reason to exist, but to keep them alive, significant evolution is inevitable.

During the recent decade, mutation techniques are no longer used only as a tool for crop improvement of traditional traits, e.g. yield, resistance to disease and pests, but more frequently for diversified uses of crop end-products, enhancing quality and nutritional values and tolerances to abiotic stresses. Thanks to the massive progress in research of plant molecular biology and biotechnology, particularly plant genomics, we are witnessing new impulses in plant mutation research, from fundamental studies on mutagenesis to reverse genetics. Breeders are now able to use mutation techniques with more sophistication and efficiency than ever before dreamed possible.

With all these on-going developments, we are confident that MBNL and MNR should and could evolve, step by step, towards a periodical of higher scientific value, possibly as an international journal on mutation research and application in plants. We already have some tentative names for this journal such as “Plant Mutation Digest”, or “Plant Mutation Reports”. We also look forward to your constructive proposals on this. We fused the MBNL and MBR in this issue into Mutation Breeding Newsletter and Reviews (MBN&R), and will try to publish it for a couple of years under this format, before we attempt a final upgrade.

Your continuing contributions are highly appreciated and positively encouraged.

*Qingyao Shu*

*Pierre J.L. Lagoda*

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# Induced Mutation Variability in *Linum grandiflorum* Desp.

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*Linum grandiflorum* is a popular crop on the European market of ornamental plants. It is commonly used as a flower-bed crop. *L. grandiflorum* is known for its limited genetic variability. Up to now its colour spectrum remains poor and mutations of flower shape, which are not of small importance for any ornamental crop advancement on the consumer market, are unknown. It was supposed in this connection, that the method of chemical mutagenesis had to broaden the mutation spectrum and to increase the frequency of useful mutations in comparison with natural variability.

Seeds of annual wild species of *L. grandiflorum* with a large ruby corolla and a dark red spot at the centre of the flower (var. *rubrum*) were treated with 0.01, 0.05, 0.1 and 0.5% of ethyl methanesulphonate (EMS) for 18 hours. After treatment the seeds were washed to remove the mutagen. Seeds of every M<sub>1</sub> plant were sown separately in individual plant-to-progeny rows. In the M<sub>2</sub> generation the majority of visually selected mutants were selfed and advanced from M<sub>2</sub> to M<sub>3</sub>, where every mutant line was raised along with the parental genotype.

The results of screening M<sub>2</sub> segregating material indicated that *L. grandiflorum* was characterized by a high frequency of morphological mutations. Six types of mutants, namely chlorophyll deficiency, leaf waxy bloom, corolla colour, flower shape and size, and plant habit were selected from the M<sub>2</sub> generation (Table). EMS concentrations of 0.05 and 0.1% were the most optimal to induce the greatest number of mutants, taking into account the high survival rate of plants in M<sub>1</sub>. Half of the mutants were found with modified chlorophyll content and one-third had a new habit. Under 0.05 and 0.1% EMS treatments only super-dwarf plants and plants with the modified corolla colour were found.

The spectrum of chlorophyll mutants isolated in the present study comprised of *albina*, *viridis* and *xantha* mutations. The variant super-dwarf “*sd*” possessed a phenotype of small (several centimetres in height only), half-spheric bush. Chlorophyll-deficient mutations were often observed among this variant. All the selected “*sd*” plants were not viable. Compared with the wild type plants, the dwarf mutants (“*d*”) had shorter internodes, leaves were also shortened and thickened petals were of darker colour.

Mutations of corolla colour and corolla shape were of the greatest interest with a view to obtain new ornamental varieties. The unique variant “*apr*” possessed a phenotype with apricot petal colour and a mahogany spot at the center of the flower. The mutation of corolla shape was represented by the star-shaped flower (“star” mutant) [1]. Both these mutants had a high viability.

The mutant with an apricot coloured corolla successfully passed variety testing and under the name of “Aurora” was included in the State Register of Plant Varieties in the Ukraine for 2002. Another mutant with a star-shaped flower is now under State Variety Testing.

Type of mutation	Treatment – EMS (%)								Control
	0.1		0.5		0.1		0.5		
	No. of mutant families	%							
Chlorophyll deficiency	0	0	21	33.9	17	25.0	0	0	0
Leaf waxy bloom	1	1.5	1	1.6	1	1.5	0	0	0
Corolla colour	0	0	3	4.8	1	1.5	0	0	0
Corolla shape	0	0	1	1.6	0	0	1	25.0	0
Flower size	2	2.9	4	6.6	5	7.3	0	0	0
Plant habitus: dwarf	1	1.5	5	8.1	4	5.9	0	0	0
Superdwarf	0	0	4	6.5	1	1.5	0	0	0
Low-growing	3	4.5	4	6.5	6	8.8	0	0	0
TOTAL	7	10.3	43	69.5	35	51.5	1	25.0	0

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Lagron, V., Lyakh, V. 2002. Mutation variability in  $M_2$  in *Linum grandiflorum* Desf. Under influence of ethylmethane-sulfonate. Mutation spectrum. Visnyk Zaporizhzhia State University. 34(1): 124-129.

# Heritable Variability Induced in $M_2$ and $M_3$ with Gamma-Rays of Oil Flax

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Oil flax is an important oilseed crop, of which oil is widely used not only for industrial purposes but human nutrition as well. It is well known that both chemical and physical mutagens could induce various mutants with a large spectrum. However, information on the difference of sensitivity to gamma irradiation between parent and mutant is still very limited. This article addressed this issue by using one parent and its two mutant lines of oil flax as experimental material.

The seeds of oil flax, c.v. "Tsian", were irradiated with gamma-rays at the doses of 400 and 700 Gy. Two mutants, M12 and M24, among others, were found in  $M_2$ . M12 had white corolla and star type flower and M24 had white corolla and yellow seeds. Both are different from their parent Tsian that had blue corolla, open type flower and brown seeds. Both mutants had a good viability and agronomical characteristics. Several years later, seeds of M12 and M24 were treated with gamma-rays at doses of 400 and 700 Gy, together with their parent variety Tsian.  $M_2$  seeds of every  $M_1$  plant were sown separately in individual plant-to-progeny rows. Every  $M_3$  family was the progeny of an  $M_2$  family. In the  $M_2$  and  $M_3$  generations, the majority of visually selected mutants was selfed and advanced from  $M_2$  to  $M_3$  or from  $M_3$  to  $M_4$ . Every mutant line was raised along with its parent. Mutation frequency was calculated as a percent of families with mutant plants selected in  $M_2$  and  $M_3$  to the total number of analyzed families. The type of mutation observed within the plants of  $M_3$  family was not taken into account if it was observed earlier in the same  $M_2$  family.

It was found that after irradiation of radiomutants with the doses of both 400 and 700 Gy the frequency of morphological and physiological mutations was lower and their spectrum was narrower in comparison with the irradiated variety. Genotypic differences were revealed for mutation variability in the radiomutant lines studied. For the radiomutants a considerable increase in the mutation frequency and broadening of the spectrum of inheritable changes was revealed at the irradiation dose of 700 Gy while in the Tsian variety the differences between two doses were not found (Table).

Seven types of mutations, namely, chlorophyll deficiency, corolla and anther colour, flower shape and size, seed colour, plant habitus, sterility, plant growth and development were found in the  $M_2$  and  $M_3$  generations of the studied genotypes (Table). Higher frequency of mutations of corolla and anther colour, as well as chlorophyll deficiency, was noted after variety Tsian was irradiated [1]. However, the mutation frequency of seed colour and chlorophyll deficiency was low in radio mutants, after radiation treatments. It is very promising that after the radiomutants were irradiated, new original mutations were selected among them, such as unopening corolla of spheroidal shape, late ripening and others.

Among the morphological mutants of the Tsian variety, the mutations of plant habitus, which comprised tall stem, dwarf plants and leaf size had the most practical importance. The dwarf mutants had the shortened internodes and leaves. The mutation that influences leaf size was characterized by wider leaf plate and higher leafiness of the plants; besides that, many flowers on such plants had 6 or 7-locular ovules.

Type of mutation	Number of mutant families, %		
	Tsian	M12	M24
Chlorophyll deficiency	16.8	14.6	10.0
Corolla and anther colour	32.2	0	3.2
Shape and size of flower	7.9	0	4.8
Seed colour	7.9	0	25.3
Plant habitus	6.0	3.5	2.6
Sterility	8.7	0	2.6
Plant growth and development	0.8	1.4	1.6
Total	80.3 ± 3.01	19.5 ± 3.50 *	54.3 ± 3.95 *
Including 400 Gy	44.5 ± 4.31	5.7 ± 2.77 <sup>1</sup>	17.7 ± 3.89 <sup>1</sup>
700 Gy	35.8 ± 7.40	13.8 ± 4.49 <sup>1</sup>	36.6 ± 6.06 <sup>a</sup>

\*<sup>1</sup> = Differences from the variety Tsian are significant at the 0.05 level of probability

<sup>a</sup> = Differences between doses inside genotype are significant at the 0.05 level of probability

Thus radiomutants in comparison with the Tsian variety were characterized by a lower frequency and narrower spectrum of morphological and physiological mutations. Together with that, the treatment with a dose of 700 Gy was more effective. However, it was after irradiation of the radiomutant lines that rare original mutations were obtained.

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# Induced High Yielding Mutants in Cotton (*Gossypium hirsutum* L.)

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Mutations are known to enhance the genetic variability of crop plants as the variability at species level has reached the ceiling due to high breeding intensity and rapid erosion of genetic resources. Since spontaneous mutations occur at very low frequency, induced mutations facilitate the development of improved varieties at a swifter rate [1]. Besides the vital role in plant breeding programs, a new role of induced mutations in releasing of gene silencing in transgenic plants has been reported [2]. Cotton is an economically important crop plant and cultivated in many parts of the world and it is a leading fiber crop, the second best potential source of plant proteins and fifth best oil-producing crop. Cotton (white gold) enjoys a predominant position amongst all cash crops in India. Cotton is an important raw material for the Indian textile industry, constituting about 65% of its requirements [3]. In addition to being the world's most important textile fiber crop, cotton is also an important source of edible oil and protein. By-products such as cotton seed cakes, hulls and cotton stalks are useful in generating several industrial goods. Among the different breeding methods used, mutation induction has been used as an important tool to supplement existing variability and to create additional variability for qualitatively as well as quantitatively inherited traits in cotton, especially various degrees of resistance to biotic and abiotic stress, number of bolls, seed yield and oil content.

Well-developed, fresh, uniform and delinted seeds of two tetraploid varieties of cotton (*Gossypium hirsutum* L.) Var. MCU 5 and MCU 11, were obtained from the Cotton Breeding Centre, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. After standardization for moisture content at 16% (with glycerol), seeds were irradiated with gamma rays (GR) from a  $^{60}\text{Co}$  at the Sugarcane Breeding Institute (ICAR), Coimbatore at doses of 100, 200, 300, 400 and 500 Gy. Another set of seeds were presoaked in distilled water for 8 h and were treated with various concentrations of ethyl methane sulphonate (EMS) and sodium azide (SA) which were prepared in a phosphate buffer solution of pH 6.0 and 3.0 respectively for 4 hours. The seeds treated with chemical mutagens were thoroughly washed in running tap water for 1 h and sown in the pre-irrigated field. The dry seeds without gamma irradiation and soaked in distilled water served as control. The treated seeds along with control were sown in the experimental field of randomized block design during August 1997 with three replications. For each treatment 5 rows of seeds were sown spaced at 75 cm between rows and 45 cm between seeds in a row, with two seeds per hole. In  $M_1$  generation, groups of plants (treatment wise) were harvested on a bulk basis. In  $M_2$  generation, the plants were analyzed thoroughly and selected based on yield characters with respect to control. The seeds from those plants were collected separately. The selected seeds from  $M_2$  generation were advanced to  $M_3$  generation and examined for yield characters in February 1998. Selected superior mutant progenies were evaluated in  $M_4$  during 1998-1999 and  $M_5$  during 1999-2000.

Ten mutants were selected based on yield and plant type, by *in vivo* mutagenesis. Out of these 10 mutants, 6 belong to the variety MCU 5 and 4 belong to the variety MCU 11 (Table 1). The selected characters of each mutant show higher yield characters in each generation. These mutants are now being tested at Central Institute for Cotton Research (ICAR), Regional Station, Coimbatore, Tamil Nadu, for multilocation experiments.

**Table 1. Yield components of the isolated mutants of MCU 5 and MCU 11**

Mutant	Mutated characters	Mutagenic treatment	Number of bolls	Boll weight (g)	Yield (g/plant)
<b>MCU 5</b>					
Control	--	--	45.0 ± 3.0	3.8 ± 0.3	183.0 ± 12.0
Mutant 1	Early flowering	30 mM EMS	50.0 ± 3.0	5.2 ± 0.4*	190.4 ± 14.1
Mutant 2	Tall and high yield	30 mM EMS	55.0 ± 4.0	5.3 ± 0.5*	225.3 ± 13.4**
Mutant 3	Twin boll	100 Gy GR	70.0 ± 4.0**	4.9 ± 0.4	210.5 ± 14.6*
Mutant 4	Tall mutant	200 Gy GR	60.0 ± 5.0*	4.7 ± 0.3	200.8 ± 16.7*
Mutant 5	Short, higher yield	30 mM EMS	62.0 ± 3.0*	5.4 ± 0.4*	215.6 ± 18.9*
Mutant 6	Higher no. of seeds	30 mM EMS	64.0 ± 4.0*	5.7 ± 0.4*	212.7 ± 20.1*
<b>MCU 11</b>					
Control	--	--	48.0 ± 4.0	4.0 ± 0.4	195.4 ± 17.9
Mutant 7	Early flowering	40 mM SA	54.0 ± 3.0	6.1 ± 0.5*	216.0 ± 19.8*
Mutant 8	Higher yield	400 Gy GR	57.0 ± 4.0	6.5 ± 0.4**	225.8 ± 15.8**
Mutant 9	Bifurcated	40 mM SA	60.0 ± 4.0*	5.7 ± 0.5	217.8 ± 19.7*
Mutant 10	Lengthy branched	40 mM EMS	59.0 ± 5.0	6.2 ± 0.4*	213.8 ± 16.5*

## Acknowledgement

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## Development of an Early Maturing Chickpea Variety, BINASOLA-3

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Chickpea has higher yield potential, more important nutrients and diversified use than all other pulse crops in Bangladesh. Nevertheless, farmers are not very interested in growing this crop because the varieties have a long maturity period, small seed size and poor seed yielding. For this reason huge amounts of chickpea seeds are imported every year in exchange for valuable foreign currency.

A mutation-breeding program was undertaken at Bangladesh Institute of Nuclear Agriculture (BINA) in 1989 to develop an early maturing, large seeded and high yielding variety of chickpea. Seeds of the two exotic lines G-97 (now Binasola-2) and G-319 were treated with gamma-rays, sodium azide and a combination of the two mutagens. The following doses of gamma rays: 0, 200, 300, 400 and 500 Gy, concentrations of sodium azide: 0.4 mM, and combined doses: 200 Gy+0.4 mM, 300 Gy+0.4 mM, 400 Gy+0.4 mM and 500 Gy+0.4 mM were used. In the first year, the treated seeds were grown dose-wise and raised as M<sub>1</sub> generation during 1989. M<sub>2</sub> seeds were harvested from 4,542 individual M<sub>1</sub> plants. These were grown in plant- progeny- rows in M<sub>2</sub> generation. Eighty-seven individual M<sub>2</sub> plants were selected, from which 16 M<sub>4</sub> lines were further selected and put into preliminary observation trials in M<sub>4</sub>. Among them, one elite mutant L-84, which was derived from a single plant in the population of the 200 Gy gamma-ray treated G-97 was tested in M<sub>5</sub>, M<sub>6</sub> and M<sub>7</sub> along with other mutants and its parent. Finally the mutant was evaluated in advanced, zonal yield trials and farmers field trials in the following generations from 1995-2001 along with two selected lines (P-70 and P-34) and three released varieties (Hyprosola, Binasola-2 and Barisola-3). All the selected lines were grown at different agro-ecological zones in Bangladesh to observe the yield and other potentiality. Mutant performance was evaluated under two management practices i.e., Research management and Farmers' management. In the research management practices, NPK fertilizers were applied @ 25, 50, and 25 kg/ha, respectively. In cultural practices, weeding and irrigation were done for maximum growth and also insecticide was sprayed to control insects. On the other hand, in farmers' practices only insecticides were applied, not fertilizers.

Early maturing chickpea mutants were obtained through mutation breeding [1,2]. The mutant strain L-84 matured significantly earlier than all other lines. It matures about two weeks earlier than its parent variety Binasola-2. In Bangladesh, in most years chickpea plants get rain at the maturity stage, which reduces the seed yield drastically. Early maturing varieties are essential in our country. Seed size of the strain, L-84 is medium type i.e., 100-seed weight around 17.1g. The mutant also produced a higher seed yield per day than its parental variety, Binasola-2. It produced the second highest seed yield in both research stations and farmers fields. The strain L-84 was found early maturing, high seed yielding with medium seed size.

### Performances of an elite mutant and two selected lines grown at 13 research fields and 6 farmers fields during 1995-2001

Variety/Strain	Research Station			Farmers field	
	Days to maturity (No.)	100-seed weight (g)	Seed yield/day (kg/ha)	Seed yield (kg/ha)	Seed yield (kg/ha)
L-84 (Mutant)	120a	17.1b	13.17a	1581.4a	1284a
P-70 (Selected)	127b	21.3a	12.66b	1608.4a	1254a
P-34 (Selected)	126b	12.0c	12.89b	1625.0a	1382a
Hyprosola (Variety)	126b	07.9d	10.32c	1301.3b	952c
Binasola-2 (Variety)	135c	17.9b	10.43c	1409.0b	1098b
Barisola-3 (Variety)	131c	16.7b	10.39c	1362.3b	1096b

Based on the above characters, the mutant L-84 was proposed to the National Seed Board for registration in 2001, with a tentative name of Binasola-3.

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# Induction of Drought Tolerance in Tomato using $^{60}\text{Co}$ Gamma Ray Irradiation

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Drought is one of the environmental limitations that affects, on a higher degree, the production of different crops including tomato [1,2]. A tomato breeding program was started to develop varieties suitable for growing under low water input conditions, which is not only important for saving this valuable liquid but also for diversifying food production in drought-affected areas.

Two Cuban tomato varieties (INCA 9-1 and Amalia) were irradiated by  $^{60}\text{Co}$  gamma rays at doses of 300 and 500 Gy. In  $M_2$  generation, plants were cultivated in two zones of the country (Holguin and Havana province) in the months of lower precipitation (December-March). Irrigation was made three times at transplanting stage. Plants were grown in the short rainy season to keep suitable conditions for a promising genotype selection. During the following six generations selection was made for genotypes of high-yield, large fruit, high yield, disease resistance and fruit quality. In  $M_6$  generation, evaluation was conducted under water stress conditions for 60 plants of each of the best mutant lines, four of them from INCA 9-1 variety and three from Amalia variety. (Table).

The mutant lines M15, M17 and M19 have been further evaluated in different areas of the country and they have shown very good behavior.

## Performance of mutant lines (mean $\pm$ standard deviation) evaluated at the National Institute of Agricultural Science (Havana province) under drought conditions

Variety/mutant	Treatment (Gy)	Yield/plant (kg)	Fruit number/plant	Fruit weight/fruit (g)
INCA-9-1	Parent	1.36 $\pm$ 0.15	32.70 $\pm$ 2.9	41.60 $\pm$ 1.9
M19	500	2.69 $\pm$ 0.14	39.30 $\pm$ 1.8	68.59 $\pm$ 0.5
M4	300	2.72 $\pm$ 0.14	33.10 $\pm$ 1.5	82.15 $\pm$ 1.2
M20	300	2.16 $\pm$ 0.04	53.10 $\pm$ 1.0	40.71 $\pm$ 1.1
M16	300	2.05 $\pm$ 0.03	46.10 $\pm$ 2.5	44.64 $\pm$ 1.5
AMALIA	Parent	1.41 $\pm$ 0.17	17.60 $\pm$ 1.0	88.45 $\pm$ 2.0
M15	500	3.69 $\pm$ 0.09	46.50 $\pm$ 2.9	79.84 $\pm$ 3.8
M17	500	2.34 $\pm$ 0.12	23.30 $\pm$ 4.3	106.22 $\pm$ 8.3
M2	300	2.51 $\pm$ 0.11	26.60 $\pm$ 1.0	94.02 $\pm$ 4.7

## Acknowledgement

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# An Induced Bushy Mutant in Mungbean

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Mungbean (*Vigna radiata* (L.) Wilczek) is an important pulse crop in India. An experiment was conducted, aiming to develop novel mungbean mutants, during the summer seasons of 2000/01, 2001/02 and 2002/03 at the Agricultural Farm, Aligarh Muslim University, Aligarh, India. Three hundred uniform and healthy seeds of mungbean var. PS-16, presoaked in distilled water for 9 hours, were treated with 0.02% methylmethane sulphonate (MMS) for 6 hours and washed by using tap water for 2 hours prior to sowing. About 300 treated and control (untreated) seeds were sown in the field and seeds of M<sub>1</sub> plants and control were harvested separately and sown in plant progeny rows to raise M<sub>2</sub> population. A bushy mutant was detected in M<sub>2</sub> population. The seeds of the bushy mutant were collected separately and grown as single plant progenies in M<sub>3</sub> generation. This mutant breeds true in M<sub>3</sub> generation.

The mutant had branching and short plant height, which gave it a bush-like appearance. The reduction in plant height was due to reduced internode length. Yield and yield components were also found to have been adversely affected in the bushy mutant (Table). However, the flowering time and maturity period of the mutant showed no significant difference from the parental variety PS-16 (Table). Genotypic coefficient of variation (GCV) provides a means to study the genetic variability generated in quantitative traits. In the present study GCV and the genetic advance (Gs) for plant height, number of fertile branches, number of pods and total plant yield increased considerably in the bushy mutant. Therefore, the bushy mutant might be useful in breeding programmes for these traits.

## Characteristics of the bushy mutant and its parental variety PS-16 of *Vigna radiata*

Characters	Parental var. PS-16			Bushy mutant		
	Mean±S.E.	GCV (%)	Gs (% of X)	Mean±S.E.	GCV (%)	Gs (% of X)
Plant height (cm)	46.50±0.13	1.51	1.69	31.80±0.15*	5.09	8.49
Days to flowering	38.43±0.27	1.68	1.81	37.90±0.22	1.71	2.24
Days to maturity	62.73±0.85	2.00	3.25	61.20±0.57	3.02	3.62
Fertile branches / plant	5.00±0.22	17.81	35.36	2.00±0.20*	24.19	42.50
Pods / plant	52.00±0.25	1.45	1.58	29.00±0.25*	7.09	16.71
Seeds / pod	8.00±0.35	10.21	16.64	7.50±0.63	11.33	16.00
100-seed weight (g)	3.45±0.20	4.50	6.58	2.64±0.10	6.81	7.95
Yield / plant (g)	12.90±0.22	1.59	2.22	8.25±0.09*	4.24	8.13

\*Significant at 5% level; GCV = Genotypic coefficient of variation; Gs = Genetic advance

# Trombay Groundnut Recombinants Resistant to Foliar Diseases

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Groundnut (*Arachis hypogaea*) is an economically important oilseed and food crop of India, grown in an area of about 6.8 million hectares with a productivity of 794 kg/ha during 2003. Its low productivity is partially due to the incidence of foliar diseases e.g. rust and late leaf spot (LLS) caused by *Puccinia arachidis* and *Phaeoisariopsis personata*, respectively. Both fungal diseases cause severe yield losses over 50% in groundnut. Although fungicidal application is effective to control diseases, cost and residual effect are of concern. Hence, the alternative is to identify resistant groundnut sources and incorporate them into adapted cultivars.

Genetic improvement of groundnut through induced mutagenesis in combination with recombination breeding has been carried out at this institute for five decades, leading to the release of ten Trombay groundnut (TG) varieties for commercial cultivation, besides maintaining TG germplasm comprising of more than 200 mutants and breeding lines which act as genetic source material in various breeding projects. One of the TG varieties, TAG 24 is a high yielding and widely adapted national check variety. It has semi-dwarf height, enhanced harvest index, partitioning efficiency and water use efficiency, which is widely accepted by farmers [1, 2, 3]. However, it is susceptible to LLS and rust. An inter-specific derivative, VG 9514 developed at Tamil Nadu Agricultural University, Vriddhachalam is resistant to both diseases [4]. However, it has certain undesirable traits such as low harvest index, low yield, thick and reticulated pod shell and small seeds.

With an objective to incorporate disease resistance, TAG 24 was hybridized with V 9514 as the male parent in 2000. During 2001, F<sub>2</sub> seeds from three F<sub>1</sub> plants out of five were irradiated with 200 Gy of gamma rays from a <sup>60</sup>Co source. Both the treated and untreated F<sub>2</sub> seeds were sown in the field. Selection was practiced in F<sub>3</sub> and F<sub>3</sub>M<sub>2</sub> and subsequent generations for higher yield, desired plant type, pod features and seed size as well as field resistance to LLS and rust diseases. At the end of F<sub>6</sub> and F<sub>6</sub>M<sub>5</sub> generations, five ideal recombinants named as Trombay foliar disease resistant groundnut (TFDRG) 1 to 5 were established. TFDRG 1 is the selection from the untreated F<sub>2</sub> population while the rest are from gamma ray treated F<sub>2</sub> populations.

The morphologic, agronomic and disease score traits observed are shown in Table 1 and 2. TAG 24 belonged to ssp. *fastigiata* var. *vulgaris* and VG 9514 to ssp. *hypogaea* var. *hypogaea*. TFDRG 3 belonged to var. *hypogaea* while the rest of the recombinants belonged to var. *vulgaris*. Testa color in TFDRG 3 and 5 was red and in others it was rose. Days to 50% flowering in selections were closer to TAG 24. At Trombay conditions, VG 9514 scored 1 for LLS and rust and TAG 24 scored 7 on 1-9 scale indicating resistance and susceptible reaction, respectively. Recombinants, TFDRG 1 to 5-recorded 1 to 3 score for LLS showing resistance to this disease. For rust disease, TFDRG 1, 3, 4 and 5 showed resistance with 1 to 3 disease score (Table 1). Another important viral disease, peanut bud necrosis is prevalent in summer in the majority of the groundnut areas and in late-rainy season in a few areas. All selections showed either enhanced or similar tolerance level compared to their parents for this disease.

All TFDRG selections recorded significantly superior pod and seed yields over the disease resistant parent VG 9514 (Table 2). Further, TFDRG 1 also showed superiority for pod and seed yield and TFDRG 2 for seed yield alone as compared to TAG 24. TFDRG 5 had better shelling turnout and TFDRG 2 had higher 100-seed weight over TAG 24. Oil content of the recombinants was maintained as that of TAG 24 except TFDRG 3 where it was lower. Some of these genotypes will be further evaluated in the National/State multi-location trials. The present study emphasizes the importance of gamma rays in evolving ideal recombinants along with high yield, disease resistance and other superior agronomic traits.

**Table 1. Morphological traits and disease response of Trombay groundnut recombinants**

Genotypes	Botanical variety	Testa colour	Days to 50 % flowering	Peanut bud Necrosis (%) <sup>#</sup>	Late leaf spot <sup>*</sup>	Rust <sup>*</sup>
TFDRG 1	<i>vulgaris</i>	Rose	25	10.0	2	1
TFDRG 2	<i>vulgaris</i>	Rose	25	12.2	3	5
TFDRG 3	<i>hypogaea</i>	Red	26	4.9	2	3
TFDRG 4	<i>vulgaris</i>	Rose	26	4.9	3	2
TFDRG 5	<i>vulgaris</i>	Red	23	10.0	1	3
TAG 24	<i>vulgaris</i>	Rose	23	9.9	7	7
VG 9514	<i>hypogaea</i>	Red	30	6.2	1	1

\* 1-9 scale, where 1= 0% and 9 = 81-100 % disease severity; <sup>#</sup> % of infected plants

**Table 2. Field performance\* of Trombay groundnut recombinants**

Genotypes	Pod weight (g/plant)	Seed weight (g/plant)	Shelling out turn (%)	100-seed weight (g)	Oil content (%)
TFDRG 1	30.9	21.5	70.0	53	48.4
TFDRG 2	25.8	19.8	76.8	58	48.2
TFDRG 3	25.0	17.7	71.9	47	46.7
TFDRG 4	26.5	19.7	74.1	55	48.3
TFDRG 5	24.1	18.5	77.8	52	48.7
TAG 24	24.8	18.4	74.2	51	48.1
VG 9514	11.4	7.7	68.4	37	45.5
CV %	4.4	4.3	1.4	3.6	1.1
CD (0.05)	1.9	1.3	2.6	4.5	1.3

\* Pooled mean from two seasons

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## Root Hair Mutants of Barley

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Barley mutants without root hairs or with short or reduced root hairs were isolated among M<sub>2</sub> seeds of "Lux" barley (*Hordeum vulgare L.*) after acidified sodium azide mutagenesis. Root hair mutants are investigated intensively in *Arabidopsis* where about 40 genes are known [1,2,3]. A few root hair mutants are known in maize, rice, barley and tomato [4]. Many plants without root hairs grow quite well with good plant nutrition, and mutants have been used for investigations of uptake of strongly bound nutrients like phosphorus, iron, zinc and silicon.

Seed of "Lux" barley (Sejet Plant Breeding, Denmark) were soaked overnight, and then treated with 1.5-millimolar-sodium azide in 0.1 molar sodium phosphate buffer, pH 3, for 2.5 hours according to the IAEA Manual on Mutation Breeding (2<sup>nd</sup> Ed.). After rinsing in tap water and air-drying, the M<sub>2</sub> seeds were sown in the field the same day. Spikes, 4-6 per M<sub>1</sub> plant, were harvested. The mutation frequency was similar to that obtained with other barley cultivars from

which low-phytate mutants were isolated [5]. Seeds were germinated on black filter paper in tap water for 3 or 4 days before scoring for root hair mutants. The agar growth technique used for *Arabidopsis* mutants did not work well for barley.

**Table 1. Number of root hair mutants of barley selected among 2000 M<sub>1</sub> progenies (about 20,000 M<sub>2</sub> seedlings. The chlorophyll mutation frequency was 0.9% of M<sub>2</sub> seeds**

Minus root hairs	Short root hairs	Few root hairs	Tufts, unstable, segregating
8	16	2	32

The results show that root hair mutants can be quite common in plants other than *Arabidopsis*. The mutants are viable and can be used as genetic markers, in investigations of root hair physiology, root architecture, nutrient uptake, and the function and importance of mycorrhiza. Some of the mutants show unstable, variable or poorly visible phenotypes. Some of the instabilities may be caused by lack of control of gases such as ethylene which influences root hair formation.

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# Development of a Short Duration Upland Rice Mutant Line through Anther Culture of Gamma Irradiated Plants

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This experiment was conducted in the field and at the tissue culture laboratory of the Plant Physiology Division, CARI, Yezin from 1994 to 1997. Upland rice, Yar-2 was used as the test variety.

Dried seeds (14% moisture content) were treated with gamma rays at doses of 0, 300, 350, 400 and 450 Gy respectively. These seeds were planted separately according to the gamma-ray treatment they received. At booting stage, tillers were cut. Anthers from the top and middle portion of the panicle were taken. The pollen, at developmental stage in each anther, was examined using Acetocarmine dye. Pollens at the uninucleate to early binucleate stage were selected. Tillers having pollens at the above-mentioned stage were placed in a dark room at 25(±) 1 C and 16 hours photoperiod. When plantlets were obtained from these media, well-developed green plantlets were selected and planted in Yoshida solution to attain vigorous root growth. Diploid and haploid plants were formed from the anther culture method. At the heading stage, haploid plants were treated with colchicines to promote development into diploid plants. At maturity, plants produced from materials treated with different gamma doses were harvested separately. These homozygous lines were planted in the field and the characters were compared with their parents grown at the same time.

The effects of gamma ray irradiation on callus formation was observed and recorded in Table 1 and on green plantlet regeneration in Table 2.

**Table 1. Callus formation at different gamma ray treatment**

r-ray dose (Gy)	No. of anther cultures	No. of callus formed	Callus induction (%)
0	77400	261	0.3372
300	57150	58	0.1015
350	70100	62	0.0872
400	36300	17	0.0468
450	38900	528	0.7663
<b>Total</b>	<b>300850</b>	-	-
<b>Average</b>	-	-	<b>0.30</b>

**Table 2. The effect of gamma ray treatments on green plantlet regeneration**

r-ray dose (Gy)	Transferred calli (no.)	Regenerated green plants (no.)	Plant regeneration (%)	Total line (no.)
0	261	68	26	10
300	58	16	27	8
350	62	10	16	3
400	17	5	29	-
450	528	38	7	24
<b>Total</b>	926	137	-	45
<b>Average</b>	-	-	21	-

The highest callus induction rate was found in materials treated with 450 Gy of gamma rays, but the lowest green plant regeneration rate was also observed at this dose.

Among the forty-five homozygous lines obtained of the above materials, 7 lines, all from the 450 Gy gamma rays treated material, flowered earlier than the parents. One of the 7 early flowering lines, mutant line No 18, matured 19 days earlier but had the same yield as the parent. All other lines had a lower yield than the parent.

Comparisons of yield and yield components of mutant line 18 and Yar-2 (parent) is shown in Table 3, and comparison of quality characters in Table 4.

**Table 3. Yield and yield component of Yar-2 and Yar-2 mutant**

Varieties	Plant height (cm)	Tillering with panicle	Panicle length (cm)	No. of filled grain with one panicle	Filled grain (%)	1000 grain weight (gm)	50% flowering date	Yield basket/acre
Yar-2	106.4	10.4	23.0	85.4	70.3	23.4	96	55
Mutant (line No. 18)	82.0	12.8	21.7	100.3	71.1	21.9	77	57

**Table 4. Comparison of quality of Yar-2 and Yar-2 mutant**

Sample	Elongation ratio	Gel. Temp.	Protein (%)	Amylose (%)	Gel consistency
Yar-2	1.76	High/Inter	5.36	24.05	Hard
Yar-2 mutant	1.52	Inter	6.53	28.98	Medium

# Stable Dwarf Mutants of a High Quality Tall Rice Variety White Ponni

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White Ponni has been a commercially superior rice variety for over a decade in South India. Even though it is a poor yielder with a yield potential of 3.0 Tons/ha. it is valued for its high quality rice with slender grain. Due to this, White Ponni fetches a very high price for the farmer. It is a medium duration rice variety with a growing period of about 150 days and is highly fertilizer non-responsive. Any seasonal heavy wind, rainfall and/or high fertilizer leads to lodging and results in a heavy loss to the farmer. Any improvement in this variety is a boon to the farmer. Hence an attempt has been made to reduce plant height using mutation techniques.

The dried seeds of White Ponni rice were exposed to gamma rays irradiation with 100, 200 and 400 Gy doses. The  $M_1$  and  $M_2$  generations were raised. In  $M_2$  generation, the numbers of dwarf mutant plants were identified in different doses. These plants were harvested individually and the seeds were divided into five different groups. From July to September 1999, these seeds were raised for  $M_3$  generation, in monthly intervals of five months. A total of 16 families were raised in randomized block design with three replications. The various biometrical observations were recorded, including height. The data was subjected for identification of stable dwarf mutants through stability analysis of Eberhart and Russel [1966].

The different dwarf mutants showed the mean performance from 112 to 132 cm (Table 1). Two mutants from 100 Gy flowered 15 days earlier than the control. It also maintained the dwarf stature. Other traits like tiller number, panicle length and yield per plant were also more or less similar to that of White Ponni.

The seasonal difference in plant height expression was noticed (Table 2). Instability, genotypes with lowest standard deviation coupled with unit regression are considered as the most stable ones [2]. Accordingly, the mutants viz., 10kR-1 and 10kR-4, 20 kR-2 and 20 kR-14 and 40 kR-1 and 40 kR-4, had unit regression along with non-significant deviation. The dwarfness of these mutants was stable over season and these mutants had a mean height reduction of 17.2% over the check, White Ponni. The individual stable mutants were raised family-wise for identification of dwarfing gene and further exploitation is in progress.

**Table 1. Mean performance of mutants for different economic traits over seasons**

Mutants/Parent	50% Flowering (days)	Plant height (cm)	No. of tillers	Panicle length (cm)	Single plant yield (g)
W.Ponni 10kR-1	95.2	116.4	12.2	21.4	19.3
W.Ponni 10kR-4	95.6	112.2	13.3	23.2	21.5
W.Ponni 10kR-5	104.7	130.2	11.2	19.7	20.5
W.Ponni 10kR-8	108.3	128.2	9.8	22.8	23.2
W.Ponni 10kR-10	107.5	130.1	9.1	18.9	22.1
W.Ponni 20kR-1	108.2	125.4	14.8	23.5	26.2
W.Ponni 20kR-2	109.6	115.2	15.1	24.3	23.7
W.Ponni 20kR-5	110.0	125.0	11.9	21.2	17.2
W.Ponni 20kR-14	112.1	115.2	9.7	20.5	19.1
W.Ponni 20kR-16	113.7	121.0	12.3	18.3	18.2
W.Ponni 40kR-1	108.8	120.0	12.9	25.2	24.2
W.Ponni 40kR-2	108.4	128.0	13.7	24.8	26.1
W.Ponni 40kR-4	111.2	121.2	8.7	19.2	18.0
W.Ponni 40kR-8	115.8	125.9	9.1	21.5	19.2
W.Ponni 40kR-11	116.5	132.0	10.5	23.1	21.8
White Ponni (ck)	110.3	138.2	9.2	20.5	18.2

**Table 2. Performance of plant height over season and stability parameters**

Mutants	Plant height (cm) for different seasons of 1999						'b'	Sd <sup>2</sup>
	July	Aug.	Sept.	Oct.	Nov.	Mean		
W.Ponni 10kR-1	117.6	113.6	120.3	117.3	113.2	116.4	1.05	58.5
W.Ponni 10kR-4	108.4	112.3	117.3	112.4	112.6	112.2	0.95	64.3
W.Ponni 10kR-5	122.1	142.8	123.2	145.3	117.6	130.2	1.41	131.6*
W.Ponni 10kR-8	124.5	141.2	114.6	139.3	121.4	128.2	1.54	178.1**
W.Ponni 10kR-10	117.9	139.2	139.8	137.3	116.3	130.1	1.23	213.2**
W.Ponni 20kR-1	118.8	139.2	115.1	136.5	119.3	125.4	1.32	-215.2**
W.Ponni 20kR-2	114.3	137.4	113.2	119.2	114.6	115.2	1.03	78.2
W.Ponni 20kR-5	120.7	114.7	105.9	138.8	120.2	125.0	1.02	-527.8**
W.Ponni 20kR-14	113.9	139.4	117.3	118.3	111.2	115.2	0.98	60.8
W.Ponni 20kR-16	116.3	115.3	103.3	135.2	118.1	121.0	0.54	198.2**
W.Ponni 40kR-1	118.7	132.1	120.5	122.1	119.3	120.0	1.04	32.8
W.Ponni 40kR-2	119.3	119.4	121.7	141.2	121.2	128.0	1.23	-625.2**
W.Ponni 40kR-4	118.2	136.6	120.2	123.6	121.7	121.2	1.08	48.7
W.Ponni 40kR-8	115.2	122.3	118.7	138.2	117.3	125.9	0.82	148.6*
W.Ponni 40kR-11	119.9	140.1	141.1	140.1	119.7	132.0	0.91	128.3*
White Ponni control	139.2	139.2	136.7	145.6	139.3	138.2	1.04	89.8
Mean	119.06	129.92	120.55	131.9	118.93		1.04	
SE	6.02	11.08	10.36	10.69	6.12			

1. Unit, 2. Standard deviation

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# High Yielding Lines of 'Basmati' Rice Evolved through Cross Breeding with Induced Mutants

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A number of tall *indica* scented rice varieties including "Basmati" are traditionally cultivated in different agro-climatic regions of India. Consumer demand of these varieties is very high. However, the varieties are susceptible to lodging and have poor yield. Out of these varieties "Basmati" is the most popular with high demand in the international market for its pleasant aroma, fine long slender grains with superior cooking and eating qualities. In a research project sponsored by the Department of Atomic Energy, Government of India, three short culm, high yielding mutants were hybridised with three "Basmati" varieties vsz. Basmati 370, Pakistan Basmati and Pusa Basmati 1 to develop short statured, high yielding varieties of aromatic rice with "Basmati" type grain quality for cultivation in subhumid and humid regions of eastern India. The mutant lines were induced earlier through gamma rays from two local aromatic rice cultivars: Tulai-panja [1] and Gobindabhog [2]. The object of hybridisation was to combine high yield, short stature and adaptability of the mutants and the superior grain characters of "Basmati" varieties. Selection of the hybrid materials was done following the pedigree method.

Four-selected short height, high yielding lines, with aroma and grain dimension similar to "Basmati" type produced 30% - 40% higher yield in replicated trials of F<sub>7</sub>. The performance of these lines and their parents are presented in the table.

**Table. Performance of four selected lines of aromatic rice in F<sub>7</sub> and their parents**

Selected lines of the cross combination	Plant height (cm)	Filled grains/panicle	Grain length (mm)	Grain length/breadth ratio	Yield tons/ha
IET 14143 x Pakistan Basmati	93	73	10.5	4.59	5.1
IET 14142 x Pakistan Basmati	125	98	10.0	4.92	4.2
IET 13541 x Basmati 370	131	79	11.0	5.03	3.9
IET 13541 x Pakistan Basmati	124	82	10.6	5.01	4.8
Parents					
IET 14143 (mutant of Tulaipanja)	110	107	8.0	3.90	3.8
IET 14142 (mutant of Tulaipanja)	103	62	7.9	3.02	3.7
IET 13541 (mutant of Gobindabhog)	132	207	6.0	2.69	4.1
Pakistan Basmati	146	87	10.5	4.59	2.9
Basmati 370	143	60	11.2	5.24	2.6
Pusa Basmati I (check)	113	89	10.7	5.03	2.8

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# Induction of Bacterial Blight Resistance in Elite Indian Rice (*Oryza sativa* L.) Cultivars using Gamma Irradiation and Ethyl Methane Sulfonate

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Rice is one of the most important crops in the world, feeding more than 50% of the human population. During the last 30 years, mutation breeding has played a significant role in rice breeding programmes. Rice mutants with higher yield, greater tolerance to diseases and pests and other agronomic qualities have been released for commercial cultivation in many countries. By the year 1991, as many as 278 rice cultivars had been released world-wide out of which 24 were from India [1].

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv *oryzae* is the second most important disease in southeast Asia, causing an average of 21 kg/ha yield loss [2]. In the Basmati field yield loss can reach up to 100%. Moreover, there is no resistant source of Basmati rice known for its quality and aroma. Induction of bacterial blight resistance in basmati rice will not only help in developing high yielding Basmati cultivars without compromising the quality but will also be a good source of resistance for other Basmati rice varieties. Therefore, seeds of two Indian rice varieties viz. PR106 and Pusa Basmati 1 were treated with EMS (0.25% and 0.5%) at pH 7.0 at 25 ± 1°C for 12 h and gamma rays (100 and 200 Gy) (Table 1). A 3500-curie <sup>60</sup>Co gamma cell with a dose rate of 3200 radians per minute was used for gamma irradiation of the paddy seeds containing 13% moisture. After mutagenic treatment seeds were germinated along with corresponding controls in petri dishes lined with wet filter paper. The seeds from the M<sub>1</sub> generation were grown in the plant-to-progeny method for the M<sub>2</sub> generation at Kapurthala, Punjab Agricultural University. Each progeny had 22-25 plants. The plant-to-plant distance was 20 cm and row-to-row distance was 30 cm. For every 20 lines, one line of check (parent variety) was grown.

Screening against BB was made in the M<sub>2</sub> generation by inoculating the plants at maximum tillering stage, following Kauffman *et al.* (1973) [3]. Observations for disease severity were recorded after 14 days of inoculation following standard techniques. Plants with a lesion length of less than 2.5 cm were scored as resistant.

In M<sub>2</sub> out of a population of 89045 plants, a total of 40 lines comprising 145 plants (2.46%) were resistant to bacterial blight. A maximum of 16 BB resistant mutant lines were observed in Pusa Basmati 1, whereas there were 24 in PR 106.

On a line basis, 24 out of 1876 lines of PR106 segregated for BB resistance leading to a frequency of 1.279%, whereas 16 out of 1953 segregated for BB resistance leading to a frequency of 0.819% in the case of Pusa Basmati 1. When observed in terms of mutagens, 17 out of 24 mutants were induced by EMS in the case of PR 106 whereas for Pusa Basmati 1, 12 out of 16 cases the resistance was induced by gamma irradiation. In a study by Padmanabhan *et al* (1976) [4], 0.36% resistant and 0.65% moderately resistant plants were obtained in the M<sub>2</sub> population derived from EMS treated variety. The rate of successful induction of resistance during the present investigation is comparatively low since the moderately resistant and moderately susceptible plants in the M<sub>2</sub> were considered susceptible. The newness of resistance developed in the present study needs to be tested by going for allelic testing and testing against different pathotypes.

Induced mutants have been used to develop new BB resistant gene(s) viz. Xa-nm(t) by Nakai *et al* (1990) [5] and xa-19 by Taura *et al* (1991) [6]. The productive BB resistant lines developed here were grown in plant progeny methods by screening in every generation. Those advanced lines are under field trials and would be used to study the genetics of BB resistance in the future and the release of commercial cultivars for the farming community of this region.

**Table 1. Data on treatments, viability in M<sub>1</sub> and number of lines and plants screened in M<sub>2</sub> for PR 106 and Pusa Basmati 1**

Varieties	Treatments	No. of seeds treated	Final stand in M <sub>1</sub>	% Viability (up to maturity) M <sub>1</sub>	No. of lines in M <sub>2</sub>	Total plants in M <sub>2</sub>
PR 106	EMS 0.25%	1100	608	55.3	598	13635
	EMS 0.50%	1100	481	43.7	481	10510
	Gamma rays 100 Gy	1100	323	29.4	322	06730
	Gamma rays 200 Gy	1100	479	43.6	475	10380
Pussa Basmati 1	EMS 0.25%	1100	362	32.9	349	08290
	EMS 0.50%	1100	354	32.2	337	08005
	Gamma rays 100 Gy	1100	642	58.4	636	15010
	Gamma rays 200 Gy	1100	661	60.0	631	15585
<b>TOTAL</b>					3829	89045

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# “SAKATA-MEZURU” – A New Lodging-Resistant Glutinous Rice Mutant Cultivar

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Because of its excellent quality in the manufacture of rice cake (e.g. stickiness and extendability), Mezuru, a local glutinous rice cultivar developed in the early 20<sup>th</sup> century, was grown indigenously in the Sakata and Akumi Region. More than 700 ha were under cultivation. The long culm, however, made it susceptible to lodging and unsuitable for mechanical harvesting. Thus, its cultivation has been restricted to a few rural districts of Sakata city, and has been dependent on manual cultivation. Recently the excellent quality of rice cake made from Mezuru has been re-evaluated in comparison to the products from present glutinous varieties.

It was attempted to improve the defective lodging susceptibility by shortening the culm length and/or the lower internodes. Radiation-induced mutations and selection were used as effective breeding methods. In 1989, dry seeds of Mezuru were exposed to gamma rays (100, 200 and 300 Gy) at the Institute of Radiation Breeding, MAAF. M<sub>1</sub> and M<sub>2</sub> plants in bulk and afterwards progenies from M<sub>3</sub> generation were grown on the experimental paddy fields.

At maturity of M<sub>3</sub>, the culm lengths of 1,340 plants of each dose treatment were measured for progenies from doses. In the field, 206 short culm plants having about 80 cm length (the average culm length of Sasanishiki, a previously leading variety) were selected for laboratory studies. The lengths of the upper five internodes were measured on each short culm plant to obtain further information on the lodging resistance. After investigating total grains of each plant, thirty plants with short culm and promising lodging resistance were selected. Thirty lines raised in M<sub>4</sub> generation were recognized as short culm mutant lines, showing to be practically true breeding. Based on the result of the preliminary test, 13 lines were selected with potential agronomic value. In M<sub>5</sub> to M<sub>8</sub> the yield and adaptability were further tested along with the parental variety and the other glutinous variety Dewaminori, being recommended for cultivation in Yamagata prefecture, for four years in the five districts of Sakata.

From the results it was recognized that one short culm mutant with more lodging resistance, deriving from 300 Gy irradiation treatment, would be widely adaptable in all Sakata districts where the parent Mezuru had formerly been grown. The mutant was called Sakata-Mezuru, a new cultivar in 1996. Sakata-Mezuru is mainly characterized by shortened internodes, especially the lowest one. Sakata-Mezuru was similar to the parental variety – it had better performance but reduced culm length. The mutant variety was more productive than the parent in 1998, but comparable to the parent in 1999. Sakara-Mezuru was more adaptable to mechanical harvesting than the parent Mezuru because of increased lodging resistance. Harvest index of Sakata-Mezuru was increased due to shortening of most culm internodes. When the quality of rice cake was investigated by panel testing, more panellists recognized that the quality of Sakata-Mezuru equals or slightly surpasses that of Mezuru.

This variety was registered in February 2001 and officially released. The first cultivation acreage was about three ha of paddy fields in Sakata.

In conclusion, this study confirms that the induction of short culm mutations, often accompanied by straw stiffness, while retaining the other desirable traits of the original variety, is one of the most promising applications of ionising radiation in plant breeding. Indeed, we have attained our aim to obtain the short culm mutant cultivar Sakata-Mezuru with superior lodging resistance and yielding ability to its parental variety.

**Table. Mean performance and productivity of parental mutant and check varieties (5-6 plants/hill; 18.2 hills/m<sup>2</sup>)**

Variety	Mezuru	Sakata-Mezuru	Dewaminori
Heading date (August)	9	8	11
Culm length (cm)	84.9	75.8	68.2
Panicle length (cm)	17.5	16.8	16.5
Panicle numbers (no./m <sup>2</sup> )	299	325	340
Leaf numbers	14.0	13.6	13.8
Lodging score	3	2	1
Grain yield (kg/a)	40.5	45.5	52.8
1000 seeds wt (g)	21.0	20.5	18.9

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# Gamma Ray Induced Small Flower Mutant in *Hibiscus rosa-sinensis*

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Induced somatic mutation breeding has been highly effective in the improvement of vegetatively propagated ornamental crops (1, 2), which has greatly contributed to India's economic growth. Most ornamentals are in great demand, for their aesthetic appeal as well as for their multifarious uses.

*Hibiscus rosa-sinensis*, family Malvaceae, a native of China, is grown as an ornamental plant throughout India. It is an evergreen, woody, glabrous, showy shrub, 5-8 feet high, leaves bright green, flowers solitary axillary, bell shaped, large, 4-6 inches in diameter with pistil and stamens projecting from the center, capsule roundish and many seeded. Cuttings propagate this plant because it never produces seed in India. The flowers are eaten either raw or pickled in China and the Philippines. Crushed flowers produce a dark purplish dye that was used for polishing shoes in China and other countries, and also used for coloring hair and eyebrows. The flowers contain carotene and plants are also used as fodder (3). Recent research has shown that the flowers have post-coital antifertility activity. The presence of potent anti-estrogenic activity in flowers may be responsible for terminating pregnancy, as tested and reported for rats (4).

The cuttings (size 12 cm) of *Hibiscus rosa-sinensis* were irradiated with <sup>60</sup>Co source of gamma rays at 5, 10, 20, 30, 50, 70 and 100 Gy to induce mutation. Three replicates of 20 cuttings each for every dose were separately planted in 6-inch earthen pots with equal number of cuttings for control to raise the VM<sub>1</sub> generation and all replicates were maintained under glasshouse condition. All flowers produced throughout the blooming period in control and irradiated plants were carefully examined to detect the somatic mutations in flower shape and size. No changes were observed in control population. The variants for different morphological characters were isolated from VM<sub>1</sub> generation.

At 10 Gy a mutant plant was detected, which produced 5 flowers in the first blooming season and all of them were significantly small in size as compared to the control. The average length of petals in control was 5.2 cm while that of the mutant was 3.3 cm and the width at the broadest point was 3.0 cm for control and 1.6 cms for the mutant. The color and

aestivation of the mutant was like the control. The mutant was a chimera, as out of 22 cuttings of the mutant plant used to raise VM<sub>2</sub> population, only 2 plants (9%) produced small flowers and these are being further multiplied. Floral variants obtained at other doses showed variation in the length and orientation of the style, number and size of anthers and also flower colour and are being studied.

Flower dimensions (VM <sub>1</sub> )	Control	Small flower mutant (10 Gy)	Extension
Length of petal (cm)	5.2	3.3	3.89**
Width of petal (cm)	3.0	1.6	11.6**
Length of pedicel (cm)	5.9	3.9	17.0**
Length of staminal tube (cm)	5.8	4.2	8.3**
No. of anthers/flower	5.9	32	16.0**

\*\* Significant at .05 and .01 probability

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# Development of a Dwarf Mutant of Deshi Jute (*Corchorus capsularis*) to be used as Vegetables

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Jute leaves have been used as vegetables in India-Bangladesh for a long time. The jute plant is currently grown commercially for vegetable purpose in Bangladesh and exported to earn foreign currency. There is still no released variety of jute for commercial cultivation as vegetables in our country. Seeds of a wild cultivated variety, CVL-1 were treated with  $\gamma$ - rays and EMS to create variability. The treated seeds were grown dose-wise in M<sub>1</sub> generation. Seeds were collected from each plant and kept separately for growing M<sub>2</sub> population. A total of 266 individual plants were selected on the basis of plant height and vegetative growth from M<sub>2</sub> population. Seeds of the individual plants were kept separately and grown in plant-progeny-rows in M<sub>3</sub> generation. From this generation 48 plants were selected on the basis of plant height and base diameter. Only a few extreme dwarf plants were selected on the basis of extra vegetative growth with maximum number of green leaves. The selected mutant CM-18 (dwarf mutant) along with the parent CVL-1 and two other check varieties, Atompat-38 and D-154, were grown in a randomized block design with three replications at Mymensingh, Rangpur, Magura, Satkhira and Ishurdi during 2001. Unit plot size was 5m x 1.5m. Fertilizers were applied @ 65 kg Urea, 30 kg MP and 5000 kg cowdung per hectare. Normal cultural practices were followed. Spacing between lines and plants were 15 cm and 3 cm, respectively. The experiments were harvested 30 to 35 days after sowing. Data on seedling height, number of leaves/plant, leaf weight of edible portion/m<sup>2</sup> and leaf area were recorded. Edible portion means young leaves and about 1cm soft stem of upper part of the seedling. Leaf area was measured only from the experiment conducted at Mymensingh by a leaf area meter. Yield data were recorded from sample area and then converted into kg/ha. The analyzed data and increased edible portion (%) are presented in Table 1.

Due to the high number of leaves and leaf area/plant the mutant CM-18 showed the highest edible portion yield. On average, edible portion (*Patshak*) yield of the mutant line CM-18 was found 45.3 and 40.3% increase over CVL-1 and Atompat-38, respectively. The leaf was also found attractive due to its deep green colour. The mutant will be tested on farmers' fields in the coming season. Farmers' field trials are a prerequisite for registration of a variety with the Na-

tional Seed Board. It is hoped that the export of green leaves as vegetables (*Patshak*) and as well as for local consumption, will contribute to the country's economy.

Mutant/varieties	No. of leaves/plant	Leaf area/plant (cm <sup>2</sup> )	Edible portion (kg/ha)	Edible portion over (%)
CM-18 (Mutant)	12.16a	107.0a	1562.5a	-
CVL-1 (Parent)	8.92b	54.5b	1075.0c	45.3
Atompat-38 (Cultivar)	8.07c	56.7b	1113.0b	40.3

## 'NIFA-Mustard Canola' – First Mutant Variety of Oilseed Mustard (*Brassica juncea* COSS & CZERN.) in Pakistan

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The edible oil production in the country is short of requirements to the tune of 70%. The gap is filled by heavy imports costing the national exchequer over 400 million US\$ annually. Increasing domestic production of edible oil is thus of high priority for the Government of Pakistan. Rapeseed and mustard (*Brassica juncea*), being indigenous oilseed crops and well entrenched in the cropping system of the growers, have the potential to bridge the gap between production and consumption. Mustard is the predominantly grown species of oilseed Brassica in Pakistan as it has superior adaptability to semi-arid conditions and tolerance to drought as well as heat and seed shattering. However, no *juncea* cultivar of canola quality is available in Pakistan so far. In this backdrop, a comprehensive research program was launched in 1994-95 at NIFA, with the help of IAEA, through a Technical Cooperation Project to develop improved varieties of mustard.

Gamma irradiation and fast neutrons were used to induce genetic variability in traits of economic importance, thus diversifying the genetic base of indigenous/exotic cultivars (cvs.) of Brassica oilseeds. About 15,000 dry seeds of exotic mustard line (DLJ-3) having about 10% moisture were irradiated at 1.0, 1.2 and 1.4 kGy gamma rays (<sup>60</sup>Co gamma source) in 1994-95. The treated seeds were planted directly in the field in isolation of M<sub>1</sub> generation. Selection for desirable mutants was carried out in M<sub>2</sub> and useful mutants, including MM-1266, were selected. The stability of selected traits of MM-1266 was confirmed in M<sub>3</sub> and M<sub>4</sub> generations. MM-1266 was thoroughly assessed for yield in different replicated trials at NIFA and other stations in Pakistan and the results of MM-1266 in comparison with the control cv. is presented in Table 1. In the preliminary yield trial in rabi 1997-98d at NIFA, MM-1266 produced the highest yield (1908 kg/ha) and significantly outyielded the parent by 100%. The following year, MM-1266 was tested in advanced yield trials (AYT) at NIFA. The line exhibited sustainable yield performance (2042 kg/ha) and produced over 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 multi-location yield test in North West Frontier Province (NWFP) during 1999-2000. The pooled data over sites indicated that MM-1266 significantly outyielded the parent variety that was used as a check (in 3 irrigated and 2 rainfed environments). MM-1266 was assessed for adaptability in diversified environments throughout Pakistan in national Uniform Mustard Yield Trials (NUMYT), which were conducted by the Pakistan Agriculture Research Council, Islamabad. MM-1266 produced 1.5 t ha<sup>-1</sup> yields and outyielded the non-canola check (BARD-1) in 3 out of 7 sites in NUMYT 2000-01. In NUMYT 2002-2002, MM-1266 repeated its excellent performance and produced again 1.5 t ha<sup>-1</sup> yields and outyielded the check in 3 of 7 sites, indicating broader adaptability and genetic stability.

**Table 1. Yield data (kg/Ha) of NIFA-mustard canola (MM-1266) and control cvs. in different yield trials, 1997-98 to 2001-2002**

Yield test/year	Cultivars		(%) Yield increase of MM-1266
	MM-1266	Control	
PYT, 1997-98	1908.0	950.0 (parent)	100.84
AYT, 1998-99	2041.8	1104.5 (parent)	84.86
MYT, 1999-2000	1464.5	895.8 (parent)	63.49
NUMYT, 2000-01, Selected sites Mean	1716.7	1643.3 (commercial cv)	4.5
NUYT, 2001-02, Selected sites Mean	1370.3	1188.0 (commercial cv)	15.35

MM-1266 matured significantly earlier than parent and commercial cv. with a margin of 23 and 9 days respectively at different sites. Significant reduction in plant height of exotic mustard line (DLJ-3) was induced through gamma rays irradiation. A reduction of 60-70 cm in plant height of MM-1266 from the parent was observed. The mutant is also moderately resistant to *Alternaria* blight (*Alternaria brassicae*), *Sclerotinia* stem rot (*Sclerotinia sclerotiorum*) and downy mildew (*Peronospora parasitica*) diseases.

Results regarding oil content, erucic acid and glucosinolates are shown in Table 2. MM-1266 possesses 47% oil as against 41% of control; an increase of 14.6% over controls cv. It contains less than 1.3% erucic acid (C<sub>22:1</sub>) and 22 micromoles total glucosinolates per gram seed. Based on its quality characteristics, oil of MM-1266 (NIFA – MUSTARD CANOLA) is suitable for human consumption and its meal is fit for animal use as part of their ration.

**Table 2. Oil, erucic acid and total glucosinolate contents of MM-1266 (NIFA – MUSTARD CANOLA), DLJ-3 (Parent) and BARD-1 (commercial cv.), analysed by GC-UNICAM 6010**

Entry name	Oil content (%)	Erucic acid (% of total fatty acids)	Glucosinolates (µM/gm)
MM-1266	47.0	1.3	22.0
BARD-1 (Control)	41.0	49.7	104
DLJ-3 (Parent/control)	39.2	0.9	18

MM-1266 is uniform, stable and morphologically distinct from the parent cultivar. Based on its superb performance in different yield trials and its wider adaptability to diversified climates, MM-1266 was approved by the NWFP Provincial Seed Council on 8 October 2003 for normal Rabi (winter) planting in irrigated and rainfed areas of NWFP under the name of NIFA-Mustard Canola.

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