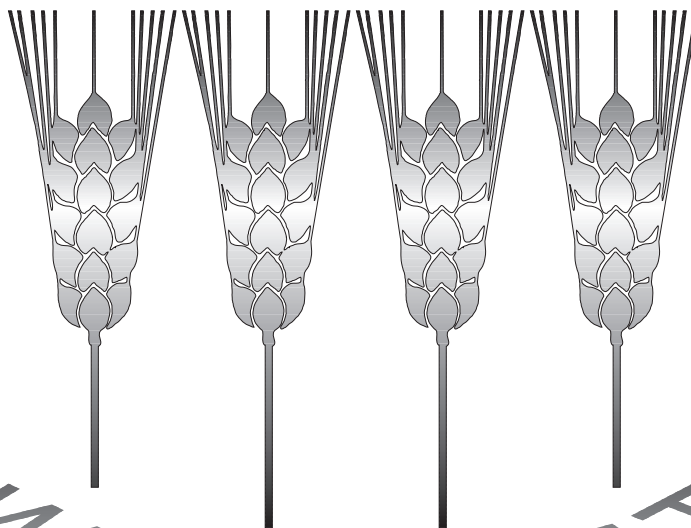


MUTATION BREEDING

NEWSLETTER



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'ABASIN-95', A NEW OILSEED RAPE CULTIVAR DEVELOPED THROUGH INDUCED MUTATIONS

Brassica oilseeds are the second most important source of vegetable oil in Pakistan. Due to the low priority attached to these crops in the past, no systematic breeding work was undertaken to develop improved varieties of rapeseed/mustard, resulting in a narrow genetic base of these crops. At the Nuclear Institute for Food and Agriculture (NIFA), gamma radiation was used to induce genetic variability in traits of economic importance thus diversifying the genetic base of indigenous/exotic cultivars of Brassica oilseeds. About 10,000-15,000 dry seeds of oilseed rape (*Brassica napus* L.) cultivar 'Tower', having about 10% moisture, were irradiated at 1.0, 1.2 and 1.4 kGy gamma rays (^{60}Co) in 1988. The treated seeds were planted directly in the field in isolation as M_1 generation. Selection for desirable mutants was carried out in M_2/M_3 generations and a useful mutant, RM-152-2, was selected in M_3 . To speed up the breeding process two generations a year were raised, one at NIFA in winter and the other in summer at Hill Agricultural Research Station, Kaghan,. This shuttle breeding programme resulted in significant achievements in a short time span. The M_5 mutants were tested in preliminary yield trials during 1990-91, at NIFA and mutant RM-152-2 significantly outyielded the parent variety and a commercial cultivar 'PR-7', by producing 2 t/ha grain yield, against 1.4 t/ha of control. This mutant was tested in advanced yield trials under irrigated as well as rainfed conditions during 1991-92. RM-152-2 again significantly outyielded the control cultivars under both environments and produced 1.8 and 1.67 t/ha yield of the control. Based on its excellent performance in these trials, RM-152-2 was assessed simultaneously in a multilocation yield trial in North West Frontier Province (NWFP), and in a 30-entry National Uniform Rapeseed Yield Trial (NURYT) for two consecutive years i.e. 1992-93 and 1993-94. In the multilocation trial in NWFP, RM-152-2 significantly outyielded the control cultivar at all locations in both years, producing 1.8 t/ha grain yield (Table 1). It is very clear that RM-152-2 significantly outyielded the control cultivars under both irrigated and rainfed conditions in both years. The mutant line produced 3rd highest yield of 1.62 t/ha (average of two years) amongst 30 entries (candidate varieties) consecutively for two years in the National trials. These results clearly indicated genetic stability of RM-152-2 over years and locations. RM-152-2 outclassed all the commercially grown cultivars by wide margins i.e. 'Pak cheen' by 13.3% and 'DGL' by 5.2% (two non canola cultivars), and 'Shiralee' and 'Westar' (both canola type) by 10.2% and 18.4% respectively.

Table 1. Yield and other characteristics of Abasin-95 and commercial cultivars in NURYT, 1992-93 and 1993-94 (average of 15 sites)

Cultivars	Maturity (days)	Plant height (cm)	1000 seed wt. (g)	Yield (kg/ha)	Yield increase of Abasin-95 (%)
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)	-
Pak cheen	175.9 (160-184.5)	156.3 (133-175.7)	3.8 (2.8-5.0)	1416.3 (potential 2.0 t)	13.3
DGL	177.0 (166-186)	151.6 (124.1-175.1)	4.1 (3.1-5.7)	1526.3 (potential 2.5 t)	5.2
Shiralee	179.3 (168-188)	159.6 (134-197)	3.4 (2.9-4.2)	1456.9 (potential 2.4 t)	10.2
Westar	178.7 (158-187)	156.9 (131-185)	3.6 (2.9-4.9)	1355.2 (potential 2.2 t)	18.4

* Ranges are given within parenthesis

RM-152-2 matured significantly earlier than control cultivars at different irrigated and rainfed sites in the National trials. The earliness in maturity ranged from 6 to 18 days at different sites, however, it matured a week earlier than the controls (average of 15 sites for two years). Plant height of RM-152-2 (162 cm) is almost the same as that of parent and other check cultivars. The mutant is also moderately resistant to *Alternaria blight* (*Alternaria brassicae*) and *Sclerotinia stem rot* (*Sclerotinia sclerotiorum*) and completely resistant to downy mildew (*Peronospora parasitica*) and white rust (*Albugo cruciferarum*).

Results regarding oil content, erucic acid and glucosinolates (courtesy: NARC, Islamabad) indicated that RM-152-2 possesses 46% oil (range 43-47% at different sites) as against 42% of Tower and 43% of Pak Cheen (Table 2). It contains less than 3% erucic acid (C_{22:1}) and 25 micromoles total glucosinolates per gram of oil free meal. Based on its quality characteristics, RM-152-2 falls in to the Canola standard for Pakistan (which require less than 5% erucic acid in oil and less than 40 micromoles of total glucosinolates per gram of oil free meal).

Table 2. Oil content, erucic acid and total glucosinolate content of RM-152-2 (Abasin-95), Pak Cheen and Tower varieties analysed by the Oilseed Analytical Laboratory, NARC, Islamabad)

Entry name	Oil content (%) [*]	Erucic acid (%) [*]	Glucosinolates (μ mole/g)
RM-152-2 (Abasin-95)	46.0 (43-47)	2.98	25.0
Pak Cheen	43.5 (41-44)	33.52	68.8
Tower (Parent variety)	42.3 (41-43)	10.31	41.9

* Ranges are given within parenthesis

RM-152-2 is uniform, stable and morphologically distinct from the parent cultivar Tower. Based on its superb performance in different yield trials and its wide adaptability to diversified climates, RM-152-2 has recently been approved by the NWFP Provincial Seed Council for normal Rabi (winter) planting in irrigated and rainfed areas of NWFP under the name of Abasin-95.

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‘GORNOORIAHOVSKA KAPIA F₁’* - A NEW HYBRID PEPPER VARIETY BASED ON RADIATION INDUCED MALE STERILITY

The female parent line ‘Zlaten medal ms-8’ was obtained by Daskalov [1] as a gamma rays induced mutant of pepper (*Capsicum annum* L.). Dry seeds of the initial variety ‘Zlaten medal’ were irradiated with 135 Gy gamma rays and after screening of a large M₂ population

(57 000 plants) 3 male sterile mutants were obtained. After an allelic test the genes responsible for male sterility were denoted as *ms-6*, *ms-7*, and *ms-8*. After many years of testing various male sterile sources the gene *ms-8* proved to be the most suitable for hybrid seed production because it determines 100% male sterility, independent of the climatic condition or the genotype. The mutation causes the highest reduction of anthers, which allows easy distinction between male sterile and fertile flowers. The development of the mutant male sterile line Zlaten medal *ms-8* is a good example of how mutation techniques can be very useful for adding or changing only one trait without altering the basic genotype.

The male parent line 'GO-250B' was developed at the Vegetable Research Station at *G. Oriahoviza* and is characterized by a good combining ability and many outstanding agronomic traits. The new hybrid variety 'Gornooriahovska kapia F₁' is suitable for early and middle early field production as well as for cultivation under plastic or glasshouses (Table 1). The vegetation period from emergence to maturity is approximately 105 days. The heterotic plants are 60-70 cm high and lodging resistant. The fruits are big, long (14-18 cm), "kapia" type, two to three lobbed, 70-90 g, with tender and tasty flesh (4.5-5.5 mm thickness of the pericarp), 9-10% dry matter, 220-250 mg% vitamin C, 4.5 % sugars. The immature fruits are green and the mature ones – dark red. The variety is resistant to TMV and possesses high field resistance to *Verticillium* and CMV. Hybrid seed production will be performed according to the techniques described by Daskalov [2].

Table 1. Performance of Gornooriahovska kapia F₁ variety under field and plastic house conditions

Variants	Early yield kg/ha				Total yield kg/ha			
	1993	1994	Average	(%)	1993	1994	Average	(%)
Field								
Albena (check)	18440	32490	25640	100.00	36880	41020	38950	100.00
G. kapia*	37500	31740	34620	135.02	73870	60490	67180	172.47
Plastic house								
Albena (check)	42340	23610	32970	100.00	8280	50960	66880	100.00
G. kapia	51000	34500	42750	129.66	10386	59760	81810	122.32

*The hybrid variety 'Gornooriahovska kapia F₁' was developed by T. Hristov, S. Daskalov, L. Milkova and E. Stoimenova.

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'CM 88' – A MULTIPLE DISEASE RESISTANT CHICKPEA MUTANT VARIETY

Chickpea is the most important grain legume crop of Pakistan. *Ascochyta* blight (*Ascochyta rabiei*) and Fusarium wilt (*Fusarium oxysporum* F. sp *cicer*) are most serious diseases, having the potential to devastate a crop [1]. A multiple disease resistant and high yielding mutant CM 88 has been developed through 100 Gy gamma irradiation treatment of

variety 'C 727'. This was once a widely grown and popular variety, which lost its resistance to *Ascochyta* and was replaced. The selection of mutants was performed in the M₂ generation grown in the *Ascochyta* blight nursery and sixteen mutants were selected [2]. In the subsequent generations CM 88 proved resistant to both *Ascochyta* blight and *Fusarium* wilt, and exhibited superiority in agronomic characteristics. CM 88 was also tested for many years in the various yield trials on research stations and farmers fields throughout the country. In these trials it out yielded both the parent and standard varieties [3]. The mutant CM 88 has been approved by the Punjab Seed Council on 27 October 1994 for general cultivation in the Punjab Province, especially the Thal area which accounts for more than 70% of the area under chickpea cultivation.

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A HIGH YIELDING, BETTER QUALITY CHICKPEA MUTANT VARIETY 'NIFA-95'

Chickpea or gram (*Cicer arietinum* L.) is an important legume crop of Pakistan, grown on over one million hectares annually. The national average yield of the crop is very low (0.5 t/ha) and thus the country had to spent about 2 billion rupees (\$ 50 million) on import of pulses. The main causes of low yield are non-availability of genetic sources for resistance to various diseases especially gram blight *Ascochyta rabiei* (Pass.) Lab., insect pest (Pod borer) and non-adoption of proper production technology by the farmers. This calls for earnest efforts of breeders to evolve high yielding and disease resistant varieties of chickpea for provision of quality seeds to the farming community to increase production of this important crop.

Seeds of a highly blight susceptible variety '6153' were irradiated at 200 Gy dose of gamma radiation in 1985 and the promising mutant line CMN-446-4 was selected in M₃ generation on the basis of disease resistance, greater number of pods and better plant type. After confirmation of its resistance to blight in M₄ and M₅, the mutant line was evaluated in various trials at different locations. In the advanced and zonal yield trials during 1993-95, the line CMN-446-4 produced the highest grain yield of 2,600 kg/ha as compared to the rest of the mutants and varieties. The line was also evaluated in the chickpea national uniform yield trial, conducted on over 11 locations in the country during 1993-94. In this trial, the mutant line ranked 3rd by producing an average yield of 1,528 kg/ha as compared to the two check varieties 'Punjab-91' (1,316 kg/ha) and 'Paidar-91' (1,391 kg/ha). The mutant line CMN-446-4 is moderately resistant to gram blight, highly resistant to stored pest (pulse beetle), contains 25.3% more protein as compared to the parental variety 6153 and is also better in nitrogen fixing capacity.

The proposal for release of the mutant line CMN-446-4 as a new variety under the name 'NIFA-95' for general cultivation in the rainfed area of North West Frontier Province was approved by the Provincial Seed Council on 8 December 1996. The characteristics of the mutant variety NIFA-95 are presented in Table 1.

Table 1. Agronomic characters of mutant variety NIFA-95 and its parent

Characteristics	Mutant variety NIFA-95	Parent variety 6153
Growth habit	Semi spreading	Semi spreading
Plant height	86 cm	84 cm
Flower colour	Pink	Pink
Days to flowering	136	130
Days to maturity	205	190
Seed coat colour	Light brown	Dark brown
Seed surface	Rough	Smooth
1000 grain wt (g)	186	220
Blight resistance	MR	H.S
Protein content (%)	25.2	20.1
Av. yield (kg/ha)	2600	400

(Contributed by *HASSAN, S., M.A. JAVED, S.U.K. KHATTAK and M.M. IQBAL, Nuclear Institute for Food and Agriculture, P.O. Box No.446, Peshawar, Pakistan*)

IMPACT OF MUTANT VARIETIES OF BLACKGRAM IN REALISING IMPROVED PRODUCTIVITY

Blackgram (*Vigna mungo* L. Hepper) is an important pulse crop extensively grown in India. It is the cheapest source of protein for millions of Indians. The seeds contain about 22% protein. The area under cultivation in India is about 3.25 million hectares with an annual production of 1.45 million tons. About 70% of the total area is in the Central and Southern part of the country, which contributes about 77% of the total production. In the past there have been attempts to increase the production and productivity of this crop using conventional breeding approaches at different Agricultural Research Centres. However, the yield remains around 500 kg/ha. We have used induced mutation techniques to break the yield barrier. The induced mutations were used in cross breeding to synthesise an ideal plant type with high yield potential suitable for different agroclimatic conditions.

Induced mutation experiments were initiated at Nuclear Agriculture and Biotechnology Division of Bhabha Atomic Research Centre, Mumbai during 1973-74 using 'No. 55', a variety popular in Maharashtra state and later during 1986 with 'EC168200' an exotic collection obtained from AVRDC, Taiwan. The seeds were exposed to gamma rays (15 to 750 Gy). Fast neutron irradiation (20 to 60 Gy) was carried out at the 'APSARA' reactor at Trombay, BARC in a specially designed Standard Neutron Irradiation Facility (SNIF) to obtain fast neutrons free from slow neutrons and gamma rays.

In all, forty-nine true breeding mutants with distinct morphological characters were established and classified on the basis of most conspicuous and easily discernible morphological and agronomic traits like chlorophyll, growth, leaf, pod, seed characters flowering and/or maturity. Though no mutant superior in yield per se compared to the parent variety No. 55 or EC 168200 was obtained, several mutants superior in one or more yield components were isolated like early flowering, dwarf, altered branching pattern, large seed,

shiny and green seed etc. Two large seed mutants UM-196 and UM-201 with 100 seed weight 5.6 and 6.9 g respectively as compared to 4.5 to 5 g of the parent variety No. 55 were used in the cross breeding programme. The desirable recombinants having increased seed size, early maturity and high yield were selected from the segregating population and this has resulted in the development of 'TAU-1', 'TUA-2' and 'TPU-4' varieties for different agroclimatic conditions in the country [2; 3] (Table 1). Later on an early maturing mutant TAU-5 of yellow mosaic virus resistant EC168200 was used in the cross breeding programme. 'TU 94-2' with high yield and resistance to yellow mosaic virus disease was developed and released for commercial cultivation. TAU-5 has also been identified as donor parent for yellow mosaic virus resistance by the All India Pulse Improvement Programme [1].

The development, introduction and later popularization of Trombay blackgram varieties in many states have made a significant impact in increasing the production. Blackgram varieties developed at BARC are well suited to Central and Southern parts of the country. Among the blackgram varieties released, TAU-1 with large seed size has become the most popular variety in Maharashtra, Karnataka and Andhra Pradesh. Presently TAU-1 is grown on an area of 500,000 ha (95% of the total area under blackgram) in Maharashtra state. It was also released for adjoining state Karnataka during 1996. About 20,000 tons of certified seed have been distributed by Maharashtra State Seed Corporation, Akola to the farmers of Maharashtra since 1990.

Though the area under blackgram increased marginally after TAU-1 release, the production and the productivity of blackgram in Maharashtra has increased dramatically in 1999 by 60 and 50% respectively (Table 2). The additional production of 129,400 tons of blackgram achieved in 1999 in the state as compared to 1989 is due to increase in the productivity of TAU-1. The national income generated due to this increased production amounts to a considerate estimate of 67 million dollars annually. The other three varieties TAU-2, TPU-4 and TU94-4 developed under induced mutation approaches are becoming popular in Central and Southern States and the breeder seed indent of the Ministry of Agriculture, Government of India for all four varieties is almost 48% of the total indent of blackgram breeders seed during 2000-2001.

Table 1. Released and notified varieties of blackgram developed at BARC

Variety	Pedigree	Year of release	Area of adaptation	Yield (kg/ha)	Yield increase (%) and character
TAU-1	T-9 x UM-196 (Mutant of No. 55)	1985	Maharashtra Karnataka	975	27; large seed size
TAU-2	T-9 x UM-196	1992	Maharashtra	1158	18 (over TAU-1)
TPU-4	UM-201 (Mutant of No. 55) x T-9	1992	Madhya Pradesh Gujarat Maharashtra	884	22 (over check PU-30)
TU-94-2	TPU-3 x TAU-5 (Mutant of EC-168200)	1999	Andhra Pradesh Karnataka Tamil Nadu Kerala	962	35 (over PU-30); Resistance to YMV

Table 2. Area, production and productivity of blackgram in Maharashtra

Year	Area (thousand ha)	Production (thousand tons)	Productivity kg/ha
1989	513.0	215.0	419.0
1999	546.0	344.4	631.0
Increase (%)	6.4	60.2	50.6

Source: The Economics and Statistical Survey of India, Ministry of Agriculture, Govt. of India.

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AN EARLY MATURING RICE MUTANT RELEASED AS A VARIETY

In the context of food grain production deficiency (about 1.0 – 1.5 million tons of rice per year according to the Bangladesh Bureau of Statistics, 1998) an induced mutation programme was undertaken in 1985. One moderate early maturing and high yielding rice mutant line (BINA6-84-4-115) has been developed by irradiating F₂ seeds of the cross 'BR4' x 'Iratom 38'. Three treatments viz., 250, 300 and 350 Gy were given to the F₂ seeds. Finally, this line was selected in M₆ generation for advanced yield trial. The line was evaluated in comparative trials with another mutant line BINA6-84-4-163. These two mutant lines had been selected earlier from 300 Gy originated lines. The two check varieties, 'BR 11' and 'BR 22' were also included in the trial, which was conducted in two consecutive T. aman seasons (July to December) during 1994 and 1995 at five locations in Bangladesh.

From the results, it was evident that the mutant BINA6-84-4-115 did not differ much with the other mutant lines or check varieties in respect to plant height, number of effective tillers and panicle length but it was 10-18 days earlier than the other 3 entries (Table 1). It produced a similar yield as the check BR 11 in 1994 and a higher yield than the check BR 11 and BR 22 in 1995. This mutant line gave the highest yield per day among all the entries (Table 2). In addition to this, the grains are long, fine and possess a high L/B ratio, which are of high commercial value. This line has been released by the National Seed Board of Bangladesh in 1998 as a commercial variety under the name "BINADHAN-4" for cultivation throughout Bangladesh.

Table 1. Some important traits of BINA6-84-4-115 (BINADHAN-4) compared to two check varieties

Variety/line	Plant height (cm)	No. of effective tillers	Panicle length (cm)	No. of grains/panicle	Days to maturity	1000-grain wt. (g)	Grain length (mm)	Grain breadth (mm)	L/B ratio
BINA6-84-4-115	117	9.0	25.6	118	130	24.60	9.9	2.85	3.47
BINA6-84-4-163	108	9.1	25.5	116	140	25.65	7.9	2.70	2.93
BR 11 (check)	116	9.2	24.0	122	138	24.53	8.0	2.75	2.91
BR 22 (check)	118	9.7	27.0	137	148	20.02	7.9	2.40	3.29

Table 2. Grain yield performance of BINA6-84-4-115 (BINADHAN-4) compared to two check varieties

Variety/line	Grain yield (kg/ha)		Average yield (kg/ha)	Average yield/day (kg/ha)
	1994	1995		
BINA6-84-4-115	4897b	4670b	4783.5b	36.80
BINA6-84-4-163	5140a	4882a	5011.0a	35.79
BR 11 (check)	4990b	4619c	48.4.5b	34.81
BR 22 (check)	5094a	4555d	4824.5b	32.59

Same letters in a column did not differ significantly at 5% level

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INDUCTION OF RESISTANCE TO BLAST DISEASE IN AN ELITE RICE CULTIVAR 'IR 50'

One of the most promising techniques for producing disease resistant forms of plants is the use of mutagenic agents. It has been demonstrated by several workers that genetic variability for several desired characters can be induced successfully through mutations and its practical value in plant improvement programmes has been well established. The main advantage of mutation breeding is the possibility of improving one or two characters without changing the rest of the genotype.

The elite cultivar, 'IR 50' (IR 2153-14-1-6-2/IR 28//IR 36) was developed at IRRI, Los Banos, The Philippines and was released in India for the State of Tamil Nadu in 1982. It is highly responsive to fertilizer, records high yields and possesses good grain characters. It matured in just more than 100 days and was ideal for both samba and navarai seasons in Tamil Nadu. But, the cultivar was shown to be highly susceptible to blast (causative organism *Magnaportha grisea*) causing extensive losses year after year. With the objective of developing high yielding, blast tolerant mutant lines from IR 50, the mutation approach was adopted and both physical (gamma-rays from ^{60}Co) and chemical mutagens (EMS - ethyl methanesulphonate and sodium azide) were employed on dry seeds. The M_1 generation was grown in closely spaced plants. One hundred and sixty-eight derived families were grown in M_2 . In M_3 generation, 128 M_3 families were further selected for evaluation in M_4 and M_5 . Based on evaluation of yield and other attributes, a total of 85 mutants were finally selected and evaluated for their stability. In selection of the mutants, it was ensured that all the selected mutants resemble the parent for both agronomic and quality characteristics.

The evaluation of these mutant lines for the level of tolerance to blast disease was conducted at CRRRI over a number of years under both artificial and natural conditions. These mutant lines showed varied levels of tolerance to blast in comparison to total susceptibility of

the parent to the disease. The mutants were tested at different 'hot spot' locations of blast like Hazaribag in Bihar, Maruteru in Andhra Pradesh and Jagdalpur in Madhya Pradesh. In addition, they were also screened under greenhouse conditions at the Directorate of Rice Research, Hyderabad. Experimental data from all these centers support the earlier finding that variation for tolerance to blast exist in these mutant lines.

The relatively highly tolerant mutant lines were further evaluated under artificial screening at CRRRI and highly tolerant individual plants with individual scores of 1 and 2 as against the parent variety score of 7 to 9 (in the IRRI disease score scale of 1 to 9) were selected. After seed multiplication, yield evaluation trials were conducted on fourteen different individual plant derived lines. The field evaluation data on the selected fourteen mutant lines i.e. CRM 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54 and 58) indicate that all these mutant lines yielded either at par or higher than the parent.

The mutants were further tested for their suitability in the replacement of the parent variety in the State of Assam. In the yield evaluation and adaptation trials conducted at Kokilabari Farm, Assam, the mutants performed consistently with a yield of over 3 t/ha. Further evaluation of CRM mutant lines over a four year period at Regional Agricultural Research Station, Assam Agricultural University, Diphu, Assam revealed that three mutant selections, i.e. CRM 49, 51 and 53, consistently yielded double that of the parent (2.5t/ha in comparison to 1.25t/ha for parent). Further, in the trials conducted at Zonal Agricultural Research Station of Indira Gandhi Krishi Viswa Vidyalaya, Jagdalpur, the CRM mutants performed well for both yield and the disease scores. Based on the performance of these mutants, the Government of Assam is proposing the release of three mutants namely, CRM 49, 51 and 53 and wishes to replace the parent cultivar IR 50 with these high yielding and blast tolerant mutants.

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INDUCTION OF DROUGHT TOLERANT MUTANTS OF RICE

The ultimate goal of crop breeding is to develop varieties with a high yield potential and desirable agronomic characteristics. In Egypt, the most important qualities sought by breeders have been high yield potential, resistance to major diseases and insects, and improved grain and eating quality. However, breeding efforts should concentrate on varieties with the potential to minimize yield losses under unfavorable conditions such as drought, and to maximize yields when conditions are favorable. Rice (*Oryza sativa* L.) in Egypt is completely irrigated and a significant portion of the rice cultivated area is subject to water deficit resulting from an inadequate or insufficient irrigation supply. Drought tolerance is a complex trait in that it results from the interaction of histological and physiological characters of plant with environmental factors, both above-ground and under-ground [2]. Accordingly, root characters are closely related to drought tolerance. Little attention has been paid in Egyptian breeding programs to root characters and their relation to shoot characters. Furthermore, induced mutations are considered as one of the most important methods to induce useful mutants, especially with improved root characters, to overcome the drought problem. The present investigation aimed to study the effect of different doses of gamma rays on several characters of three Egyptian rice varieties, i.e. 'Giza 171', 'Giza 175' and 'Giza 176' and to induce one or more mutants possessing drought tolerance.

In the 1991 season, five gamma-rays doses i.e., 100, 200, 300, 400 and 500 Gy, were used to treat the seeds of the above varieties from ^{60}Co source at the National Center for Radiation Research and Technology, Cairo, Egypt. Treated seeds, together with untreated ones, were directly grown in the nursery and all surviving seedlings were individually transplanted in the permanent field. At heading, the first emerged panicle of each plant was bagged and harvested individually. Viable mutations were selected in M_2 according to the significant differences between these mutants and the respective parent variety in visual drought symptoms, shoot dry weight, root dry weight, and root/shoot ratio. The procedures were followed till M_7 in 1997 when some selected mutants were tested in a randomized complete block design experiment with three replicates and irrigation water was applied every 14 days. The visual score of drought symptoms were recorded on the basis of visual scoring systems [1].

Seven mutants were selected from the three rice varieties (2 mutants from Giza 171, 3 mutants from Giza 175, and 2 mutants from Giza 176), which proved to be tolerant to drought conditions in comparison to the respective parent variety. Selected mutants showed an improved trend regarding the studied characters (Table 1).

Table 1. Means of studied characters for the selected mutants from the three rice varieties under drought conditions in M_7 generation

Entries	Visual drought symptoms	Shoot dry weight (g)	Root dry weight (g)	Root/shoot ratio
Giza 171 (Control)	7.31 ± 0.19	6.64 ± 0.55	1.35 ± 0.61	0.20 ± 0.01
G 171 M7-1	3.54 ± 0.81	10.81 ± 0.15	4.03 ± 0.31	0.37 ± 0.28
G 171 M7-2	3.02 ± 0.07	10.07 ± 0.15	3.99 ± 0.70	0.40 ± 0.22
Giza 175 (Control)	6.92 ± 0.08	7.92 ± 0.45	2.03 ± 0.11	0.26 ± 0.08
G 175 M7-1	2.56 ± 0.13	11.35 ± 0.17	5.61 ± 0.15	0.49 ± 0.42
G 175 M7-2	3.01 ± 0.24	10.92 ± 0.25	4.93 ± 0.07	0.45 ± 0.15
G 175 M7-3	2.67 ± 0.57	10.84 ± 0.11	5.88 ± 0.63	0.54 ± 0.24
Giza 176 (Control)	7.94 ± 0.22	5.83 ± 0.11	1.05 ± 0.15	0.18 ± 0.03
G 176 M7-1	3.15 ± 0.44	10.73 ± 0.66	4.89 ± 0.28	0.46 ± 0.73
G 176 M7-2	2.62 ± 0.06	10.89 ± 0.43	5.07 ± 0.57	0.47 ± 0.56

The visual drought symptoms ranged between 2.56 and 3.54 for the mutants G175 M7-1 and G 171 M7-1, respectively. However the same score was 7.31 for Giza 171, 6.92 for Giza 175 and 7.94 for Giza 176 indicating that all these selected mutants were more tolerant to drought conditions. In these mutants the highest shoot dry weight (11.35 g) was observed in G175 M7-1 and the lowest (10.07 g) in G171 M7-1. The same character differed from 5.83 g to 7.92 g for Giza 176 and Giza 175, respectively. In addition, the highest root dry weight (5.88 g) was found in the mutant G175 M7-3, which also showed the highest root/shoot ratio (0.54). In general, these selected mutants could be utilized as drought tolerant varieties and/or as a source of drought tolerance in the hybridization breeding program.

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INDUCED MUTATION FOR TUNGRO RESISTANCE IN RICE

Tungro is the most serious virus disease of rice in South and Southeast Asia. It is a composite disease of two kinds of viruses, rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). Damage to the plant is mostly caused by RTBV, while RTSV acts to facilitate RTBV acquisition and transmission by insect vector. Both viruses are transmitted mainly by green leafhopper (GLH). Resistance to GLH is common in rice germplasm but extremely rare for the two viruses. To induce mutations for tungro resistance, a susceptible variety IR22 was treated with N-methyl-N-nitrosourea (MNH) following the procedure of Satoh and Omura [1]. The panicles of rice variety 'IR22' were soaked in 1 mM MNH solution for 45 minutes at 16 to 18 hours after flowering.

Two thousand six hundred and forty fertile M₁ plants were produced. From these plants M₂ lines with 10 or more seedlings were planted in the field to evaluate their reaction against tungro under natural conditions in the 1990 dry season on the IRRI central research farm, Los Banos, the Philippines. Of these, 124 M₂ lines were selected by visual evaluation. Five plants were harvested individually from each selected line. A bulk was also made from all the remaining plants in the line. In the M₃ generation, each family consisted of five sister lines and one bulked line. One line (M₃-723) showed no tungro symptoms and its related bulk segregated for resistance but all other M₃ lines from the same family were susceptible to tungro. The resistant line, M₃-723, showed low infection with RTBV and RTSV when leaves were tested by enzyme-linked immunosorbent assay (ELISA) to diagnose tungro infection. All M₄ lines from M₃-723 showed uniform resistance in the field. They were not infected with RTBV and were resistant to RTSV infection (Table 1). The reaction of these plants to the virus vector GLH was variable.

To investigate the resistance of the M₄ lines in the field, they were inoculated with viruliferous GLH at the 10-day-old seedling stage in the laboratory. The rate of tungro infection of these selections, at 14 days after inoculation, was as high as the susceptible variety IR22 (Table 1). When the lines were diagnosed at 30, 60 and 90 days after inoculation, the infection rate did not decrease with the sampling date (Table 1). This indicates that the mutant lines are susceptible to tungro infection at the seedling stage and the infected plants do not recover from the disease. To determine whether the resistant reaction in the mutants differ among the growth stage, the progeny of M₄ lines, that showed no infection with either RTBV or RTSV in the field but showed low level of antibiosis to GLH, were used. They were inoculated with viruliferous GLH at 10, 24, 38, 52, 66 and 88 days after seeding, respectively. Both RTBV and RTSV infection rate decreased rapidly with age in selected M₅ lines (Table 2). Those mutant lines slightly susceptible to RTSV at the seedling stage were resistant at early tillering stage 24 days after seeding. All the mutant lines were susceptible to RTBV at the young seedling stage but became resistant at maximum tillering stage (52-66 days after seeding). It was therefore concluded that these mutant lines have resistance to both RTBV and RTSV infection at the maximum tillering stage, and they possess adult plant resistance to tungro.

Table 1. Reaction of mutant lines resistant to tungro in the field and in the laboratory on different sampling stage

M ₄ line and variety	Plant ¹ derived from M ₃	Infection in field (%)		Infection in laboratory when 10-day-old seedlings were tested (%)													
		At maturity ²		14 DAI ³				30 DAI				60 DAI				90 DAI	
		Plants tested (No.)	RTBV	RTSV	Plants tested (No.)	RTBV	RTSV	RTBV	RTSV	RTBV	RTSV	RTBV	RTSV	RTBV	RTSV	RTBV	RTSV
453	723-1	24	5	19	25	92	4	92	4	92	4	92	4	92	8	92	8
454	723-2	11	0	9	3	100	0	100	0	100	0	100	0	100	0	100	0
455	723-3	18	0	11	26	89	4	92	15	96	15	96	15	96	15	96	15
456	723-4	19	0	26	24	83	0	83	12	88	17	88	17	88	12	88	12
457	723-5	20	0	10	12	92	0	92	8	92	8	92	8	92	0	92	0
458	723-6	23	0	17	9	78	11	89	22	100	22	100	22	89	22	89	22
459	723-7	18	0	11	9	89	0	100	0	100	0	100	0	100	0	100	0
460	723-8	22	0	23	14	100	0	100	21	100	21	100	29	100	21	100	21
461	723-9	24	0	25	13	92	0	100	8	100	8	100	8	100	8	100	8
IR22		24	92	92	16	94	19	100	81	100	81	100	-	100	100	100	100

¹ Line and plant number of M₃ generation; ² Leaves were sampled at maturing stage in the field to diagnose for tungro infection, on the other hand, they were sampled on different days after inoculation in the laboratory; ³ DAI; days after inoculation

Table 2. Percentage of tungro infection in 20 plants each of M₅ lines on different inoculation dates for ELISA test

M ₅ line and variety	Plant ¹ derived from	10 DAS ²		24 DAS		38 DAS		52 DAS		66 DAS		80 DAS	
		RTBV	RTSV	RTBV	RTSV	RTBV	RTSV	RTBV	RTSV	RTBV	RTSV	RTBV	RTSV
H 6	M ₄ 460-6	100	5	40	0	55	0	25	0	5	0	5	0
H 10	460-10	100	40	45	0	30	0	20	0	15	5	5	0
I 7	461-7	95	5	50	0	30	0	35	0	5	0	0	0
I 9	461-9	90	10	55	0	45	0	30	0	5	0	0	0
IR22		95	45	100	55	100	25	100	55	50	0	65	0
TN1		100	90	80	70	80	50	100	25	50	15	35	10
ARC11554		15	5	5	0	0	0	0	0	0	0	0	0

¹ Line and plant number of M₄ generation; ² DAS; days after seedling

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RICE MUTANTS OBTAINED THROUGH SODIUM AZIDE (NaN₃) TREATMENT

The successful utilization of sodium azide to generate genetic variability in plant breeding has been reported in barley [3], [4], rice [1], [2] and other crops. Rice seeds of 'Dourado Precoce', Brazilian upland cultivar, were treated with 5×10^{-3} M of sodium azide, prepared in buffer solution of pH 3,0, for 8 hours at laboratory temperature. Ten short culm mutant lines were selected in the M₂, M₃ and M₄ generations. They were denominated 1, 2, 3, 4, 5, 6, 8, 9, 10 and 13. In the M₅ generation, the mutant lines were evaluated for flowering and maturing cycles, tiller number per plant, plant height, panicle number per m², panicle length, fertility of panicle, weight of 1.000 grains, productivity, percentage of intact grains after milling, width and thickness of peeled and polished grains and length/width grain ratio. The experiment was conducted in the Centro Experimental of Instituto Agronômico, Campinas, São Paulo, Brazil, during the period of 1993/94, utilizing randomized block design with four replications. Each experimental plot consisted of five rows of four meters in length, 50 cm between rows, with 75 seeds sown per meter. The cultivar 'IAC 201' and the original Dourado Precoce were planted as checks. All observations were made on the three central rows of each experimental plot. The data was analysed by the SANEST statistical program and the mean values were discriminated by the Tukey's test at the level 5% of probability (Table 1).

Table 1. Mean values of various characters of 10 selected mutant lines, compared with original variety Dourado Precoce using Tukey's test

Mutant/lines	TNP	PH	PL	NFSP	PSSP	WTG	P	GW	GT	GLW R
1	2,16	124,64	20,50	80,84*	11,31	37,23*	5,587	2,53	2,02	3,00*
2	2,10	132,34	22,57	98,41	9,70	36,86	5,953	2,57*	2,05*	2,85
3	1,98	133,74	22,48	101,16	11,90	37,52*	6,433	2,56	2,03	2,84
4	2,53	106,60*	20,71	84,74*	7,47	31,16*	5,703	2,48	1,81*	2,79
5	2,75	107,70*	20,57	80,73*	6,17	30,53*	5,978	2,58	1,87*	2,76*
7	2,85*	112,55*	21,43	84,35*	4,49	31,16*	6,712	2,49	1,84*	2,79
8	2,81*	83,50*	16,75*	49,84*	20,05*	29,93*	2,833*	2,28*	1,95	3,04*
9	2,46	112,00*	20,13*	79,04*	4,69	30,00*	5,908	2,46	1,83*	2,78
10	2,56	107,60*	18,76*	68,42*	6,58	30,68*	5,545	2,48	1,83*	2,77*
13	2,16	128,25	20,66	89,20	11,61	37,28*	5,275	2,55	2,02	2,83
Dourado Precoce	1,99	135,84	23,00	114,84	6,70	34,36	6,278	2,48	1,96	2,86
IAC 201	2,25	119,94*	23,11	169,53*	6,58	24,21*	6,217	1,97*	1,69*	3,59*

*/ significant at the level of 5%

TNP = tiller number per plant

PH = planta height

WTG = weight of 1.000 grains

PL = panicle length

NFSP = number of fertile spikelets per panicle

PSSP = Percentage of sterile spikelets per panicle

P = Productivity

GW = grain width

GT = grain thickness

GLWR = grains length/width ratio

There was no significant difference among treatments for flowering cycle, panicle number per m², length of peeled and polished grains and intact grain percentage after milling. All ten tested lines were different from the parent variety in at least one of the evaluated characters, and only the mutant lines 4, 5, 7, 8, 9 and 10 showed a reduction in plant height compared to parent. An increase was observed in the tiller number per plant in mutant lines 7 and 8, reduction in panicle length in mutants 8, 9, 10, reduction in number of fertile spikelets per panicle in mutants 1, 4, 5, 7, 8, 9 and 10, reduction in productivity and increase in the percentage of sterile spikelets in the mutant lines 1, 3 and 13, increase in grain width in the mutant line 2 and reduction in grain thickness in 4, 5, 7, 9 and 10. In relation to the length/width ratio, which determines the grain type, the reduction was observed in mutant lines 5 and 10 and an increase in mutants 1 and 8. The mutant line 7 was the most promising because it showed reduction in culm length and increase in tiller number, without changing its panicle length and productivity in comparison to parent variety Douado Precoce.

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INDUCTION OF BACTERIAL BLIGHT RESISTANCE IN ELITE INDIAN RICE CULTIVARS USING GAMMA-RAYS AND ETHYL METHANESULFONATE

Rice is the most important cereal crop in the world feeding more than 50 percent of the human population. During the last 30 years, induced 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. Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* is the second most important disease in Southeast Asia. In the Basmati field sometime the yield loss is up to 100%. Moreover, there is no resistance source available in Basmati rice, which is known for its quality and aroma. Induction of bacterial blight resistance in Basmati will help in developing high yielding Basmati type cultivars without compromising the quality. Therefore, seeds of two Indian rice varieties viz. 'PR 106' and 'Pusa Basmati 1' were treated with ethyl methanesulfonate (EMS - 0.25% and 0.5%) at pH 7.0 at 25±1⁰C for 12 h and gamma rays (1 and 2 Gy) (Table 1). A 3500 curie Co⁶⁰ gamma cell with a dose rate of 3200 rads per minute was used for gamma irradiation of the paddy seeds containing 13±1% moisture. The mutagen treated seeds were germinated along with corresponding parent variety in petri dishes lined with wet filter paper. The seeds from the M₁ generation were grown in plant-to-progeny method for the M₂ 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 corresponding parent variety was grown.

Screening against BB was made in the M_2 generation by inoculating the plants at maximum tillering stage, following Kauffman *et al.* [1]. Observations for disease severity were recorded after 14 days of inoculation following standard techniques. Plants with a lesion length up to 2.5 cm were scored as resistant. In M_2 , out of 89,045 plants, a total of 40 lines comprising 145 plants (2.47%) were resistant to bacterial blight. The gene action for resistance was observed in M_2 . It was found that in 34 lines, the resistant plants segregated as controlled by recessive gene. In four cases the ratio of resistant and susceptible plants did not give a clear picture (Table 2). The M_3 generation was raised by the plant-progeny method for all plants of lines screened in M_2 . Observations were made to see whether the resistant lines in M_2 were true breeding in M_3 .

Table 1. Treatments, viability of M_1 and number of lines and plants screened in M_2 for PR 106 and Pusa Basmati 1

Variety	Treatments	No. of seeds treated	Final stand in M_1	M_1 viability up to maturity (%)	No. of lines in M_2	Total plants in M_2
PR 106	EMS 0.25 %	1100	608	55.27	598	13,635
	EMS 0.50 %	1100	481	43.73	481	10,510
	Gamma rays 1Gy	1100	323	29.36	322	6,730
	Gamma rays 2Gy	1100	479	43.55	475	10,380
Pusa Basmati 1	EMS 0.25 %	1100	362	32.91	349	8,290
	EMS 0.50 %	1100	354	32.18	337	8,005
	Gamma rays 1Gy	1100	642	58.36	636	15,910
	Gamma rays 2Gy	1100	661	60.09	631	15,585
Total					3,829	89,045

In PR 106, 24 lines out of 1,876 segregated for BB resistance (1.279%) whereas it was 16 out of 1,953 (0.819%) in the case of Pusa Basmati 1. In a study by Padmanabhan *et al.* [3], 0.36% resistant and 0.65% moderately resistant plants were obtained in the M_2 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_2 were considered as susceptible. The resistance developed in the present study needs to undergo allelic testing and testing against different pathotypes. When observed in terms of mutagens, 17 out of 24 mutants were induced by EMS in case of PR 106 whereas for Pusa Basmati 1, in 12 out of 16 cases the resistance was induced by gamma rays.

Table 2. Number of BB resistance lines and their segregation in M₂ generation

Varieties	Treatments	Gene action			Total
		Segregating 3:1	Non- segregating	Not clear segregation	
PR 106	EMS 0.25 %	5	0	0	5
	EMS 0.50 %	11	0	0	11
	Gamma rays 1Gy	2	0	0	2
	Gamma rays 2Gy	3	0	3	6
Pusa Basmati 1	EMS 0.25 %	2	0	0	2
	EMS 0.50 %	0	1	1	2
	Gamma rays 1Gy	1	0	0	1
	Gamma rays 2Gy	10	1	0	11
Total		34	2	4	40

Induced mutants have been used to develop new BB resistant gene(s) viz. *xa-nm(t)* by Nakai *et al.* [2] and *xa-19* by Taura *et al.* [4]. The productive BB resistant lines developed here were grown in plant-progeny methods by screening in every generation. Advanced lines are under field trial and would be used to study the genetics of the BB resistance or released as commercial cultivars for the farming community of this region.

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AN EXTRA EARLY MUTANT OF PIGEONPEA

The redgram (*Cajanus cajan* (L.) Huth) variety 'Prabhat DT' was gamma irradiated with 100, 200, 300 and 400 Gy doses. Several mutants have been identified viz., extra early mutants, monostem mutants, obcordifoliate mutants and bi-stigmatic mutants. The extra early mutant was obtained when treated with 100 Gy dose. The mutant was selfed and forwarded from M₂ to M₄ generation. In the M₄ generation the mutant line was raised along with the parental variety. Normal cultural practices were followed and the biometrical observations were recorded (Table 1). It was observed that for the characters viz., total number of branches per plant, number of pods per plants, seeds per pod, 100 seed weight and seed yield per plant there was no difference between the mutant and parent variety. Whereas, regarding the days to

flowering and maturity the mutants were earlier than the parents. The observation was recorded from two hundred plants each. The mutant gives the same yield in 90 days as that of the parent variety in 107 days, which make it an economic mutant.

Table 1. Comparative morphological data on mutant and parent variety Prabhat DT

Characters	Mutant		Parent	
	Range	Mean	Range	Mean
Plant height (cm)	66-71	68.60	69-75	72.50
Number of branches	4-7	5.70	4-7	6.20
Days to flowering	46-49	47.20	58-60	58.60
Days to maturity	86-90	88.20	106-112	107.60
Number of pods per plant	75-90	78.00	72-84	78.60
Seeds per pod	2-5	3.10	2-5	3.20
Hundred seed weight (g)	7.2-7.8	7.32	7.1-7.6	7.33
Seed yield per plant	9.5-13.2	10.70	9.3-13.2	10.57
*Estimated yield in kg/ha	---	2379.99	---	2388.89

*Estimated yield was calculated by keeping the same plant population per hectare.

(Contributed by **RAVIKESAVAN, R., T. KALAIMAGAL** and **R. RATHNASWAMY**, Department of Pulses, Tamil Nadu Agricultural University, Coimbatore – 3, India)

GAMMA RAYS INDUCED BOLD SEEDED HIGH YIELDING MUTANT IN CHICKPEA

In pulses especially in chickpea (*Cicer arietinum* L.), genetic variability has been exhausted due to natural selection and hence conventional breeding methods are not very fruitful. Mutation techniques are the best methods to enlarge the genetically conditioned variability of a species within a short time and have played a significant role in the development of many crop varieties [2]. Investigations on the effects of ionizing radiations and chemical mutagens in induction of macro-mutations have received much attention owing to their utmost importance in plant breeding. The present study reports a bold seeded mutant in chickpea, the most dominating pulse crop on the Indian subcontinent.

Fresh seeds of chickpea variety 'Pusa-212' were procured from IARI, New Delhi and treated with different doses/concentrations of gamma rays (^{60}Co source at NBRI, Lucknow) and ethyl methanesulphonate (EMS), individually as well as in combination, to raise the M_1 generation. Seeds of M_1 plants were sown to raise M_2 plant progenies. A bold seeded mutant was isolated from 400 Gy gamma ray treatments. The mutant was confirmed as true bred, all the mutant seeds gave rise to morphologically similar plants in M_3 , which were quite distinct from the control.

The bold seeded mutant showed "gigas" characteristics and vigorous growth. The plant remained initially straight but later on attained a trailing habit due to heavy secondary branching. The leaves, petioles, flowers, pods and seeds were almost double that of the parent variety, in size. The flowering occurred 10 days later than the parent and maturity was also delayed accordingly. Observations were recorded on various quantitative traits (Table 1). Plant height and number of primary branches showed a significant improvement over the parent. It is interesting to note that the number of pods and number of seeds per pod significantly decreased. However, the hundred seed weight ($31.73 \pm 0.59\text{g}$) in the mutant plants was more than double in the parent variety ($12.64 \pm 0.14\text{g}$). This ultimately resulted in an increase in the

overall yield of the mutant plant ($38.86 \pm 1.69\text{g}$) as compared to Pusa-212 ($30.05 \pm 0.59\text{g}$). Gamma ray induced bold seeded mutants have been reported earlier by different workers [1, 3]. The decrease in the number of seeds per pod and pods/plant and increase in seed weight is evidence of the fact that each trait is affected independently by the mutagenic treatment. Although the mutant was morphologically distinct, cytologically it was normal. There were 8 perfect bivalents at metaphase and the anaphase segregation was normal. It is concluded that bold seeded mutant may be utilized in various breeding programs as a donor parent for boldness character of the mutant. On the other hand the mutant may also itself be improved through crosses with other parents to accommodate more seeds in its large sized pod, which remained almost 50% empty.

Table 1. Seed yield and other agronomic traits of mutant and its parent variety (in brackets) in chickpea

Character	Mean \pm SE	Shift in mean	C.V. (%)
Plant height (cm)	61.66 ± 0.85 (59.21 ± 0.71)	+ 2.45* -	5.34 (4.67)
No. of branches/plant	7.46 ± 0.42 (5.73 ± 0.28)	+ 1.73* -	22.00 (19.19)
No. of pods/plant	124.33 ± 4.06 (144.53 ± 1.98)	- 20.20* -	12.65 (5.30)
No. of seeds/pod	1.35 ± 0.03 (1.58 ± 0.09)	- 0.23* -	8.33 (5.95)
100 seed weight (g)	31.73 ± 0.59 (12.64 ± 0.14)	+ 19.09* -	7.29 (4.40)
Plant yield (g)	38.86 ± 1.69 (30.05 ± 0.59)	+ 8.81* -	16.89 (7.64)

*Significant at 1%

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EVALUATION OF HIGH YIELDING MUNGBEAN MUTANTS

Mungbean is the second major (*Vigna radiata* (L.) Wilczek) pulse crop in Pakistan, after chickpea, and is the main pulse crop grown during the spring season in the province of Sindh. Its yield is very low (450 kg/ha) which is mainly due to the non-availability of pure seed of high yield potential genotypes. Keeping in view the importance of induced mutations in all field crops and particularly in the evolution of mungbean cultivars, an induced mutation programme was initiated at AEARC, Tandojam during 1985. Since then a large number of mutants have been developed and are at various stages of evaluation. Among them two mungbean mutants

(AEM 6/20 and AEM 32/20) isolated from the treated population of a local cultivar '6601' with 200 Gy gamma-ray treatment gave very encouraging performance in station as well as zonal trials [1]. On the basis of these results they were promoted in the National Trials, where they remained under evaluation for four years during spring as well as summer seasons. The pool data of four consecutive years of both seasons (Table 1) indicated that mutant lines AEM 32/20 and AEM 6/20 produced 1298 and 1246 kg/ha grain yield respectively as compared to the check variety 'NM 121-25' (1055 kg/ha) evolved at NIAB, Faisalabad through induced mutations. The seed yield increase over the check variety ranged from 18-23%. These two mungbean mutants have short stature combined with short duration and synchrony in maturity. Keeping in view the outstanding performance of these mutant lines, variety release proposals are being submitted to the Technical Sub-Committee for approval of varieties and techniques.

Table 1. Performance of mungbean mutant lines in Sindh province in national uniform yield trials

Genotypes	Mean of 4 years (Kharif 1990-93)	Mean of 4 years (Spring 1991-94)	Overall mean of two seasons	Increase over check (%)
AEM 6/20	1235	1257	1246	18.10
AEM 32/20	1269	1327	1298	23.03
NM 121-25 (Check)	1071	1039	1055	-

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GAMMA RAY INDUCED MALE STERILE MUTANT IN LENTIL

Male sterility refers to the failure of pollen grains to bring about effective fertilization, either due to structural default or physiological disfunctioning and has special significance in hybridization programmes. Male steriles have been produced in a number of crop plants like red gram [1], pigeon pea [3], mung bean [4], khesari [2] and lentil [5].

A completely male sterile mutant was isolated in *Lens culinaris* Medik, after seed treatment with 100 Gy dose of gamma rays. The male sterile mutant showed 100% pollen sterility but was morphologically more vigorous than the parent plants. It showed more branches and its leaves were bigger, more oblong and dark green. The number of flowers borne by the mutant was significantly higher than any other plant of the treatment. The size of the flowers was also increased but the anthers were smaller in size. Pollen grains were few in number, round in shape but empty and did not take up any stain, indicating that normal microsporogenesis had not taken place.

This male sterile mutant was used as the female parent and pollinated with pollen of a parent. Four pods with one seed in each were formed indicating that the mutant was female fertile. The seeds were smaller than those of the parent variety and also dark coloured. The mutant showed increased vigour and flower number as compared to parental plants. Lentil is an important pulse crop and induction of variability in its germplasm is necessary for its improvement. Male steriles can be used conveniently in lentil hybridization programmes.

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EFFECTS OF GAMMA RADIATION ON MORPHOLOGICAL TRAITS AND SEED STORAGE PROTEINS OF BEAN

The use of mutagenic agents to induce variability has been a practical tool, especially where natural variability is not available [2]. In this investigation gamma radiation (^{60}Co) was used to induce mutations to generate variability in morphological traits and electrophoretic profile of seed storage proteins in a nearly-white seed coat color bean (*Phaseolus vulgaris* L.) cultivar 'EMGOPA-Ouro'. The following characters were observed: percent germination, plant height, final stand, plant yield and yield components, the number of chlorotic and albino mutants, growth habit, earliness, alterations in seed coat color, seed coat brightness, halo color, seed size and form. Foundation seeds were submitted to 8 levels of radiation: 0, 100, 150, 200, 250, 300, 350, and 400 Gy and were planted in randomized complete-block design with four replications sowed with 100 seeds each. For biochemical analyses, 40 seeds of M_2 generation, collected randomly, were submitted to an acid saline solution, according to the method described by Romero *et al.* [1], modified by reducing the time of incubation at the extraction buffer to 1 hour. The effect of radiation dose on the protein electrophoretic profile was evaluated in 12% SDS-PAGE. Results indicated that the treatments with 200 and 250 Gy generated the highest variability. Some contrasting characters were observed on seed morphology (opaque and bright, large and small, squared and rounded, light and greenish coat color), growth habits (types I and II), and pod shape (straight and arched). Other traits such as variable leaf shape, yield components and chlorotic plants were also observed. In general, the 200 Gy treatment showed the highest variability, presenting the highest number of chlorotic plants. However, the 250 Gy treatment was the most efficient for modifying traits of agronomic interest, such as seed size, halo color and plant architecture. The main alterations in color and format of the seeds were observed in the 300 and 350 Gy treatments. The biochemical analysis demonstrated that three bands with molecular mass estimated between 40-50 **KDa**, corresponding to the globulins, are highly conserved. The mutation had a random effect related to electrophoretic profile, showing no association between the intensity of radiation and protein profile alterations.

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HYDRAZINE HYDRATE INDUCED DWARF BOLD SEEDED MUTANT IN BLACK GRAM CULTIVAR 'PU-19'

The attributes of interest to plant breeders are the quantitative traits controlled by polygenic interaction. In such cases effective selection of a desirable mutant for a specific trait becomes more difficult than the selection of a trait controlled by a single gene. Induced mutations have been utilized to achieve success in improving plant yield as well as plant architecture [1,2]. Both dwarfness and compactness of a genotype ensure more plants per unit area, thereby significantly contributing to the production and productivity.

Hydrazine hydrate (HZ) treated population of black gram (*Vigna mungo* L. Hepper) cultivar 'PU-19' was screened for certain desirable mutants in M₂ generation. A dwarf bold seeded mutant was observed in the population treated with 0.03% concentration of HZ. The seeds of the mutant were collected separately and sown in the field in plant progeny rows in next season to raise M₃ generation. The mutant was found to breed true for dwarfness as well as boldness of seeds. The mutant line was characterized by dwarf and compact stature with reduced length of internodes, petioles and peduncles. The mutant line flowered and matured earlier than the control by about 4 days. Seeds of mutants showed a marked change in size and colour (Table 1). Although the mutant line was characterized by moderate yield and desirable plant architecture, the estimation of protein content revealed that alteration in this trait was in a negative direction, indicating that the yield and protein content are negatively correlated [3]. However, increase in the protein content in some EMS and gamma ray induced mutants and decrease in others has also been demonstrated [4].

Table 1. Alteration in important characteristics of dwarf bold seeded M₃ mutants

Character	Control	M ₃ mutant line
Plant type	Trailing herbaceous	Dwarf compact
Days to flowering	42.34	38.46
No. of branches/plant	6.16	5.24
Days to maturity	78.60	74.70
Seeds/pod	6.40	5.85
Seed colour	Brownish black	Black
50-seed colour	2.12	2.83
Seed protein content (%)	23.20	22.40

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AGRONOMIC PERFORMANCE OF OLD SOYBEAN VARIETY 'ALTONA' DERIVED MUTANTS

An induced mutation program has been initiated at the Department of Genetics and Plant Breeding to develop early maturing cultivars with good yielding capacity. Some new mutants have been produced by irradiation of variety Altona with ⁶⁰Co gamma rays. Ten years of breeding resulted in two new mutant varieties named 'Noventa' and 'Gate 511'. The present study deals with agronomic performance of these mutants. Registered soybean varieties Altona and 'McCall' as well as Altona derived mutants (Gate 511 and Noventa) have been compared (Table 1).

Table 1. Some agronomic characters of early maturing mutants in comparison with the original and check cultivars (1988-1990)

Character	Altona	Gate 511 (mutant)	Noventa (mutant)	McCall (check)
Vegetation period	120-141	115-122	90-105	120-125
Date of maturity	3 September	25 August	30 July	29 August
Plant height (cm)	46-50	50-55	40-46	40-55
Hylum color	black	black	black	yellow
Seed weight (g)	5-16	5-17	8-19	4-19
1000 seed weight (g)	170	171	177	147
Seed yield (t/ha)	1,5-2,3	2,3-2,8	1,8-2,4	1,9-2,7

The vegetation period of mutant lines has been reduced considerably, Gate-511 was about one week earlier, while Noventa was about one month earlier than the parent variety Altona. The plant height of mutant Noventa was shorter while the mutant Gate-511 was higher than the parent. The seed yield of mutants showed a slight difference in comparison with the parent and check varieties. The mutant Noventa has been released as new variety in Hungary in 1993, License No. 207 922. Gate 511 is being tested in official field trials in Hungary.

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FAST NEUTRON MUTAGENESIS IN BARLEY

In order to conduct a deletion mutant analysis of the barley genome, seeds of cultivar 'Steptoe' were irradiated in 1992 with two doses of fast neutrons, 3.5 Gy and 4.0 Gy at the FAO/IAEA Seibersdorf SNIF facility by Dr. H. Brunner. M₁ seeds were grown at Pullman, Washington, USA in the field. Approximately 500 M₂ spikes were picked from each treatment and the remainder harvested in bulk. Mutation rates were determined on 1000 bulk M₂ seedlings (chlorophyll deficient) and 500 M₂ head rows (chlorophyll deficient and morphological) per treatment.

Chlorophyll-deficient mutations were observed at a frequency of 8.1% and 9.4% on M₁ spike basis and 2.2% and 2.6% on M₂ seedling basis for the 3.5 and 4.0 Gy treatments, respectively. Total mutations observed in the field were 19.0% and 20.8% on M₁ spike basis for the two treatments. Approximately 2,500 M₂ seedlings were assayed for nitrate reductase-deficient mutants and 12,000 M₂ seeds screened for waxy mutants. Although several putative mutants were identified, none have been confirmed to date.

The mutation frequencies observed are similar for both treatments and appear to be approximately the same as what we have previously observed with γ -radiation treatments. The absence of nitrate reductase-deficient and waxy mutants is most likely due to the small population size screened.

The morphological mutants recovered include dwarfs, sterile, necrotic, glossy, elongated outer glume, winter type and some very interesting floral mutants such as multi-ovary and branched inflorescence. Mutants affecting functions of genes for which cloned DNA segments are available will be sought in order to identify specific molecular changes that have been induced by fast neutron radiation.

(Contributed by KLEINHOF, A., D. KUDRNA and A. KILIAN, Crop and Soil Science and Genetics and Cell Biology Departments, Washington State, University, Pullman, WA 99164-6420, USA)

SEMIDWARF, HIGH YIELDING AND HIGH PROTEIN MUTANTS IN BARLEY

An induced mutations programme was undertaken in barley (*Hordeum vulgare* L.) with the primary objective of developing some semidwarf, short duration, high yielding types as most of the local cultivars grown in Meerut are tall statured with maturity period of 130-140 days. In recent years, the barley crop in this region, particularly at flowering/grain filling stages during February/March, has often been affected by unexpected rains accompanied by gusty winds, resulting in severe lodging of the crop and thereby reduction in crop yields. To combat this problem it is necessary to develop short statured types of barley, which can withstand lodging. If such types are associated with early maturity, that will be of greater help.

Dry seeds of three local cultivars of barley 'K-169', 'K-272' and 'DL-281' were irradiated with gamma rays at 100; 200; 300 and 400 Gy. A number of morphological mutants for different plant characters like plant height (dwarfs and semidwarfs), maturity period (early and late maturing), spike density and size (lax panicle and erectoides), high tillering and chlorophyll deficiency (virescent) types were isolated from M₂ mutated populations of the three varieties. Among these mutants, one semidwarf mutant (No. 3-20-5) of parent cultivar K-169 is of particular interest because of increased yield and significantly high protein content, besides the short stature. This mutant bred true for the altered characters and its performance

has been assessed during 1993-1994. Protein content in the seed of mutant and its control were estimated through conventional Kjeldahl method with slight modification, following rapid chromic acid procedure of Sharma and Sud [1].

The semidwarf mutant is characterized by erect, compact tillers with bright green leaves and short stature (76 cm) with good lodging resistance. The mutant also recorded improvement in biological and grain yields per plant over its parent with slightly delayed maturity (by 4 days) and medium sized seed (Table 1). No marked change in productive tiller number, spikelets per spike, seed fertility and 100 grain wt were noticed between the mutant and its control. The most significant feature of this mutant is its remarkably high seed protein content (15.46%). This mutant with its better plant type, vigorous growth, short stature, increased yield and high seed protein content is of practical utility in barley breeding and may be utilized for direct commercial cultivation as a new improved mutant variety after successful yield trials.

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Table 1. Comparative morphological data of semidwarf barley mutant and parent K-169

Character	Parent variety		Semidwarf mutant	
	Mean	Range	Mean	Range
Plant height (cm)	120.5	104 – 134	76.6	60 – 90
Culm length (cm)	95.3	80 – 105	56.3	39 – 70
Peduncle length (cm)	38.0	32 – 42	24.7	12.0 – 30.5
Spike length (cm)	25.2	18.0 – 25.0	20.3	16 – 23
Boot leaf size (cm ²)	26.1	11.2 – 41.4	31.5	84.0 – 56.7
Days to 50% heading	98.0	97 – 99	102.0	101 – 103
Days to maturity	131.0	130 – 133	135.0	134 – 136
Productive tiller number	12.5	7 – 18	12.6	5 – 25
Spikelets/spike	71.1	60 – 78	75.7	66 – 90
100 grain wt. (g)	4.6	4.6 – 4.7	4.6	4.8 – 5.0
Biological yield/plant (g)	79.3	58.0 – 106.0	92.8	60.0 – 122.0
Grain yield/plant (g)	32.0	20.0 – 46.0	35.9	29.0 – 50.0
Seed type		Bold		Medium
Seed length (cm)	1.3	1.1 – 1.5	1.23	1.0 – 1.4
Seed width (cm)	0.37	0.3 – 0.4	0.33	0.25 – 0.4
Seed protein content (%)	8.75	8.27 – 9.62	15.46	14.87 – 15.75

(Contributed by **RAMESH, B., B. KUMAR PRASAD** and **V.P. SINGH**, Department of Agricultural Botany, Ch. Charan Singh University, Meerut 250 004, India)

WINTER BARLEY MUTANTS CREATED IN THE UKRAINE

Increasing fodder and protein production is one of the objectives of the development of agriculture in Ukraine. Higher productivity of fodder crops, due to new highly productive varieties, is the means to meet this aim. Winter barley is an important crop for fodder purposes. The climate of the Ukraine is favourable for growing this crop. The areas used for the growth

of winter barley are however, small (500,000-550,000 ha) and there is a shortage of good quality varieties. The main aim of the work was therefore to create new varieties of highly productive winter barley, of good quality.

The new varieties and mutation lines of winter barley were created under the influence of water solutions of N-nitroso-N-methylurea (NMH - 0,012, 0,005%), N-nitroso-N-ethylurea (NEH - 0,05; 0,025; 0,012%) ethyleneimine (EI - 0,02; 0,01; 0,005%) on winter barley seeds of the varieties of local and foreign selections. On the basis of many years of investigations (1984-94) the following mutations were described: hard-grained, winter-hardiness, earliness, middle-maturity, late-maturity, wide and large leaves, narrow leaves, multinodal, great number of leaves, great number of flowers, strong stem (lodging resistant), tallness, semi-dwarfness, dwarfness, and high productivity. Particularly valuable are mutants with high productivity of green bulk. Their potential yield is 70 t/ha. As a result of the work two varieties of winter barley 'Shyrokolysty' and 'Kormovy' were released into the State register of plant varieties of the Ukraine. The other valuable mutant genotypes are used in cross breeding programmes.

(Contributed by ZAYATS, O.M., Institute of Agronomy and Animal Biology, Ukrainian Academy of Agricultural Science, Obroshyno Pustomyty District, Lviv Region, Ukraine 292084)

GAMMA RADIATION INDUCED MUTANT FOR IMPROVED YIELD COMPONENTS IN SUNFLOWER

Sunflower has become an important oilseed in the Indian vegetable oil pool following its introduction from Russia in 1969. It can be used for all quality products useful to humans. The need for genetic variability and new useful gene sources has necessitated that sunflower breeders and geneticists utilize a wide range of germplasm in their breeding programmes. The induction of mutations in sunflower by physical and chemical mutagens has been practiced quite intensively in the last two decades. The results recorded to date suggest that utilization of mutagenesis could be a great advantage in improving the sunflower crop.

An induced mutation programme was undertaken to generate variability in the variety 'Morden' using gamma rays. The certified and genetically pure seeds were irradiated with 50, 100, and 150 Gy gamma rays and used for further studies. Selection in M_2 generations, raised from different treatments, revealed the presence of an erectophylly leaf mutant from 50 Gy treatment. The isolated mutant showed improved yield components like head diameter, 100-seed weight and yield per plant. The mutant was a plant with short petiole length and erect leaves. This type of leaf get sunlight throughout the day. From morning to afternoon, the first half of the leaf gets sunlight, and from afternoon to evening the second half of the leaf gets sunlight. As a result of getting sunlight the whole day, the plant had more photosynthetic products and grew vigorously. Plant height, head diameter and 100-seed weight had direct effect on seed yield, and the number of leaves and stem diameter influenced the seed yield indirectly. In the M_3 generation, the mutant showed an almost two-fold increase over the parent variety for all investigated characters, except that of the yield per plant where there was a three-fold increase (Table 1). The present investigation has shown that there are remarkable possibilities of increasing the yield components in sunflower by induced mutations.

Table 1. Important characters of the control and mutant of sunflower

Variety/ Mutant	Height (cm)	No. of leaves	Stem diameter (cm)	Head diameter (cm)	100-Seed weight (g)	Yield per plant (g)
Morden	89	26	2.1	18.8	6.21	43.73
Erectophylly leaf mutant	185	33	3.2	30.0	10.74	120.75

(Contributed by **ELANGOVA**, M., Central Plantation Crops Research Institute, Kasaragod, Kerala, India)

THE VARIATION OF NITROGEN AND PHOSPHORUS CONTENTS IN M₄-GENERATION SEEDS OF AN IRRADIATED LOCAL SORGHUM VARIETY ORIGINATING FROM NORTHERN GHANA

Natural genetic variability in sorghum (*Sorghum bicolor* (L.) Moench) is very large, however, a number of attempts have been made to broaden its genetic base by induced mutations. Most authors [2; 3; 4; 5] refer to visible characters in plant and grain. Occasionally, effects on grain quality, e.g. high lysine/high protein, are reported [1].

Sorghum in northern Ghana, commonly called "guinea corn", is a widely cultivated cereal crop and can be found in three local races among which the *caudatum* race is represented by 'Naga White', an improved local variety originating from the Upper East Region of Ghana. It is characterized by short straw, earliness, good grain yield, a semi-loose head, and white grains, but with a relatively poor grain quality. The objective in several breeding programmes was the improvement of its grain quality, and an induced mutations programme was started at Nyankpala Agricultural Experiment Station (NAES) in 1988. About 10,000 seeds of Naga White were treated with 200Gy from a ⁶⁰Co gamma-rays source. The M₁-generation was planted at NAES and multiplied up to M₄ in 1991, subject to selection for agronomic value. Protein contents (N x 5.7) varied from 9 to 10% for grains of parental genotype and from 8 to 12% in M₂ grains harvested in 1990. In 1992, the agronomically best 112 seed samples of M₄ lines were analyzed for N and P contents, 1000-grain weight (GW) and protein contents. Protein contents ranged from 7.0 to 13.6%, phosphorus from 0.15 to 0.45%, and thousand grain weight varied from 11.8 to 19.0 g. The coefficient of the phenotypic correlation between N and P was + 0.337, and several lines with both high N and P contents could be identified. The coefficients of correlation between grain size and both N and P contents were slightly negative but not statistically significant. The coefficient of variation for the P content was twice as high as that for the N content. This might indicate a considerable microvariability in soil phosphorus due to its low content and availability (highly weathered oxisol, low organic matter, low pH). However, in spite of these difficulties, some of the lines tested showed the ability to accumulate normal to high phosphorus contents in their grains.

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SEARCH FOR C₄ DEVELOPMENTAL MUTANTS IN *Panicum maximum* Jacq.

Mutant plants are useful tools for studying developmental processes in defined genetic backgrounds by comparing them with their respective wild type forms. In this sense, developmental mutants or mutations involved in the establishment of certain leaf or flower specific traits are of special interest. In particular, the evolution of C₄ photosynthesis from C₃ precursors was accompanied by severe developmental changes in leaf morphology and anatomy. Our search of such mutants was followed by the idea to approach the evolution of the C₄ syndrome from a mutagenic point of view. Variants affecting normal development of the C₄ leaf anatomy may, in fact, represent possible regressive steps in C₄ photosynthesis [1].

Seeds of the C₄ grass *Panicum maximum* Jacq. were mutagenized using ethylmethanesulfonate (EMS) and putative variants were isolated in the M₂ generation by visual inspection. Main selection characteristics were whole plant, leaf morphology and pigmentation, and growth characteristics. The choice of a polyploid species for mutagenesis experiments was based on the need of detecting rare mutants, which are possibly lethal when using a diploid plant species. These variants could be of regulatory nature, affecting both morphology and physiology of C₄ photosynthesis early in leaf development. In total, nearly 100 variants were isolated and grown to maturity. Main isolated variants, which conforms to the prediction mentioned above, were as following: large interveinal space-1 and -3 (*lis1*, *lis3*), abnormal bundle sheath (*abs*), midribless (*mbl*) and variegated leaf -1 (*var1*). The variant *lis1* was a short plant with leaves smaller than the wild type, and had a leaf lamina with a crinkly surface. Photosynthetically, *lis1* indicates a clear regression from the C₄ to the C₃ photosynthesis type, which was correlated in the leaf lamina with an increase in the distance between small veins. The variant *lis3* was not similar phenotypically to *lis1*, but it also had very small leaves and reached a total plant height of maximal 0.6 meter. In leaf sections, it was characterized by an almost lack of the small veins surrounded by four bundle sheath cells. The leaf lamina of the variant *abs* showed several alterations, including doublets of veins, veins without bundle sheath, additional bundle sheath cells outside the veins or large bundle sheath cells participating in two bundle sheaths. Also the distribution of phloem and xylem cells within the bundles were quite altered in the variant compared to the wildtype. The leaves were greener, with a higher than normal chlorophyll content and with longitudinal veins not perfectly straight but following a wavy path on the leaf lamina.

Compared with wild type plants the phenotype of the *mbl* mutant was less erect and had pending leaves because of the absence of the main midrib. In wild type leaves the midrib was represented by an enlargement of the mesophyll parenchyma which included parenchymatous and sclerenchymatous cells. This structure was absent in mutant leaves, only small irregular files of parenchymatous cells were present at the base of the leaf lamina. The florets of this mutant had no carpel but one or two additional stamen.

The variant *var1* had a variegated phenotype with stripes of yellow-green and white tissues alternating the leaf laminae. In yellow-green sectors the chloroplasts were absent only in bundle sheath cells, which supports the hypothesis of different ways of development of bundle sheath and mesophyll cell chloroplasts. The adjacent mesophyll cells were less pigmented than

similar ones present in non-variant sectors. In white sectors, the chloroplasts were absent both in bundle sheath and mesophyll cells. The variant was partially fertile. Seed germination was 30 to 40%, and despite the unknown portion of apomictic seeds, 65 produced white, 20 green and 34 variegated seedlings out of a sample of 119 germinated seeds. Analysis of segregation of these green and variegated plants of the next generation is in progress.

In monocot species, leaves are divided lengthwise by three types of veins: midvein, lateral and small veins. The vascular system is established in a hierarchical fashion as the leaf develops. The midvein is established first in an acropetal direction towards the tip and basipetally into the sheath. Lateral veins develop acropetally in the leaf lamina, while small veins are initiated in this organ basipetally. The mutants in *P. maximum* demonstrate, that the complete process of vein initiation and development is apparently under complex genetic control [2].

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INDUCED MUTATIONS IN CASTOR

Castor (*Ricinus communis* L.) is an important oilseed crop in India. To create variability mutations were induced in two cultivars 'TMV5' (maturing in 130-140 days) and 'CO1' (perennial type). Gamma rays and diethyl sulphate and ethidium bromide were used for seed treatment. Ten doses, from 100 to 1000 Gy were employed. For chemical mutagenesis five concentrations of mutagens from 10 to 50 mM were tried. No economic mutants could be isolated after treatment with the chemical mutagens. The following economic mutants were identified in the dose 300 Gy of gamma rays.

Annual types from perennial CO 1 castor

CO 1 is a perennial variety (8-10 years) with bold seeds (100 seed weight 90 g) and high oil content (57%). Twenty-one lines were isolated with annual types (160-180 days) with high yield potential as well as bold seeds and high oil content. These mutants, identified in M₃ generation were bred true in subsequent generations up to M₈ generation. Critical evaluation of the mutants in yield evaluation trials is in progress.

Parental lines for development of hybrids

The inflorescence of castor is monoecious type with bottom 30-35% male flowers and top 65-70% female flowers. Four mutants were identified from the variety TMV 5 with higher proportions of female flowers ranging from 80-90%. These mutants in M₇ generation were identified as good combiners in the development of hybrid combinations. The yield of the hybrid combinations are presented in Table 1. Present investigations have clearly shown that there is a great potentiality in improving castor productivity and production by mutation induction.

Table 1. Performance of hybrid combinations

Combinations	Seed yield (kg/ha)	Increase over standard hybrid GCH 4 (%)
LRES 17 x TMV 5 – Mutant No.1	1333	13.6
LRES 17 x TMV 5 – Mutant No.4	1396	11.9
LRES 17 x TMV 5 – Mutant No.2	1529	30.3
LRES 17 x TMV 5 – Mutant No.3	1513	28.9
SKP 24 x TMV 5 – Mutant No. 1	1349	15.0
SKP 24 x TMV 5 – Mutant No. 2	1451	23.7
SKP 24 x TMV 5 – Mutant No. 3	1481	26.3
SKP 24 x TMV 5 – Mutant No. 4	1420	21.1
GCH 4 Hybrid (Check) (VP 1 x 48-1)	1173	-

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MUTAGENESIS AS A BREEDING METHOD IN LENTIL

Mutagenesis was used to develop cultivars with good adaptability to exogenous factors and with increased productivity [1; 5]. By means of this alternative breeding procedure, increases in biological and nutritive value of the seeds were studied [2; 3]. To increase genetic variability in lentil (*Lens culinaris* Medic.) breeding material, experimental mutagenesis was applied parallel to conventional breeding methods. The aim was to characterize the mutant lines as well as determine whether some of them could be directly registered as cultivars or as gene donors in breeding programme.

Within the period 1993-1996, eight mutant lentil lines were studied under field conditions. They were obtained as a result of gamma rays (^{60}Co) and ethyl methanesulfonate (EMS) treatment of the small seeded cultivar 'Tadjikskaya 95'. Air-dried seeds were treated. During the vegetative stage, phenological observation was made. The structural elements of productivity were established by biometrical analysis of 25-30 plants from each of the variants. Phytopathological evaluations were made using the scoring procedure established by ICARDA. Protein content was determined by the Kiejdhall method. The technological qualities of the seeds were determined using the method of Tretyakova and Ustinova [4].

The mutant lines differed considerably in their biological traits from the parent cultivar. The vegetative period ranged from 84 to 89 days (Table 1). The mutant lines were later-maturing than parent variety Tadjikskaya 95 by 1-5 days. As a result of mutagen treatment, the range in plant height was expanded from 1 to 8.3 cm. Line 96-8, obtained after irradiation with gamma rays, was the tallest (40.3 cm). Lodging of the mutant lines was greater than that of the initial cultivar and ranged from 20.0 to 66.7%. The trait varied to a great extent depending on environmental conditions. Mutagenic treatments also caused changes in seed size and seed coat colour. Development of resistance to important diseases of lentil in Bulgaria is of considerable interest. In comparison to the parent variety, mutant lines 96-8 and 96-14 proved to have better resistance to Fusarium and Anthracnose. The highest resistance to Anthracnose was found in M 96-7 (candidate cultivar 'Elitsa'). Data for characters related to productivity are presented in Table 2. As a result of the mutagenic treatments, changes occurred for branching of the 1st as well as the 2nd type. The number of branches was greatly reduced in mutant line

96-6. Considerable genotypic variation was observed with regard to the number of pods per plant. Mutant 96-7 formed the greatest number of pods and seeds. The greater number of seeds produced per plant ensured higher productivity, e.g. in mutant line 96-4. Seed yield per plant varied from 1.06 g to 2.27 g. Except for mutant line 96-6, the remaining mutants were characterized by higher yield per plant as compared with the parent variety. As a result of mutagenic treatment, considerable diversity was generated with respect to 1000 seed weight. The lines were characterized by higher seed weight and exceeded the check by 1.5 g (line 96-14) to 12.4 g (line 96-6). Statistically, no correlation has been established between productivity elements and applied doses of mutagens.

The main trait for lentil selection is productivity. In investigated lines mutagenic treatments increased productivity by 25.5% to 56.5% in comparison to the parent variety. The highest yield was obtained from line 96-4 (2,270 kg/ha). Lines 96-18, 96-8 appeared to be promising for yield. The biological value of seeds is mainly determined by protein content. Mutant line 96-8 was characterized by the highest (26.2%) and the most stable protein content. Protein yield of the mutant lines was higher than that of the parent variety and ranged from 452.5 kg/ha (mutant 96-14) to 592.5 kg/ha (mutant 94-4). Cooking time of some of the mutant lines increased and there was positive correlation between the 1000 seed weight and cooking time.

The most promising mutant line 96-4, characterized by the highest seed yield and seed protein content, was registered as an original cultivar under the name M-17-MM. Line 96-7 (Elitsa) has been entered in tests conducted by the State Testing Commission. Experimental mutagenesis is a promising alternative method for creating genetic variability for selection in lentil.

As a result of treatment with physical and chemical mutagens, many changes occurred in morphological traits. New forms with good resistance to Fusarium and Anthracnose were obtained as well as forms with higher protein content. The mutants studied exceeded the parent cultivar in productivity by 25.5 to 56.5%.

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Table 1. Biological and phytopathological characteristics of eight selected mutant lines during 1993-1996

Cultivar and mutant lines	Mutagen and doses	Vegetative period (days)	Plant height (cm)	Lodging (%)	Colour			Resistance to diseases	
					Seed coat	Cotyledons	Fusarium (1-9)	Anthracnose (1-9)	
Tadgikskaya 95	Control	84	32.0	20.0	Reddish brown + f.g.s.*	Red	1-5	1-9	
M ₈ 96-11	γ rays – 10 Gy	85	38.0	83.3	Rose greenish	Red	1-5	1-9	
M ₈ 96-4	γ rays – 40 Gy	86	34.6	43.3	Rose greenish	Red	1-5	1-7	
M ₈ 96-7	γ rays – 40 Gy	86	36.0	40.0	Grayish brown	Yellow	1-5	0-1	
M ₈ 96-14	γ rays – 40 Gy	85	37.0	63.3	Rose greenish	Red	1-3	1-3	
M ₈ 96-8	γ rays – 50 Gy	86	40.3	60.0	Grayish brown	Yellow	1-3	1-3	
M ₅ 96-15	γ rays – 120 Gy	89	36.7	86.7	Rose greenish	Red	1-5	1-5	
M ₈ 96-6	EMS – 0,05%	85	33.0	70.0	Rose greenish	Red	1-5	1-5	
M ₉ 96-18	EMS – 0,1%	86	37.3	63.3	Yellow greenish	Red	1-5	1-3	

*/fine gray spot

Table 2. Elements of productivity of the mutant lines

Mutant lines	Number of branches	Number of pods	Number of seeds	Seed weight (g)	Weight 1000 seeds (g)
Control	9.3	25.8	34.2	1.27	29.6
96-1	8.4	36.6	41.4	2.02	39.4
96-4	9.3	38.6	49.4	2.21	40.0
96-7	8.5	45.5	56.9	2.27	41.2
96-14	7.3	31.9	44.0	1.65	31.1
96-8	9.6	27.9	35.4	1.72	40.1
96-15	6.7	24.7	32.0	1.43	39.3
96-6	5.8	17.9	21.8	1.06	42.0
96-18	8.3	41.1	44.8	1.88	38.6

DEVELOPMENT OF HIGH YIELDING MUTANTS IN LENTIL

Lentil (*Lens culinaris* Medik.) locally known as Masoor, is the second most important rabi pulse crop, after chickpea, in Pakistan. It is cultivated on an area of over 63,400 ha, which constitutes about 4.83% of the total area under pulses. The annual production of the crop is 28,200 tones with an average yield of 445 kg/ha. Yield at the national level is very low, about one-half of the world's yield, which is mainly due to non-availability of high yield potential genotypes. Keeping in view the importance of mutants in developing a large number of new varieties [1], an induced mutations programme was initiated at AEARC, Tandojam during 1987-88, to develop high yielding varieties in lentil. For this, seeds of two lentil varieties, 'Masoor-85' and 'ICARDA-8' had been irradiated with gamma-rays ranging from 100-600 Gy in NIAB, Faisalabad during 1990. Selections were made in M₂ on the basis of earliness, plant height, branches/plant and 100 grain weight. After confirming these mutants in M₃ they were promoted in station yield trials and studied continuously for three consecutive years (1993-1995). Overall results revealed that these mutants have consistent improvement of earliness in flowering and maturity. Plant height also increased in all mutant lines except AEL 23/40/91 where reduction in this attribute was observed as compared to parent variety (Table 1). Mutant lines AEL 49/20/91 and AEL 13/30/91 showed improvement in 100 grain weight. The improvement of some agronomic characters enhanced the yield of mutant lines in comparison to parent varieties (Masoor-85 and ICARDA-8). The diversity in yield over the respective parents was computed from 6.94 to 60.12%. From these encouraging results it is hoped that mutant lines like AEL 12/30/91 and AEL 49/20/91 may serve as potential lentil genotypes in future.

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Table 1. Performance of high yielding mutants of lentil developed at AEARC, Tandojam

Genotype	Parent	Radiation dose (Gy)	Days to flowering	Days to maturity	Plant height (cm)	100 grain weight (g)	Grain yield (kg/ha)	Increase over parent (%)
AEL 2/20/91	ICARDA	200	69.2	129.6	36.4	1.47	529	11.36
AEL 49/20/91	Masoor-85	200	64.7	131.0	39.7	1.75	452	27.32
AEL 12/30/91	ICARDA-8	300	65.7	128.7	38.1	1.39	761	60.21
AEL 13/30/91	ICARDA-8	300	72.0	128.7	41.0	1.75	508	6.94
AEL 23/40/91	ICARDA-8	400	65.7	125.7	32.7	1.26	538	13.26
AEL 57/50/91	Masoor-85	500	88.5	134.5	35.4	1.44	443	24.78
Masoor-85	Check	-	91.0	137.5	36.3	1.28	355	-
ICARDA-8	Check	-	91.2	137.2	35.7	1.34	475	-

OBSERVATION ON GAMMA RAY INDUCED VIABLE MUTATIONS IN VEGETABLE COWPEA

Two cowpea (*Vigna unguiculata* L. Walp) varieties 'Pusa Komal' and 'Co 2' were irradiated with gamma rays at 200, 300, 400 and 500 Gy. After mutagenic treatment, M₁ generation was raised. Ten M₁ plants in each treatment and in the control were advanced to M₂ generation. The M₂ seedling progenies were examined for viable mutants. In a population of 3,199 and 3,538 plants in Pusa Komal and Co 2 respectively, a total number of 140 mutants were observed for variation in cotyledonary leaves in the early stage of growth to abberants possessing modified plant structure, leaf morphology, pod size, pod color and seed coat color. In the progeny of Pusa Komal variety, the percentage of viable mutation was the highest at 300 Gy while in Co 2, at 500 Gy (Table 1). Mutants for plant habit have also been reported in greengram [1]. The data on mutagenic effectiveness and efficiency are given in Table 2. In Pusa Komal, the effectiveness for viable mutation ranged from 4.22 to 12.45 and in Co 2 ranged from 6.60 to 13.35 (Table 2). The effectiveness and efficiency were decreased with increased doses of gamma rays as was also noted for mungbean [2].

Table 1. Viable mutations in M₂ generation of vegetable cowpea

Variety/ treatments (Gy)	Population	Viable mutation	
		Number	Percentage
Pusa Komal			
200	802	20	2.49
300	642	23	3.58
400	542	12	2.21
500	378	8	2.11
Co 2			
200	784	21	2.67
300	713	17	2.38
400	633	20	3.16
500	576	19	3.30

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Table 2. Mutagenic effectiveness and efficiency of viable mutations of vegetable cowpea

Variety/ treatments	Survival reduction (L) (%)	Height reduction (I) (%)	Seed fertility reduction (S) (%)	Mutants per 100 M ₂ (M) seedlings	Effectiveness M x 100/kR	Efficiency		
						M x 100/L	M x 100/I	
Pusa Komal								
20 kR	12.71	8.68	6.52	2.49	12.45	19.59	28.68	38.19
30 kR	27.74	12.08	10.64	3.58	11.93	12.90	29.63	33.64
40 kR	57.25	19.86	26.40	2.21	5.53	3.86	11.12	8.37
50 kR	67.73	27.94	36.30	2.11	4.22	3.12	7.55	5.81
Co 2								
20 kR	5.35	8.75	7.54	2.67	13.35	49.91	30.51	35.41
30 kR	9.95	13.15	11.49	2.38	7.93	23.92	18.09	20.71
40 kR	48.18	21.46	20.84	3.16	7.90	6.56	14.72	15.13
50 kR	54.15	28.80	26.96	3.30	6.60	6.09	11.45	12.24

HIGH YIELDING MUTANTS OF BLACKGRAM VARIETY 'PH-25'

Seeds of blackgram (*Vigna mungo* L.) variety 'PH-5' were treated with chemical mutagens ethyl methanesulfonate (EMS), nitrosoguanidine (NG), maleic hydrazide (MH) and sodium azide (NaN₃), each at 3 different concentrations. Thirty six mutant lines developed from mutagenic treatments along with parent varieties were tested in M₄ generation. The mutants showed wide variation in most of the traits and multivariate D² analysis showed genetic divergence among themselves. Twenty of the thirty mutants showed genetic divergence from parent. Ten selected high yielding mutants were tested in M₅. Yield and other productive traits of five high yielding mutants in M₄ and M₅ are presented in Table 1. The mutants, their mutagenic treatment origin and significant changes in productive traits from parent variety PH-25 are as follows:

- PE2-1: (EMS, 0.4%). Increase in plant height, bunches/plant, pods/plant, seeds/pod and 100-seed weight.
 PS1-3: (NaN₃, 0.05%). Increase in bunches/plant and pods/plant.
 PE1-2: (EMS, 0.2%). Early maturity, increase in pods/plant and 100-seed weight.
 PS2-1: (NaN₃, 0.03%). Increase in bunches/plant and pods/plant and 100-seed weight.
 PM2-3: (MH, 0.02%). Early maturity, increase in bunches/plant and pods/plant.

Table 1. Yield and productive traits of high yielding mutants of blackgram variety PH-25 in M₄ and M₅ generations

Mutant		Days to maturity	Plant ht. (cm)	Bunches/plant (No.)	Pods/plant (No.)	Seeds/pod (No.)	100-seed weight (g)	Yield (q/ha)
PE2-1	M ₄	89.3	38.2	9.9	26.6	3.87	4.24	10.92
	M ₅	93.7	41.3	9.9	21.6	3.67	4.36	9.12
PS1-3	M ₄	87.7	37.6	9.6	28.4	3.76	4.01	10.78
	M ₅	92.3	40.1	9.8	23.7	3.53	4.18	8.89
PE1-2	M ₄	86.0	33.2	8.2	27.3	3.69	4.04	10.20
	M ₅	88.0	36.1	8.7	22.5	3.48	4.33	8.84
PS2-1	M ₄	86.7	35.7	10.1	27.9	3.60	4.01	9.98
	M ₅	90.7	38.6	10.1	24.9	3.47	4.24	8.61
PM2-3	M ₄	86.0	35.7	10.2	28.7	3.67	3.82	9.95
	M ₅	88.7	38.8	10.1	24.7	3.47	4.03	8.43
PH-25 (Parent)	M ₄	88.7	34.4	7.5	20.5	3.64	3.88	7.25
	M ₅	91.7	38.0	8.2	17.6	3.41	4.09	7.36
C.D (5%)	M ₄	1.7	3.1	1.2	3.1	0.21	0.14	0.52
	M ₅	2.4	3.3	1.6	2.7	0.21	0.23	0.92

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STUDIES ON INDUCED MUTATIONS IN GARLIC

Garlic (*Allium sativum* L.) is the second most widely cultivated *Allium* - after onion. It has been recognised world-wide as a valuable spice for foods and a popular remedy for various ailments and physiological disorders. The available types of garlic exhibit low variability due to repeated vegetative propagation. As garlic flowers are mostly sterile, restoration of fertility is a difficult process and hence there exists little scope for genetic improvement through hybridization. Induced mutagenesis with gamma rays has helped to overcome these genetic barriers. Ethyl methanesulphonate (EMS) and their combination treatments attempted to improve bulb yield in garlic varieties 'Mettupalayam' and 'Ooty-1' at the Horticultural Research Station, Ooty in Nilgiris. Based on radiosensitivity studies, two doses of gamma rays (2.5 and 5.0 Gy), four concentrations of EMS (15, 20, 25 and 30 mM for 8 h at temperature 25±2°C) and four combined treatments (2.5 Gy + 20 mM, 2.5 Gy + 25 mM, 5.0 Gy + 20 mM and 5.0 Gy + 25 mM) were employed.

Table 1. Frequency and spectrum of viable mutations in V₂M₁ generation of parent variety Mettupalayam

V ₂ /M ₁ plants/characters	Gamma rays (Gy)		Treatments EMS (mM)				Gamma rays (Gy) + EMS (mM)			
	2.5	5.0	15	20	25	30	2.5+20	2.5+25	5.0+20	5.0+25
No. of plants scored	1024	632	904	664	540	436	898	878	764	412
No. of viable mutants observed	366	96	96	177	66	48	308	95	72	37
Mutation frequency per 100 VM plants	35.74	15.19	10.62	26.66	12.22	10.55	34.30	10.82	9.42	8.98
Tall types	2.73	0.00	1.00	4.21	1.48	0.91	4.12	0.46	0.00	0.00
Dwarf	1.37	3.16	0.30	0.45	1.11	1.61	0.45	0.57	1.31	2.91
Luxuriant types	5.08	0.00	1.99	3.16	0.37	0.46	5.46	0.57	0.00	0.00
Needle leaves	2.54	1.27	0.00	0.45	0.19	0.23	0.33	1.14	2.62	2.67
Short leaves	0.19	0.00	0.11	0.00	0.00	0.00	0.78	0.68	0.39	0.49
Loose culm types	3.81	4.11	1.99	2.56	2.78	2.52	4.00	1.59	0.92	0.24
Flower stalk with two tier aerial bulbs	0.39	0.00	0.00	0.00	0.00	0.00	0.67	0.00	0.00	0.00
Early maturing	2.34	1.42	1.33	2.26	0.93	0.69	3.12	1.71	0.26	0.49
White cloves	2.73	0.95	0.00	2.26	0.00	0.00	3.45	0.68	0.13	0.49
Golden yellow colour cloves	2.05	0.00	0.00	2.26	0.00	0.00	2.45	0.00	0.39	0.49
Pink coloured cloves	1.95	0.00	0.55	1.96	0.00	0.00	1.61	0.86	0.13	0.49
Fat bulbs	0.39	1.11	0.49	0.60	0.37	0.23	0.67	0.57	0.00	0.00
Double bulbs	0.78	0.00	0.00	0.15	0.93	0.00	0.89	0.00	0.00	0.00
Triple bulbs	0.39	0.00	0.00	0.60	0.00	0.00	0.67	0.00	0.00	0.00
Multiple bulbs	0.19	0.00	0.00	0.00	0.00	0.00	0.45	0.00	0.00	0.00
Rubberised bulbs	2.54	3.16	2.88	4.70	4.07	3.90	5.12	2.05	1.96	0.73

Garlic bulb and clove characteristics and the varietal response were significantly influenced by the physical, chemical mutagens and their combination treatments. The spectrum of chlorophyll mutants identified in the present study are comprised of, *albina*, *chlorina*, *straita*, *viridis* and *xantha*. The proportion of the various mutants varied with the varieties and mutagen treatments. Increasing doses of gamma rays, EMS or combination treatments increased the rate of lethality, injury and clove sterility of treated populations. Mutations for plant, leaf and shoot morphology were more frequent than bulb characters in both varieties. Non-viable mutants were dose dependant and this increased with higher doses. Gamma treatments caused more non-viable mutants (mottled and crinkled leaves) followed by combined and EMS treatments.

The magnitude of the positive or negative shift of the vegetative traits (number, length and breadth of leaves and diameter of pseudostem) was greater in both V₂M₁ and V₃M₁ generations in progenies after EMS treatments in both varieties. Genetic parameters, association analysis and regression analysis revealed the strong association of the bulb characters (diameter and volume) and clove characters (volume, weight and diameter) on bulb yield. For most of the traits stronger regression was exhibited by 2.5 Gy + 20 mM combination treatments in all generations. Consistently higher variability, skewness and kurtosis for the bulb and above characteristics in all generations indicated that top priority should be given to these traits when selecting mutants during crop improvement programmes. Continuous significant positive regression value indicated that bulb diameter is the best selection index. Four hundred and seventy-one viable economic mutants in Mettupalayam (Table 1) and seventy-seven in Ooty-1 (Table 2) with higher bulb yield contributing traits, besides tolerance to rubberisation, have been isolated for further evaluation.

Table 2. Frequency and spectrum of viable mutations in V₂M₁ generation of parent variety Ooty - 1

Treatments	Gamma (Gy)		EMS (mM)				Gamma (Gy) + EMS (mM)			
	2.5	5.0	15	20	25	30	2.5+20	2.5+25	5.0+20	5.0+25
No. of plants scored	864	408	1136	1048	528	396	560	468	412	348
No. of viable mutants observed	104	48	62	123	91	121	135	56	26	25
Total mutation frequency per 100 V ₂ M ₁ plants	12.04	11.76	5.46	11.74	17.23	30.56	24.11	11.97	6.31	7.18
Tall types	0.00	0.00	0.00	2.19	2.84	3.54	5.36	0.85	0.00	0.00
Dwarf	2.55	4.90	0.79	0.38	0.19	0.51	0.89	1.07	1.46	3.16
Luxuriant types	0.00	0.00	0.00	0.38	0.76	2.78	2.86	0.85	0.00	0.00
Needle leaves	8.47	1.72	0.00	0.38	0.19	0.25	0.18	0.64	1.46	2.59
Short leaves	0.00	0.00	0.00	0.00	0.00	0.25	0.36	0.00	0.00	0.00
Loose culm types	2.78	3.19	3.17	5.25	7.95	9.60	4.29	3.21	1.94	1.15
Flower stalk with two tier aerial bulbs	0.00	0.00	0.00	0.00	0.00	0.00	0.89	0.00	0.00	0.00
Early maturing	0.81	0.00	0.00	0.00	0.00	2.02	0.89	1.07	0.00	0.00
Pink tunicated bulbs with white cloves	0.35	0.25	0.00	0.29	0.76	1.26	1.79	0.43	0.00	0.00
Flat bulbs	0.35	0.00	0.00	0.00	0.00	0.00	0.89	0.43	0.24	0.00
Double bulbs	0.35	0.00	0.00	0.19	0.76	1.01	1.25	0.21	0.00	0.00
Triple bulbs	0.00	0.00	0.00	0.00	0.00	0.25	1.07	0.43	0.00	0.00
Multiple bulbs	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rubberised bulbs	1.39	1.72	1.50	2.61	3.19	9.10	3.39	2.78	1.21	0.29

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INDUCED MUTANT FOR MALE STERILITY IN NIGER

Niger (*Guizotia abyssinica* Cass.), an important oilseed crop of the family Compositae is highly cross-pollinated due to the twin mechanisms of protandry and incompatibility. Studies revealed the functional nature of protandry and the breakdown of incompatibility with alteration in temperature [1]. It has very small flowers (disc florets) arranged in a capitulum that open on 3-4 consecutive days which pose problems in emasculation for cross-breeding. To

induce mutations, seeds of variety 'IGP-76' were irradiated with γ -rays 200 to 1000 Gy. All seeds of M_1 plants were sown separately in individual plant-to progeny rows. The results of screening of M_2 segregating material indicated that γ -ray treatment was effective in induction of male sterility. Frequency of visible mutations were higher in sibbed progeny as compared to open pollinated population and male sterile plants were observed only in sibbed population (1000 Gy). Male sterile plants could easily be identified at the flowering stage by their altered floral morphology (disc florets transformed into ligulate ray florets) and complete absence or presence of a rudimentary anther column. Seeds were collected following sib-mating with the fertile counterparts. Progeny segregated in a ratio of 3 normal : 1 male sterile. Further work on the mechanism of sterility, maintenance and linkage relationships with associated characters is under progress. This is the first report of induction of male sterility in niger through the use of physical mutagens. The availability of this mutant will be of great value for exploitation of heterosis on commercial basis.

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PRODUCTIVE MUTANTS OF NIGER

Seeds of six niger (*Guizotia abyssinica* Cass.) varieties ('GA-10', 'ONS-8', 'IGP-72', 'N-71', 'NB-9' and 'UN-4') were treated with 0.5, 0.75 and 1% ethyl methanesulphonate. After four generations of selection, 29 mutant lines were developed and those were evaluated from 1990-92 during Kharif (July to October) and Rabi (December to March) seasons. Average plant characteristics and yield data of four high yielding mutants along with 'IGP-76' (National Check), GA-10 (Zonal Check) and 'Semiliguda Local' (Local Check) are presented in the Table 1. The high yielding mutants, their parental varieties, mutagenic origin and major characteristic improvement over check varieties are as follows:

- ONS-107: (GA-10, 0.75% EMS) - more capitula/plant and seeds/capitulum with high yield
- ONS-114: (ONS-8, 1% EMS) - moderately high capitula/plant, high seeds/capitulum and 1000-seed weight and high yield
- ONS-125: (NB-g, 1% EMS) - larger capitula with more seeds/capitulum, high 1000-seed weight and high yield
- ONS-130: (UN-4, 0.25% EMS) - early flowering and maturity short plant height with more seeds/capitulum, height 1000-seed weight and high yield.

Table 1. Performance of mutant and check varieties of niger during Kharif and Rabi seasons average over 1990-91 and 1991-92

Mutant/ variety/season		Days to maturity	Plant height (cm)	Capitula/plant	Seeds/capitulum	1000-seed weight (g)	Seed yield (q/ha)
ONS-107	K	115	168	25.8	18.4	3.82	5.04
	R	108	78	26.2	19.0	3.85	5.18
ONS-114	K	115	172	22.1	18.8	3.89	4.27
	R	106	81	22.3	19.9	3.98	5.27
ONS-125	K	110	156	18.9	18.8	4.06	3.86
	R	103	80	22.1	20.4	4.23	5.52
ONS-130	K	107	138	20.4	18.2	3.96	4.44
	R	99	71	20.9	19.6	4.18	4.56
IGP-76 (NC)	K	111	163	16.3	17.4	3.63	3.16
	R	103	73	19.6	18.7	3.92	4.46
GA-10 (ZC)	K	118	184	18.4	17.4	3.79	3.53
	R	107	83	20.9	19.9	3.94	4.73
S.Local (LC)	K	120	190	20.2	16.4	3.46	3.56
	R	110	85	21.2	16.6	3.55	3.92
C.D. (5%)	K	5.4	11.4	2.6	2.2	0.26	0.85
	R	4.1	7.2	2.2	1.9	0.22	0.72

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SEED MUTAGENESIS IN *Portulaca grandiflora* (Hook)

Betalain pigments have been used as natural additives. Despite their importance, the biochemistry and genetics of betalain synthesis remain relatively undetermined [1]. *Portulaca grandiflora* represents an ideal material for genetic analysis [2]. In the present work, seed mutagenesis was examined with a view to enhance the chance of detection of new genetic markers in this species.

White ('PgBmj') and red ('PgR') seeds of *Portulaca grandiflora* were treated with ethyl methanesulfonate (EMS) at concentrations ranging from 1.2 to 40% in 0.1 M phosphate buffer (pH 7.0 at $22 \pm 2^\circ\text{C}$) for 4 hours, or with sodium azide (NaN_3) at concentrations ranging from 2.5 to 30 mM in 0.1 M phosphate buffer (pH 3.0 at $22 \pm 2^\circ\text{C}$) for 1 hour. In another set of experiments the presoaked seeds (sterile water, 6, 16, 28 hours at room temperature 22°C) were treated with 1.2% EMS and 2.5 mM NaN_3 for two hours with shaking of the seeds at 30°C . After the mutagen treatments, the seeds were washed to removing the mutagens.

In the M_1 generation, germination and survival percentage of both cultivars decreased. For the selection of mutants, M_2 segregation progenies were raised from seeds of M_1 plants. The frequency of mutated plants was evaluated in the M_2 generation (Table 1).

Table 1. Frequency of viable mutations in the M₂ generation of *Portulaca grandiflora*

Cultivar	Mutant types	Total No. of plants	Mutated M ₁ plants
PgBmj	dwarf	938	217
	late flowering	938	99
	female-sterile	252	2
	male-sterile	162	6
Pfr	dwarf	459	144
	dwarf (altered leaves)	409	1
	late flowering	459	53
	female-sterile	370	7
	male-sterile	459	5

Five morphological mutations, namely, dwarf (Dw₁), late flowering (Flt), male-sterile (Sm), female-sterile (Sf) and dwarf mutant with altered leaves (Dwr₂) were selected from the M₂ population. Some mutants, identical with those scored in *Portulaca* were identified in another species [3, 4].

The higher frequency of mutants was obtained from treatment of seeds with 4.8% EMS concentration in variety PgR and from 20 mM NaN₃ treatment in variety PgBmj. In the M₃ generation, segregation for *Dwr*₁, *Dwr*₂, *Sm* and *Sf* revealed that these mutations are recessive to normal plants. The experiment proved that EMS and NaN₃ were effective in inducing phenotypic variation. This is the first report on inducing variability in *Portulaca* through mutagenic treatment. The new gene markers identified will be of great value for studying the genetics of *Portulaca grandiflora*.

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GENETIC ANALYSIS OF SUNFLOWER CHLOROPHYLL MUTANTS

The method of getting the chlorophyll mutations in sunflower was developed by Y.D. Beletskii in 1969 with the use of N-nitroso-N-methylurea (NMH) [1; 2]. Certain concentrations of NMH are known to induce plastid mutations in growing seeds, and their yield depends on the duration of the exposure [3]. The given work presented studies on the influence of rifampicin (R) and 2,4-dinitrophenol (DNP) on the genetic activity NMH, as an inductor of plastid and nuclear mutations.

Seeds of a sunflower line 3629 were soaked under anaerobic conditions for 18 h and then treated with 0,015% NMH in combination with either 5×10^{-5} M DNP or 10^{-2} M rifampicin. Treatment with NMH was performed at 12-15 h of growth, for 3 h. DNP and R were applied at 0-6, 6-12, 0-12, 12-18 and 15-21 h of growth. Treated and control seedlings were washed with running water and seeded in the field. The frequency of plants with altered chlorophyll content in M_1 , inheritance of traits in M_2 and in M_3 was studied. To analyze the genetic nature of chlorophyll mutants, reciprocal crosses with the original inbred line 3629 were made.

Used concentrations of DNP and R did not induce genetic changes. MNH induce variegated forms already in M_1 with the frequency 2,4-8% (in different years).

Pretreatment with R or DNP increased the effect of MNH (i.e., the frequency of chlorophyll mutants in M_2) (Table 1). After self-pollination of surviving M_2 chlorophyll mutants segregation in their progeny was observed. Variegated forms induced by MNH in combination with 6 h pre-treatment of R (variants 1, 2, 4 and 5) segregated to three types of seedlings: green, variegated and yellow-white (inviolate). Non-Mendelian segregation was observed, which indicated the plastome nature of variegated forms. In the progeny of variegated plants, no *chlorina*-type forms were ever recorded.

Table 1. Frequency of chlorophyll mutations induced by MNH in combination with DNP or R

Variant of experiments	M_2 chlorophyll mutants (%)							
	Total		Variegation		<i>Chlorina</i>		Inviolate	
	1995	1996	1995	1996	1995	1996	1995	1996
1. R(0-6)+MNH	7.2	1.5	2.9	1.5	1.4	0	2.9	0
2. R(6-12)+MNH	2.7	0.4	2.7	0	0	0	0	0.4
3. R(0-12)+MNH	2.8	0	0	0	1.4	0	1.4	0
4. R(12-18)+MNH	0	1.1	0	0.3	0	0	0	0.8
5. R(15-21)+MNH	0	15.9	0	1.2	0	13.5	0	1.2
6. DNP(0-6)+MNH	4.0	0	0	0	4	0	0	0
7. DNP(6-12)+MNH	0	0	0	0	0	0	0	0
8. DNP(0-12)+MNH	7.7	0	0	0	6.7	0	0.9	0
9. DNP(12-18)+MNH	0	2.6	0	0	0	0	0	2.6
10. DNP(15-21)+MNH	0	1.0	0	0.7	0	0	0	0.3
11. MNH(12-15)	2.2	2.9	1.1	1.5	1.1	0	0	1.5
12. Control	0	0	0	0	0	0	0	0

Chlorophyll *chlorina*-type mutations induced by MNH in combinations with R represent a heterogeneous group, although all plants were phenotypically similar. In variant 3, *chlorina*-type mutants segregated to only *chlorina* and green seedlings after self-pollination. The proportion of *chlorina* plants in M_3 was 96%. In $M_4 - M_6$ all progenies expressed the mutant phenotype. In progeny of *chlorina* variant 1, *chlorina* forms constituted only 53% in M_3 . It is noteworthy that, only in this variant, a single variegated form was noted in the *chlorina* progeny in addition to green and *chlorina* plants. In general, plants of this variant showed a decreased viability, most of them perished before flowering. This made a detailed genetic analysis of these mutants impossible. In variant 5, *chlorina* plants produced only green and *chlorina* forms in M_3 after self-pollination. The proportion of *chlorina* plants was about 73%. *Chlorina* from variants 6, 8 phenotypically differed from each other. In M_3 the proportion of mutants was from 82 to 88%, while in $M_4 - M_6$ it was 100% in both variants. The absence of segregation indicated plastom homogeneity of these plants.

Variegated forms induced by MNH are most likely of different origin. The forms developed in 1995 produced variegated, green, and yellow-white plants after self-pollination in M₃ and M₄. The variegated M₂ forms developed in 1996 produced *chlorina* plants in addition to variegated and green forms in M₃ (5,6%). The proportion of variegated forms was 25%. This was a unique variant in which *chlorina*-type mutants appeared in the progeny of variegated plants after self-pollination. The mutational changes in cytoplasmic organelles are manifested due to plastid sorting during the period necessary to attain near-homoplastomic conditions. Segregation into two phenotypic classes after self-pollination – the original mutant and normal green – indicated the existence of two plastid types. In *chlorina*-type mutants, altered chloroplasts prevail and their proportion in a cell increases in generations. The appearance of a single variegated form in the *chlorina* progeny (M₃ in the variant 1) and *chlorina* plants in the progeny of variegated forms (M₃ in the variant 11) may indicate the existence of several types of plastids in the cells of these plants. The reciprocal crosses between variegated plants (variant 2) and the line 3629 showed that, when a variegated form was used as a female, segregation in F₁ hybrid progeny was similar to that after self-pollination. In reciprocal crosses, all progeny were green, both in F₁ and F₂ (Table 2). Results indicate the plastid nature of this mutation. Cross of *chlorina* mutants (variant 6) to the green line 3629 revealed the dominance of green phenotype in F₁, irrespective of cross direction. This provides evidence for the nuclear origin of these mutations. In F₂ segregation for green and *chlorina* plants was observed. This segregation corresponded to the expected 3:1 ratio.

Table 2. Result of crosses between mutants and the original line 3629

Cross combination	F ₁ plants				F ₂ plants			
	Green	<i>Chlorina</i>	Variegated	Inviabile	Green	<i>Chlorina</i>	Variegated	Inviabile
DNP(0-12)-MNH(var.8)x3629	74	29	0	0	55	13	0	6
3629xDNP(0-12)-MNH(var.8)	174	0	0	0	363	42	4	12
R(0-12)-MNH(var.3)x3629	91	29	0	0	41	13	0	2
3629xR(0-12)-MNH(var.3)	74	0	0	0	46	7	0	1
R(6-12)-MNH(var.2)x3629	18	0	20	2	-	-	-	-
3629xR(6-12)-MNH(var.2)	73	0	0	0	72	0	0	0
DNP(0-6)-MNH(var.6)x3629	97	0	0	0	99	22	0	0
3629xDNP(0-6)-MNH(var.6)	41	0	0	0	92	22	0	0

Different results were observed in reciprocal crosses between the line 3629 and *chlorina* plants induced by MNH in combination with pretreatment with DNP or R for 12 h (variants 8 and 3 accordingly). When plants of the line 3629 were used in crosses as females, only green plants were observed in F₁. In reciprocal crosses, segregation of *chlorina* and green plants was observed in F₁. The proportion of *chlorina* in progeny was 24-28%. The obtained data demonstrate that these two *chlorina* mutations are of complex nature due to nuclear-cytoplasmic interactions. The genetic analysis of induced mutations in sunflower revealed that MNH induces variegated sunflower forms of the plastome nature. The mutagen in combination with DNP induces *chlorina*-type mutations. Beletskii succeeded in isolating *chlorina* mutations of extra nuclear origin in sunflower using MNH at concentrations of 0,01% and 0,015%. Nuclear *chlorina* mutants were generated only at the mutagen concentration above 0,02% [3]. In the presented experiment, the pretreatment with DNP for 6 h (variant 6) induced nuclear *chlorina* mutations at MNH concentration of 0,015%.

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IN VITRO MUTAGENESIS AND PRODUCTION OF AGRONOMICALLY USEFUL POTATO VARIANTS

In vitro grown shoot cultures of two Indian potato varieties 'Kufri jyoti' and 'Kufri Chandramukhi' were subjected to gamma irradiation at 20 and 40 Gy. The irradiated shoot cultures were subcultured to yield a generation of plantlets. After 4-6 weeks of incubation, these shoots were transferred onto MS medium supplemented with benzylaminopurine, BAP (10mg/l) and sucrose (8% w/v) and incubated at 20°C. The M₁V₃ plants were screened *in vitro* for late blight resistance by detached leaf method [4; 5]. The resistant plants were screened in M₁V₄ generation by artificial inoculation of sporangial inoculum on the pot sown plants. Chlorophyll persistence is a simple screening method for heat tolerance [3]. Chlorophyll persistence of different plantlets showed that the percentage of injury was less in the case of plants, which had been obtained from irradiated material. In the case of control plants, there was one hundred-percent damage to the plants. The mutation frequency was calculated for characters like late blight resistance and heat tolerance (*in vitro* microtuberisation and chlorophyll persistence). The gamma ray dose of 40 Gy was observed to produce a higher mutation frequency (Tab. 1).

Table 1. Radiation induced mutation frequency in potato microtuber progeny

Variety	Character	Treatment	Variation observed (%)
Kufri Chandramukhi	Late blight resistance	40 Gy	26.3
		20 Gy	14.5
Kufri Jyoti		40 Gy	17.6
		20 Gy	20.0
Kufri Chandramukhi	Heat tolerance (<i>in vitro</i>)	40 Gy	28.0
		20 Gy	12.0
Kufri Jyoti		40 Gy	22.0
		20 Gy	8.0
Kufri Chandramukhi	Heat tolerance (Chlorophyll resistance)	40 Gy	46.3
		20 Gy	28.0
Kufri Jyoti		40 Gy	34.0
		20 Gy	30.6

Among the two cultivars studied Kufri Chandramukhi was observed to be more amenable to induction of mutations as it showed a higher mutation frequency than Kufri jyoti. A dose of 100-200 Gy was sufficient to induce mutations *in vivo* while for *in vitro* the dose was considerably reduced to 30-50 Gy [2]. The mutation frequency observed in the present investigation ranged from 1.2% to 46.34%. The mutation frequency was different for two doses of irradiation for the two cultivars. Hence genotypic differences were detected with regard to the response to mutation induction. A mutation frequency of 4.2 to 9.4 percent for various characters, after plantlet irradiation *in vitro*, has been observed and a small population was found to be sufficient to get useful variants through this technique [1].

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EFFECT OF *IN VITRO* MUTAGENESIS ON PLANT REGENERATION IN *Citrus aurantifolia* S.

Callus was induced from different explants excised from *in vitro* raised seedlings on MS medium enriched with naphthalene acetic acid (NAA) (10 mg/l) and kinetin (0.2 mg/l). The cultures were maintained on the same media for 30 days. Part of the 30-day-old calli were exposed to gamma radiation (5 and 10 Gy) and the rest were treated with ethyl methanesulphonate (EMS) (0.1 to 0.4%) for 8 hours. All the treated calli were immediately transferred to regeneration medium [1/2 MS+Benzyl Amino Purine (BAP) (5 mg/l)] along with the untreated control. The cultures were maintained under conditions of 25± 2°C, 16/8 hours day and night regime and 2500-3000 lux light intensity. The results indicated a significant effect of mutagenic agent on callus regeneration (Fig. 1 and 2) and regenerants' morphological features. The same phenomenon was observed in *Triticum aestivum* and *Zea mays* [1; 2]. Regenerated mutants showed variation in morphological traits like, plant height, leaf length and breadth. Moreover, the mutants are being screened for resistance against citrus canker. However, the genetic origin of the mutants has not been determined.

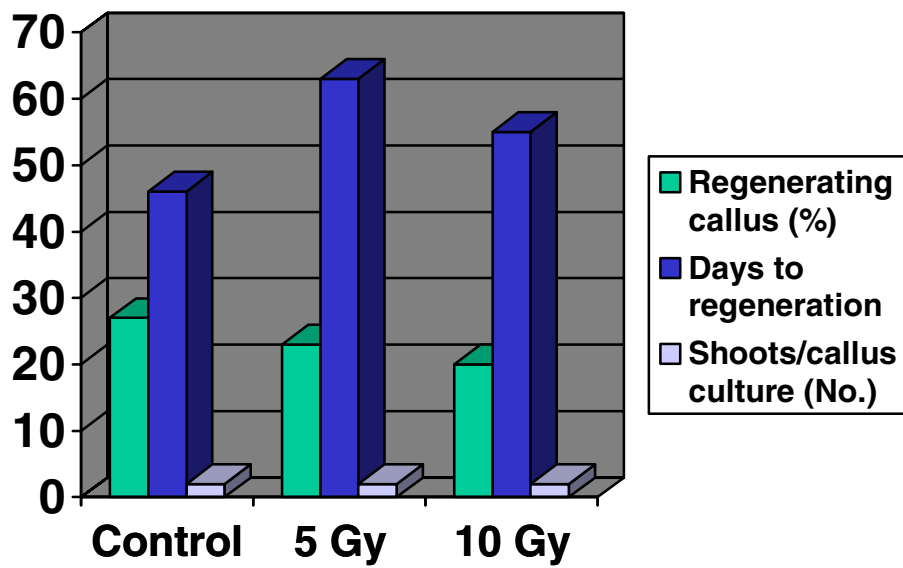


Fig. 1. Regeneration Response of 30 day old gamma irradiated callus [medium: 1/2 MS + BAP (5 mg/l) + sucrose (3%)]

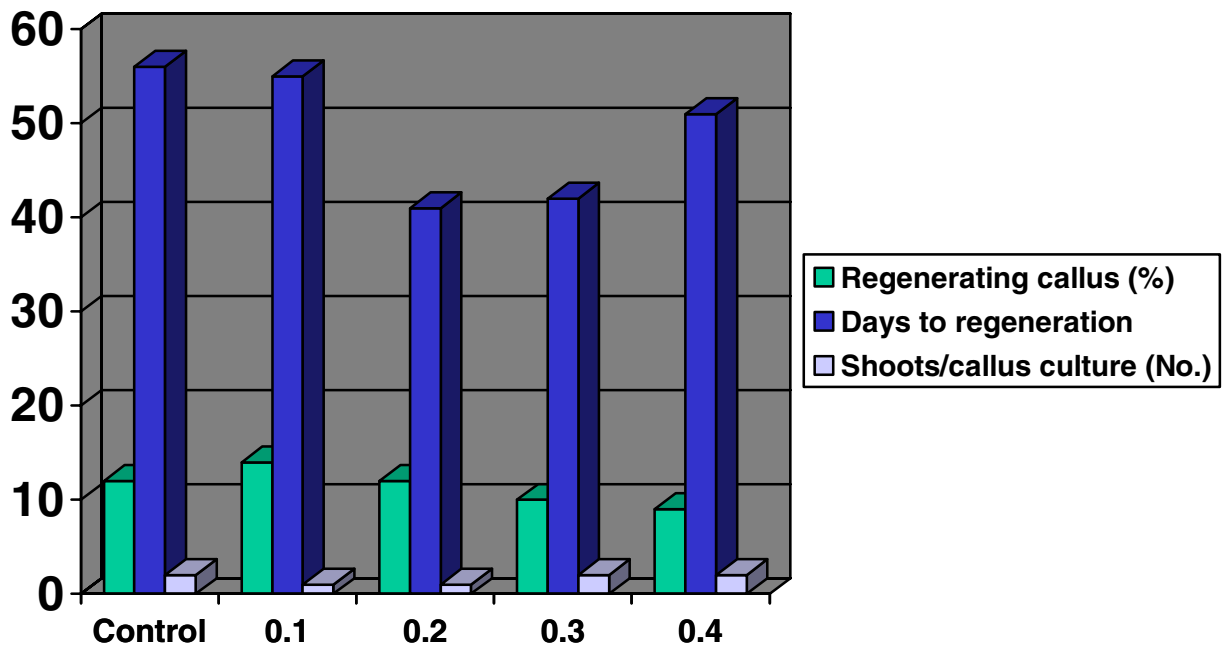


Fig. 2. Callus regeneration after EMS treatment [medium: 1/2 MS + BAP (5 mg/l) + sucrose (3%)]

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OBTAINING UNIQUE LARGE KERNEL RICE USING CHEMICAL MUTAGENESIS IN TISSUE CULTURE

Lines with improved characters have been received by chemical mutagenesis in rice tissue culture. The *japonica* rice (*Oryza sativa* L.) varieties 'Krasnodarskii 424', 'Dubovskii 129', 'Slavyanetz', 'Liman', 'Lomello', 'VNIIR 2471' were used for mutation induction. N-nitroso-N-methylurea (NMH) has been used as a mutagen. Two approaches were applied:

1. Development mutants by mutagenic treatment of seeds
2. Development regenerants from somatic tissue culture.

In the first case, dry seeds with removed covering glumes have been treated with a solution of NMH (exposure 24 hours, tested concentrations 0.05%; 0.1%; 0.2%). After treatment seeds have been rinsed and planted into the soil in vessels. The effect of mutagen was very much genotype dependant. The highest frequency of mutants were observed in the following concentrations of NMH: for variety VNIIR 2471 – 0.05-0.1%, for variety Slavyanetz – 0.1%; for Lomello – 0.2%; for Linman – 0.05% and 0.2%.

The mutant N 95, which has been selected from variety Liman after treatment with 0.2% concentration of mutagen, had the following improved characters: vegetation period 103 days (110 days for the parent variety); plant height 93.2 cm (98.2 cm - parent variety); length of the main panicle 17.2 cm; 1000 grain mass 44.9 g (39.2 g - parent variety). Mutant line N 101 selected from the same variety Liman after treatment with 0.05% concentration of mutagen mutated also in many characters: vegetation period 103 days; plant height 106 cm; 1000 grain mass was 47.0 g. In the second experiment, a somatic callus of the 2nd passage from varieties Krasnodarskii 424, Dubovskii 129, Slavyanetz, Liman were treated with the solution of mutagen NMH (concentration: 0.05%; 0.1%; 0.2% + 0.1% PABA by 40 minutes at Certomat shaking machine (100 rev./min). The treated callus has been cultivated at MS regeneration media (4 mg 2.4 D + 20 mg /l of sucrose) and MS intermediate media (non-hormonal + PABA) to obtain regenerants. Plant regeneration was carried out with the lighting 2000 lux at temperature 28-30°C. Regenerants at 2-3 leaf stage (with roots) were transplanted to liquid nutritive media of Yoshida (ph 5.0 – 5.4) and after 5-7 days they were transplanted to the vessels with soil.

The best results were obtained for the following treatments: 0.05% NMH + 0.01% PABA for varieties Krasnodarskii 424, Dubovskii 129 and Liman; and 0.01% NMH + 0.01 PABA for variety Slavyanetz. Line N 70 from callus Krasnodarskii 424 was characterized by the best indices as compared to plants from non-treated callus: vegetation period 107 days (120 days of control); panicle length 18.5 cm (16.6 cm of control); and 1000 grains weight 43.2 g (25.9 g of control).

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ISOLATION OF CARROT PLANT LINES WITH ALTERED CAROTENE CONTENTS FROM GAMMA IRRADIATED EXPLANTS

Dietary vitamin A is mainly obtained from carotenes of vegetables and fruits. Carrot (*Daucus carota* L.) is one of the major sources of carotene. Carrot cultivars have been obtained mainly through classical breeding, and genetic selection has permitted the creation of new varieties with high carotene contents. The fact that in several crops agronomically important mutants/variants have been generated by *in vitro* culture techniques prompted us to combine gamma irradiation and *in vitro* somatic embryogenesis to obtain regenerants with variations in carotene content in carrot.

To test the effect of gamma rays on somatic embryogenesis and on the carotene level, aseptically germinated seedlings of 8 carrot varieties were exposed to 5; 10 and 500 Gy before culturing petiole segments on LN1 medium [1]. Non-irradiated petioles produced calli with somatic embryos, while irradiated explants reacted differently according to radiation dose (Table 1). After 4 weeks of culture on LN1 medium, petiole segments of different varieties irradiated with 5 and 10 Gy gave more callus with embryos than those with non-irradiated segments. However, after the subculture on LN medium, the development of embryos into plantlets was rare. It was also noted that after irradiation with 5 Gy, the petiole segments gave voluminous calli. Further, in variety 'Chantenay', the irradiated calli were deep orange while non-irradiated calli were green. However, embryo formation was not observed in these calli. This orange coloration suggests an appreciable synthesis of carotene in the calli. Gamma rays, probably produced cell lines with different colors and carotene content.

Of the 8 cultivars tested, normal plantlets of 3 varieties were regenerated from somatic embryos irradiated with 10 Gy, and were transferred to greenhouse to develop roots. For each assay, the carotene analysis was carried out on 2 roots, and compared with plants produced from non-irradiated somatic embryos (Table 2). Carotene level in the plants, derived from irradiated embryos, were always lower compared to controls of the same variety except for one plant in variety 'Scarla' (SC₁₁). The mean carotene level in SC₁₁, based on 10 analyses, was 142.8 µg/g compared with 55.9 µg/g in other irradiated plant SC₁₂, 65.1 µg/g for the plant regenerated from control somatic embryo (SC₁). The difference between SC₁ and SC₁₂ was not significant, at 5% level, but were significant between SC₁, and SC₁₁. The roots of irradiated somatic embryos of the other two cultivars 'Boltex' and 'Tantal' showed similar carotene levels, mean BOI=117.6 µg/g, and TAI=489.3 µg/g, respectively, but lower than those in plants from non-irradiated somatic embryos.

It is concluded that a wide variation in carotene production exists in somaclonal populations derived through somatic embryogenesis with or without gamma-irradiation of carrot. The result is in agreement with the reports that somaclonal variation represents a new source of variability, and therefore, constitutes an additional tool for the breeder.

Table 1. Effect of gamma rays on growth of calli and initiation of somatic embryogenesis in four varieties of carrot

Dose	Intensity of callogenesis (a)				Intensity of somatic embryogenesis (b)			
	Scarla	Tantal	De Chant.	Boltex	Scarla	Tantal	De Chant.	Boltex
0 Gy	++	++	++	++	++	++	++	++
5 Gy	+++	+++	+++	+++	++	++	+	++
10 Gy	+++	+++	++	+++	++	+++	++	+++
50 Gy	++++	++++	++++	++++	0	0	0	0

a: Intensity of callogenesis based on the fresh weight of calli after 4 weeks of culture on LN1 medium
 ++:<300 mg +++:300-500 mg ++++:>500 mg

b: Number of developed somatic embryos obtained on calli after 4 weeks of culture on LN medium
 0:Nul +:<10 ++:10-25 +++:>25

Table 2: Carotene contents in taproots of plants derived from irradiated somatic embryos in 3 varieties of carrots

Varieties	Sources of samples	Carotenes ⁽¹⁾ (mg/g dry mater)
Scarla	Somatic Emb. (SC ₁)	56.1 ± 0.99
	Irrad ¹ (SC ₁₁)	142.8 ± 1.3
	Irrad ² (SC ₁₂)	55.9 ± 1.20
Boltex	Somatic Emb. (B01)	383.0 ± 2.31
	Somatic Emb. (B02)	198.9 ± 1.20
	Irrad ¹ (B0 ₁₁)	118.2 ± 1.49
Tantal	Irrad ² (B0 ₁₂)	117.0 ± 1.20
	Somatic Emb. (TA)	534.7 ± 1.03
	Irrad ¹ (TA ₁₁)	488.7 ± 1.49
	Irrad ² (TA ₁₂)	489.9 ± 1.20

⁽¹⁾Average of 10 replicates for each taproot. ± standard deviation. For the non-irradiated Boltex, 2 taproots of 2 plants were analysed.

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DEVELOPMENT OF NEW VARIETIES OF CHRYSANTHEMUM BY MUTAGENESIS *IN VITRO*

At present, the industry of flower cultivation in Mexico has been demanding new varieties produced locally. There are 6,000 hectares dedicated to the cultivation of flowers for domestic use, however the export is very low. The main production area is located in Villa

Guerrero, a small town near Mexico City, where 80% of the total national production is grown. In addition, approximately 10 hectares of greenhouses are dedicated to the production of flowers for export, mainly in the Peninsula de Baja California and the Altiplano Central (Central Plateau). Unfortunately, the production of flowers in Mexico has been affected by two factors: the first, stock plants must be imported from Holland, France and the United States; and the second, there are some government restrictions on their import. Due to these factors, producers are behind in recent innovations related to new varieties. An alternative to solve this problem would be meristem *in vitro* culture. Plantlets from two varieties 'Polaris Yellow' pom-pom type and 'Dramatic' margarita type, were obtained through the meristems tip culture in the MS culture medium, to which kinetin 1.0 mg/l and NAA 0.05 mg/l were added.

In preliminary studies, the plant material was irradiated with doses between 10 to 60 Gy and it was possible to determine that doses higher than 35 Gy were lethal for both varieties. In this experiment, plantlets were irradiated with seven doses (7.5, 10, 15, 17.5, 20, 22.5 Gy) of ⁶⁰Co gamma rays. They were then subcultured using three types of explants: bud, leaf and internode.

The best variety for production of direct organogenesis was Polaris Yellow in a range of doses between 7.5 and 15 Gy, the buds being the best explant, while the internodes and leaves were not so suitable. In contrary, the leaf was considered to be the best explant for the induction of indirect organogenesis in the variety Dramatic, in a range of doses between 10 and 20 Gy. It was possible to obtain some mutants for color, size and shape of flowers from these materials and it is expected that in the near future they will rise to new varieties.

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TO THE READER

This month the seventh issue of the PLANT BREEDING AND GENETICS NEWSLETTER was also published. The Newsletter will inform you about current activities of the FAO/IAEA sub-programme on plant breeding and genetics which is implemented by the Plant Breeding and Genetics Section of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture (Vienna) in close collaboration with the Plant Breeding Unit of the FAO/IAEA Agriculture and Biotechnology Laboratory (Seibersdorf). The Newsletter (PBGN) is published every 6 months so that you have more information about current and planned activities of the FAO/IAEA Plant Breeding and Genetics sub-Programme. They are also available on the Joint Division's home pages on the Internet. I would like to emphasize, however, that this Newsletter **does not replace the MUTATION BREEDING NEWSLETTER**. In fact, the MBNL will continue to publish scientific papers related to the application of mutation techniques in plant breeding and genetics. Requests for inclusion on the mailing list of the PBGN should be sent to the address indicated on the back cover.

Mirosław MALUSZYNSKI

LAST BUT NOT LEAST

This Newsletter is distributed free of charge. To have your name added to our mailing list, please send your request to the address shown on the back cover. In addition to your full name, the request should indicate the detailed name of your institute, university or plant breeding station. Please note that if a copy is available in your library, a duplicate cannot be sent.

All published papers are reviewed. Please submit your contribution to the Mutation Breeding Newsletter by 1 June and 1 December of each year. Authors are kindly requested to take into account that readers want to learn about new findings and new methods but would also like to see the most relevant data on which statements and contributions should not exceed 2-3 double-spaced typewritten pages including tables. We regret that for technical reasons photographs cannot be accepted. References to publications containing a more detailed description of methods for evaluation of findings are welcome but should generally be limited.

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