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EVALUATION OF GAMMA RADIATION INDUCED MORPHOLOGICAL MUTANTS IN DOLICHOS BEAN (*Lablab Purpureus* L.) IN M_2 GENERATION

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ABSTRACT

The mutagenic sensitivity was evaluated in dolichos bean cultivars Deepali and Konkan Bushan by treating the seeds with 25kR, 35kR, 45kR, 55kR and 65kR of gamma radiations. All the treatments of both cultivars with gamma radiations had shown tremendous variations in morphological and yield traits in both the generations. In M_1 generation, treatments with 65kR had registered the lowest germination (62% in Deepali and 59% in Konkan Bhushan) and the LD_{50} was reported 51.2kR and 48.6kR in Deepali and Konkan Bhushan respectively. In M_2 generation mutagenic frequency, efficiency and effectiveness were increased with increasing doses of gamma radiations. The maximum frequency (19.72% in Deepali and 26.31% in Konkan Bhushan) was observed in 55 kR of both cultivars. Total 28 macromutants from Deepali and 37 macromutants from Konkan Bhushan were isolated. Among them the mutant having specific and beneficial traits from Deepali *viz.*, dwarf (55kR) and small pod (45kR) mutants and Konkan Bhushan *viz.,* dwarf (45kR and 55kR), extensive branching (55kR), early flowering (35kR and 55kR) and rachis branching (55kR) mutants were found useful and may be useful in breeding for further improvement of dolichos bean.

INTRODUCTION

Dolichos bean (*Lablab purpureus* L.*)* belongs to the family Leguminoceae, sub family faboidene, tribe phaseoleae, sub tribe phaseolineae and the genus *Lablab*. Genus *Lablab* has included several distinct species names but, it is currently regarded as monospecific and the chromosome number varies, with 2n = 20, 22, 24 (Philip, 1982) within the genus. Dolichos bean most commonly found in peninsular region of India and cultivated in a large extent in Karnataka and adjoining districts of Tamil Nadu, Andhra Pradesh and Maharashtra.

Large proportion of Indian population relies on grain legumes as a dietary source of proteins due to economic or cultural reasons. Approximately 2334 (000) MT of beans were produced in year 2015-16 (NHB, 2017) in India. The demand for food and feed is growing with increasing population, while natural resources are limited. The yield potential of crop plants has to be significantly increased to combat the increasing demand. The approach of increasing the productivity by genetically improving the crop plants appears to be viable and eco-friendly in the present scenario. Growing such genetically improved varieties along with appropriate cultural practices can significantly boost the crop productivity. Success of a crop improvement programme depends on the availability of large genetic variability, which a plant breeder can combine to generate new varieties. This variability is the outcome of naturally occurring mutations (Hazra and Som, 1999). In nature, occurrence of natural variability in the form of spontaneous mutations is extremely low, which can be enhanced to several folds by using ionizing radiations. Mutation is an important source of variability in the organisms. The variability caused by induced mutation is not necessarily different from the variability caused by the spontaneous mutation during evolution. Mutation provides us the raw material for the genetic improvement of the commercial crops and occasionally a new cultivar also.

The efforts of improving the crop by utilizing indigenous and exotic germplasm have been useful in breaking the yield barriers resulting in compact plant type, reduced duration, photo-insensitivity and high yielding types (Shivashankar *et al.,* 1993). Gamma rays are the most energetic form of electromagnetic radiation, their energy level is from ten to several hundred kilo electron volts and they are considered as the most penetrating compared to other radiations (Kovacs and Keresztes, 2002). Dolichos bean is a highly self pollinated (cleistogamous) crop and naturally variability percentage is very low. Gamma rays have tremendous capacity to create variability (Chakraborthy and Parthasarathy, 2003). Hence, this experiment is framed out to create the variability in dolichos bean with the objectives of to evaluate the effect of gamma radiations and to find out the suitable dose of gamma radiation for inducing variability in dolichos bean cultivars Deepali and Konkan Bhushan.

MATERIALS AND METHODS

The present investigation was carried out during *Kharif* season

2014-15 for M_1 generation and 2015-16 for M_2 generation at Chilli and Vegetable Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola and the gamma radiation treatment on seeds of both cultivars was done at Bhabha Atomic Research Centre (BARC), Trombay, Mumbai. The experiment on M_1 generation was laid out in the open field in progeny rows as per the treatments (25, 35, 45, 55 and 65 kR) along with control treatments and the observations on seed germination and mortality were recorded. Also the LD_{50} has been calculated for both cultivars.

$$\text{Germination (\%)} = \frac{\text{Total Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

$$\text{Mortality (\%)} = \frac{\text{Number of plants failed to survive upto maturity}}{\text{Number of seeds germinated}} \times 100$$

Determination of LD_{50} by using Probit analysis and Finney's table (Finney, 1971)

$PT = (P_0 - P_c)/100 - P_c \times 100$

Where, P_0 is observed mortality percentage, P_c is control mortality percentage and PT is corrected mortality percentage. Then the computation of LD_{50} value for both cultivars was produced.

The seeds collected from all the treatments of M_1 generation of dolichos bean were sown in progeny rows to raise the M_2 generation according to the treatments (*viz.,* 25, 35, 45 and 55 kR) of cultivars Deepali and Konkan Bhushan along with control treatments. The treatment 65 kR of both cultivars from M_1 generation was discarded because of higher morphological defects, mortality and non viable seeds. All the recommended cultural measures *viz.*, irrigation, weeding and plant protection were carried out during the growing period of crop in both generations. In field, observation on the germination and mortality were recorded. Several morphological variants *viz.,* dwarf, leaf, flower, pod and chlorophyll variants were observed in cultivar Deepali and variants *viz.*, dwarf, tall, early, extensively branched, leaf, flower, pod, seed and chlorophyll variants were observed in cultivar Konkan Bhushan along with their growth, yield and quality parameters. The chlorophyll content was estimated according to DMSO method and calculated with the help of formula suggested by Arnon, 1949. The protein content was estimated using the Kjeldahl's distillation method (Kjeldahl, 1883) and percent protein was calculated by using following formula (AOAC, 1990)

Protein (%) = N (%) x 6.25

The observation on mutagenic frequency, efficiency and effectiveness were also recorded with respect of all doses of gamma radiations. Mutation frequency means rate of mutation in given numbers of treated population was calculated with the formula suggested by Gaul, 1958.

$$\text{Mutation frequency (\%)} = \frac{\text{Number of visible mutant scored}}{\text{Total plant population in treatment}} \times 100$$

Mutagenic efficiency and effectiveness was calculated was estimated as per the formula given by Konzak *et al.*, 1965. Mutagenic efficiency gives an idea of genetic damage (mutation) in relation to the total biological damage caused in treated population.

$$\text{Mutagenic efficiency} = \frac{\text{Mutation per 100 M2 plants (MSD)}}{\text{Lethality (Mortality percentage)}}$$

Mutagenic effectiveness of radiations is a measure of the mutations induced per unit dose of gamma radiations.

Mutagenic Effectiveness = MSD/kR

Where,

MSD = Mutation per 100 M_2 plants (mutation frequency on M_2 plants basis)

kR = Radiation dose in kilo roentgens

RESULTS AND DISCUSSION

M_1 generation (2014-15)

The observation recorded from M_1 generation had shown disastrous decrease in germination percentage and increase in mortality with increasing doses of gamma radiations. The maximum germination percentage was in control treatments of both cultivars and it was the minimum (62% in Deepali and 59% in Konkan Bhushan) in 65 kR treatments (Table 1.). From the Table 2, it was revealed that the mortality rate increased linearly with increasing doses of gamma radiations, though the mortality percentage of Konkan Bhushan was higher than the Deepali. The maximum mortality percentage (74.19% in Deepali and 77.97% in Konakan Bhushan) was recorded in 65 kR treatment of both cultivars. The similar results were also obtained by Ahirwar *et al.*, 2014 and Lavanya *et al.*, 2017.

In the present investigation 55 kR and 65 kR treatments were

Table 1: Effect of gamma radiation on germination percentage of Dolichos bean cultivars Deepali and Konkan Bhushan in M_1 generation

Treatments	Germination (%)					
	Control	25 kR	35 kR	45 kR	55 kR	65 kR
Deepali	98	86	82	75	71	62
Konkan Bhushan	98	89	81	76	69	59

Table 2: Effect of gamma radiations on mortality percentage and LD_{50} value of Dolichos bean cultivars Deepali and Konkan Bhushan in M_1 generation

Cultivars	Control		25 kR		35 kR		45 kR		55 kR		65 kR		LD_{50} (kR)
	P_c	PT	P_o	PT	P_o	PT	P_o	PT	P_o	PT	P_o	PT	
Deepali	2	2	22.09	18.09	28.05	24.05	37.33	33.33	52.11	48.11	74.19	70.19	51.2
Konkan Bhushan	3	3	15.73	9.73	24.69	18.69	40.79	34.79	55.07	49.07	77.97	71.97	48.6

P_0 = observed mortality percentage; P_c = control mortality percentage and PT = corrected mortality percentage.

Table 3: Effect of gamma radiation on germination and mortality percentage of Dolichos bean cultivars Deepali and Konkan Bhushan in M_2 generation

Treatments	Deepali Germination (%)	Mortality (%)	Konkan Bhushan Germination (%)	Mortality (%)
T_1 – 25 kR	87.33	24.14	94.33	6.38
T_2 – 35 kR	90.00	14.44	92.67	8.89
T_3 – 45 kR	89.33	15.73	87.33	29.89
T_4 – 55 kR	88.33	18.39	86.67	32.94
T_5 – Control	98.67	-	98.00	-

Table 4: Effect of gamma radiation on mutagenic frequency, mutagenic efficiency and mutagenic effectiveness of Dolichos bean cultivars Deepali and Konkan Bhushan in M_2 generation

Treatment	Deepali Mutagenic frequency (%)	Mutagenic efficiency (MP/L)	Mutagenic Effectiveness (MP/kR)	Konkan Bhushan Mutagenic frequency (%)	Mutagenic efficiency (MP/L)	Mutagenic Effectiveness (MP/kR)
T_1 – 25 kR	3.03	0.125	0.121	3.41	0.534	0.136
T_2 – 35 kR	7.79	0.539	0.223	8.43	0.948	0.241
T_3 – 45 kR	10.67	0.678	0.237	19.67	0.658	0.437
T_4 – 55 kR	19.72	1.072	0.358	26.31	0.799	0.478
T_5 – Control	-	-	-	-	-	-

Table 5: The growth, yield and qualitative characters of dolichos bean cultivar Deepali and gamma radiation induced mutants

Vegetative Characters Mutant	Vine length (cm)	Branches plant^{-1}	Days to first flower	Days to first pod set	Green pod plant^{-1}	Green pod weight(g)	Green pod yield plant^{-1}(g)	Seeds pod^{-1}	Seed yield plant^{-1}	Protein content (%)	Total chlorophyll content (mg/g)
Dwarf plant variant	296.0	8.0	135.0	139.0	102.0	13.5	1377.0	5.2	142.0	18.6	1.68
Leaf variant	590.0	7.0	142.0	147.0	96.0	14.2	1363.2	5.0	105.0	18.5	1.12
Flower colour variant	605.0	6.0	152.0	158.0	105.0	13.3	1396.5	4.3	104.0	17.9	1.77
Early flowering variant	425.0	5.0	125.0	129.0	105.0	12.8	1344.0	4.9	129.0	18.3	1.48
Small pod variant-1	350.0	10.0	141.0.0	146.0	217.0	5.9	1280.0	4.1	211.0	18.8	1.75
Small pod variant-2	395.0	9.0	161.0	168.0	21.0	3.4	71.4	2.7	11.0	19.0	1.90
Pod colour variant	570.0	5.0	161.0	169.0	79.0	6.9	545.1	3.5	85.0	18.9	1.81
Chlorophyll variant	445.0	6.0	149.0	156.0	108.0	13.8	1490.4	4.8	138.0	18.2	1.07
control	626.67	5.73	141.67	146.0	116.30	14.03	1631.97	5.10	146.83	18.80	1.571

found comparatively more lethal than the lower doses for both cultivars. The lethal doses (LD_{50}) determined by the Probit analysis were 51.2 kR for Deepali and 48.6 kR for Konkan Bhushan. The results of present investigation were also been supported earlier by Priya Ranjan Tah, 2006 in mungbean, Kamau *et al.,* 2011 and Monica and Seetharaman, 2014 in dolichos bean and Ariraman *et al.,* 2014 in pigeon pea.

M_2 generation (2015-16)

From the Table 3, all the treatments had shown gradual decrease in germination percentage and increase in mortality percentage than the control. The minimum germination percentage in Deepali was observed in 25 kR and the maximum in 35 kR dose. In Konkan Bhushan, it was the minimum in 55 kR and the maximum in 25 kR.

This might be due to the fact that, an application of mutagen on seeds delayed the emergence of roots, reduction in vigour, low metabolic and enzymatic activity, losses in membrane integrity, which might leads to failure of germination. The similar results were also suggested by Kumar and Mishra, 2004 in okra, Kamau *et al.*, 2011 in dolichos bean Avinash A., 2013 in pea and Meshram *et al.,* 2013 in black gram. The highest mortality percentage was observed in 25 kR in Deepali and 55 kR in Konkan Bhushan, while it was the minimum in 35 kR in Deepali and 25 kR in Konkan Bhushan. The significant increase in mortality is attributed to the physiological disturbance or chromosomal damage caused to the cells of the plant by the mutagen (Thilagavathi and Mullainathan, 2011). Higher dosed of gamma radiation caused the higher degree of mortality (Sharma *et al.,* 2005; Kamau *et al.,* 2011).

Mutagenic Frequency, Efficiency and Effectiveness

The data pertaining to the effect of different doses of gamma radiations on mutagenic frequency, efficiency and effectiveness in dolichos bean cultivars Deepali and Konkan Bhushan in M_2 generation are presented in Table 4.

In both the cultivars, the mutation frequency was increased with increasing doses of gamma radiations and the maximum frequency was observed in 55 kR treatment which indicated that higher doses induced more macromutation and also increased the rate of mutations as compared to lower doses. However, marked difference in mutation frequency of Konkan Bhushan was noted than Deepali. This may be described due to more chromosomal aberration and disturbance in the production and distribution of growth substances incited by the higher dose of the irradiation. These results were

PLATE-1

Gamma rays induced morphological mutants observed in dolichos bean cv. Deepali

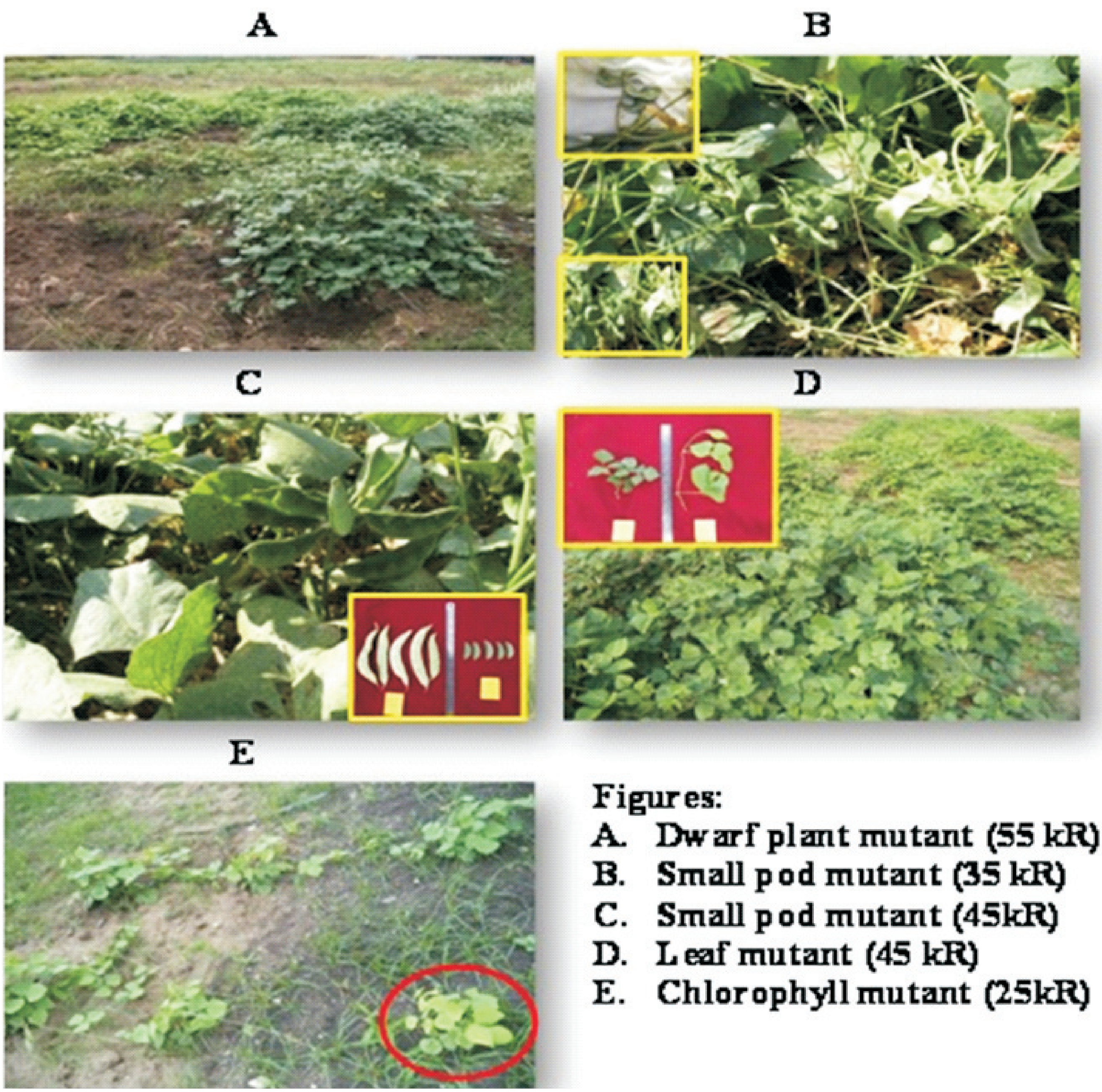
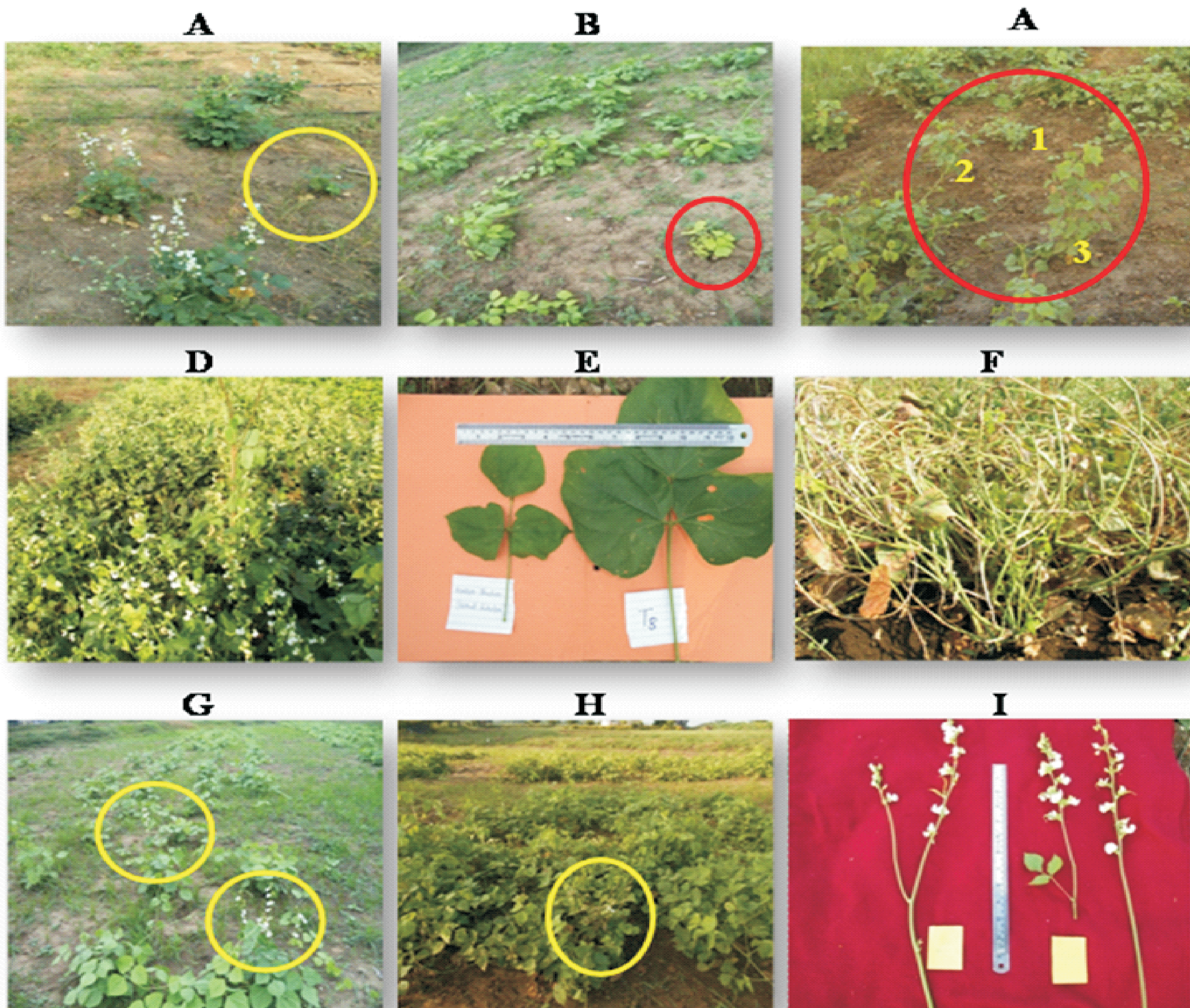


Plate 1

inconformity of findings of Ahirwar *et al.*, 2014 in microsperma lentil and Usharani and Ananda Kumar 2015 in urdbean. The mutagenic efficiency was also increased with increasing doses of gamma radiations in both the cultivars, which indicated that, despite causing higher mortality in plants, the higher doses were capable of inducing more macromutants as compared to lower doses. The mutagenic effectiveness of radiations was represented an increasing pattern with

PLATE-2

Gamma rays induced morphological mutants observed in dolichos bean cv. Konkan Bhushan



Figures:

A-Dwarf plant mutant (45 kR)
B- Dwarf plant mutant (45 kR)
C- Dwarf plant mutant (55 kR)
D- Tall plant mutant (35 kR)
E- Large leaf mutant (55 kR)
F- Extensive branching mutant (55 kR)
G- Early flowering mutant (35 kR)
H- Early flowering mutant (55 kR)
I- Rachis branching mutant (55 kR)

increasing doses of gamma radiations in both the cultivars which indicated that numbers of mutations were increased with unit increase in gamma radiation doses. The usefulness of any mutagen in the plant breeding depends not only on it's mutagenic effectiveness but, also on it's mutagenic efficiency (Konzak *et al.*, 1965). These help to find out the scope for the mutation breeding by altering the both qualitative and quantitative characters in the desirable direction with an efficient and effective dose of mutagen. Kamau *et al.*, 2011 reported that, the radio-sensitivity of a *Lablab purpureus* variety DL-002 increased with increasing dose of gamma radiations. The result obtained in the present investigation are in close conformity with the findings of Sinha and Akhaury, 1969 in pigeon pea, Singh *et al.*, 1999, Sharma *et al.*, 2005 and Sagade,

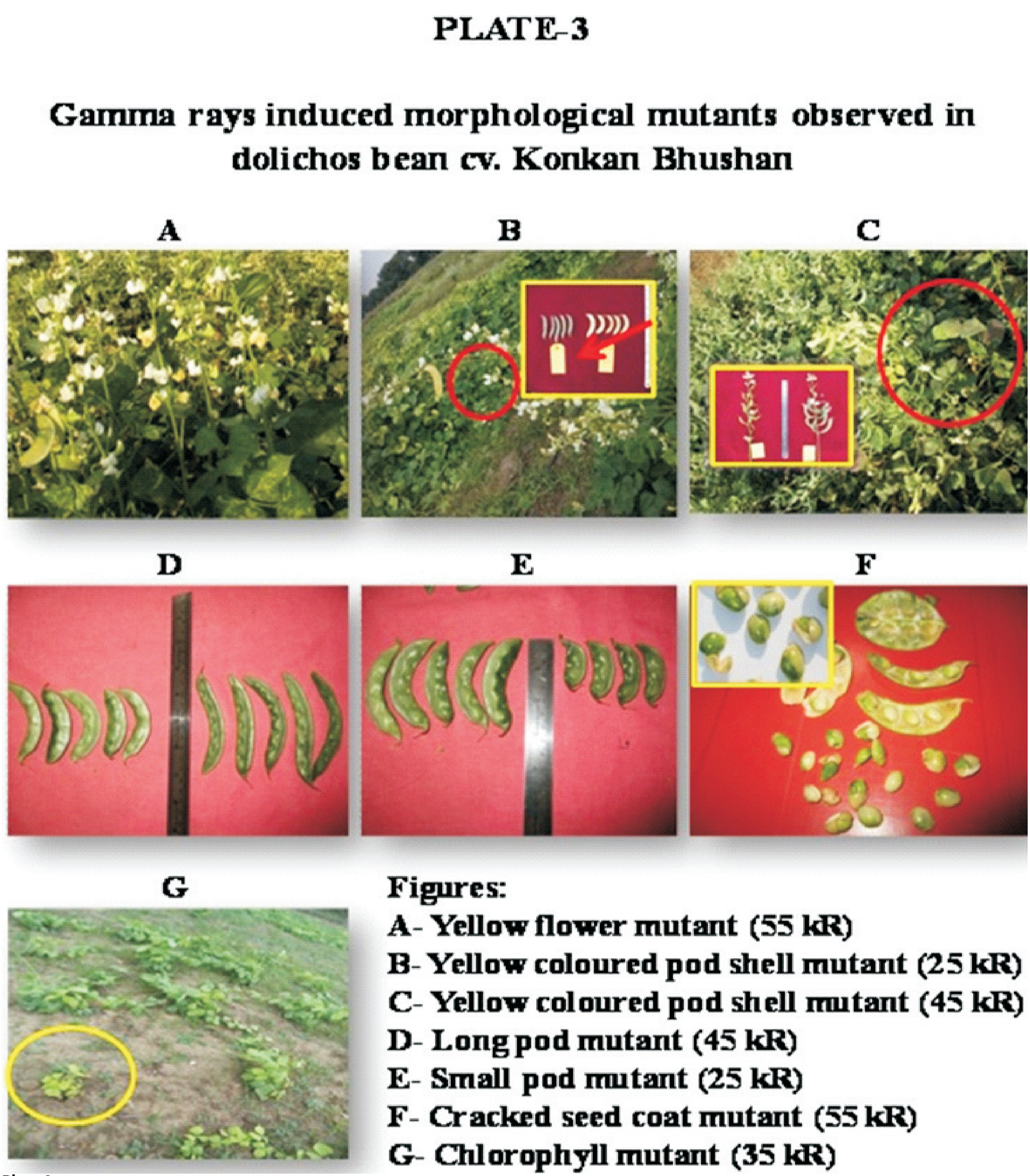


Plate 3

2008 in uradbean, Barshile *et al.*, 2006 in chickpea, Sangle and Kothekar, 2013 in pigeon pea, Gnanamurthy and Dhanavel 2014 in cowpea and Usharani and Ananda Kumar 2015 in urdbean.

Visible variants (macromutations) of cultivar Deepali

Several visible variants (macromutations) were identified and isolated from both cultivars are described below and the other relevant information of growth, yield and qualitative characters are stated in Table 5 and 6. Three dwarf plants were isolated of which one from 45 kR and two from 55 kR treatments. The dwarf plant isolated from 45 kR treatment had also been affected severally *viz.*, hardy vine, delayed flowering and smaller pod

Table 6: The growth, yield and qualitative characters of dolichos bean cultivar Konkan Bhushan and gamma radiation induced mutants

Vegetative characters Mutant	Vine length (cm)	Branches $plant^{-1}$	Days to first flower	Days to first pod set	Green pod $plant^{-1}$	Green pod weight (g)	Green pod yield $plant^{-1}$(g)	Seeds pod^{-1}	Seed yield $plant^{-1}$(g)	Protein content (%)	Chlorophyll content (mg/g)
Dwarf plant variant	98.0	8.0	89.0	93.0	151.0	5.5	830.5	4.6	8.8	22.7	1.77
Tall plant variant	352.0	6.0	156.0	160.0	145.0	4.6	667.0	3.8	78.0	20.9	1.85
Branching variant	158.0	14.0	132.0	136.0	199.0	4.8	955.2	4.9	105.0	22.0	1.91
Leaf variant	200.0	7.0	139.0	145.0	187.0	5.2	972.4	4.1	83.0	20.2	2.42
Early flower variant	218.0	5.0	46.0	51.0	169.0	4.3	726.7	4.5	95.0	22.3	1.73
Inflorescence branching	184.0	8.0	148.0	153.0	204.0	5.1	1040.4	3.7	90.0	21.3	1.97
Flower colour variant	241.0	6.0	144.0	149.0	161.0	4.6	740.6	3.7	71.0	18.7	1.86
Long pod variant	264.0	7.0	127.0	132.0	174.0	6.8	1183.2	6.3	122.0	22.4	2.21
Small pod variant	221.0	8.0	145.0	149.0	153.0	2.5	382.5	2.0	40.0	22.8	2.04
Yellow/white pod variant	234.0	7.0	151.0	158.0	149.0	3.9	581.1	3.5	67.0	17.9	1.74
Seed variant	205.0	9.0	152.0	159.0	181.0	4.7	850.7	3.9	76.0	17.6	1.69
Chlorophyll variant	168.0	6.0	141.0	147.0	176.0	3.9	686.4	4.2	97.0	21.4	1.14
Control	216.67	6.12	113.0	116.33	185.23	4.83	891.07	4.18	102.73	22.56	1.989

but higher yield than the control treatment. Seven plants were identified with variation in leaf size and shape of which one from 35 kR, three from 45 kR and three from 55 kR treatments. Only one yellow flower mutant was isolated from 45 kR treatment, later it produced defective pods along with some normal pods and seeds and also exhibited lower pod weight and yield. Only two small pod variant had been identified of which one from 35 kR and another from 45 kR treatment. The plant isolated from 35 kR treatment had shown lower yield than the control but the plant from 45 kR treatment had shown a remarkable increase in total yield.

Two early flowering plants were isolated from the 25 kR and 55 kR treatments. Both were 20 days earlier than the control treatment and total yield was remained same. Five plants with yellow coloured pods were also isolated of which two from 35 kR, one from 45 kR and two from 55 kR treatments. The other morphological characters and the pod and seed yield was remained unchanged. Total eight plants with yellow leaves and yellow leave with green patches were identified of which one from 25 kR, two from 35 kR, one from 45 kR and four from 55 kR treatments. The average pod yield and seed yield were found much lower than the control treatment in all eight plants.

Visible variants (macromutations) of cultivar Konkan Bhushan

As comparison to cultivar Deepali (28 variants), Konkan Bhushan had registered more (37 variants) numbers of visible macromutations. Among all 37 variants identified and isolated from M_2 generation of Konkan Bhushan, six dwarf plants were isolated of which one from 35 kR, three from 45 kR and two from 55 kR treatments. The mean vine length of dwarf plants was 98.00 cm, while the vine length of control was 216.67 cm. The isolated dwarf plants were also bloomed 24 days earlier but the total yield was lower as compared to control. Beside that two tall plants were also isolated of which one from 35 kR treatment and another from 45 kR treatment. The mean vine length of 352.00 cm and delayed flowering and pod set was recorded in both tall plants. Two extensively branching plants were isolated from the 55 kR treatment with fourteen branches from the vine base, while the control registered only six branches. The number of pods per plant, pod and seed yield was found increased in these two plants. Two plants with double leaf size were isolated from 45 kR and 55kR treatments but the other morphological and yield character were remained closure to the control.

Total six early flowering plants were identified of which two from 35 kR, three from 45 kR and one from 55 kR treatments. These plants started flowering in just 46 days after sowing against 113 days of control treatment. But the numbers of pods per plants were recorded lower due to the irregular and small flower stalk. Beside that, three plants with branched flower stalks were isolated of which one from 35 kR and two from 55 kR treatment. Only one yellow flowering plant was identified from 55 kR which yielded slightly lower than the control. Two plants with slightly longer pods were identified from 35 kR and 45 kR treatments which contain 6-7 seeds per pod while the control plants contain 4-5 seeds per pod. The mean pod yield and seed yield were also recorded higher than the control treatment. Two plants with small pods were isolated from 25 kR and 45 kR treatments contain only 2-3 seeds per pod. These plants registered half the yield of control plants. Three plants from 25 kR, 45 kR and 55 kR were isolated bearing pods of whitish to yellow coloured shell exhibiting lower yield than the control. Only two plants were isolated from 55 kR treatment with some defective pods and seeds having cracked cotyledons. The germination of the seeds with cracked cotyledons was recorded 26.00 percent. Six plant having light to bright yellow leaves and yellow leaves with green patches of different size were identified of which one from 25 kR, one from 35 kR, one from 45 kR and three from 55 kR treatments. All chlorophyll mutants recorded lower chlorophyll content (1.14 mg/g) as compared to control treatment (1.99 mg/g).

The results of the present investigation had shown, the wider spectrum of mutation was observed in M_2 generation in both varieties. The greater frequency and wider spectrum of mutation might be due to having the maximum number of dominant genes indicated the possibility of induction of recessive gene mutation. The different doses of gamma radiations could induced some stable and promising mutants with change length of vine (dwarf and tall mutant), early and late mutant, short and long pod mutant, colour variations in leaves, shoots, flowers and pods etc. The variations induced in these plants might be due to the chromosomal aberrations, changes in chromosome number, gene mutation and re-arrangement of different histogenic layers.

Similarly Patil *et al.,* 2004 reported that the higher doses of mutagen treatments and their combinations induced higher percentage of leaf abnormalities which were maximum upto 51.12% in 25 kR. Kamau *et al.,* 2011 reported the chlorotic spots increased with increase in doses of gamma radiation in M_1 generation of *Lablab purpureus*. Morphological variations, especially leaf abnormalities are the indicators of effective mutagen treatment. In different treatments of gamma radiations different morphological variation like trifoliate, tetrafoliate, pentafoliate, hexafoliate and fused leafs were observed by Thilagavathi and

Mullainathan, 2011 in urdbean. Gnanamurthy and Dhanavel, 2014 in cowpea and Usharani and Ananda Kumar, 2015 in urdbean were observed some viable mutant like tall, dwarf, early maturity, late maturity, leaf mutants pod mutant and flower mutants. Further, Klu *et al.,* 1989 observed a mutant in M_2 generation of gamma irradiated line UPS-122 of winged bean (*Psophocarpus tetragonolobus* L.) did not flower throughout its growth period of 5 months but developed an underground tuber weighing about 100 g.

These macromutants play an important role in a breeding programme or dolichos bean improvement programme as a source of variability. Therefore, the mutant having specific and beneficial characters from Deepali *viz.*, dwarf (55 kR) and small pod (45 kR) mutants and Konkan Bhushan *viz.,* dwarf (45 kR and 55 kR), extensive branching (55 kR), early flowering (35kR and 55 kR) and rachis branching (55 kR) mutants were found useful further improvement in dolichos bean or in a breeding program.

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