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Research Article

Induced Mutants in Locally Adapted Landraces of French Bean (*Phaseolus vulgaris* L.), their Mutagenic Sensitivity and Mutability for Crop Improvement

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Abstract

Mutagenesis is an important breeding strategy to enhance mutation frequency which enables studies of functional genomics and development of new genotypes. An attempt was made to induce mutagenesis into named landraces of french bean *viz.* Bhaderwah Local (BL) and Poonch Local (PL) with gamma rays and ethyl methane sulphonate (EMS) to determine their sensitivity and mutability. Landrace PL was more sensitive than BL to both the mutagens. The LD_{50} of germination percentage for BL and PL was determined as 248.058 Gy and 234.167 Gy, respectively. The maximum number of chlorophyll mutants was found in BL than PL to both mutagens. Gamma rays doses were found to be more effective in both the landraces for the generation of chlorophyll mutants. Mutants in M_2 generation of BL screened under 200 Gy and 250 Gy showed high resistance against anthracnose and moderate resistance of EMS induced mutants under 150 Gy and 225 Gy doses. These genetic stocks could be useful for allele mining and creation of variability for widening the genetic base of traditional landraces of french bean.

Keywords: *Phaseolus vulgaris*; LD_{50}; Chlorophyll Mutations; Mutagen Sensitivity; Radio Sensitivity; Crop Improvement

Introduction

Genetic diversity is required for any crop improvement programme, and when it is lacking in the gene pool, hybridization and mutations are the only tools available to increase allelic variations [3]. It is well known that extreme diversity exists between biological organisms in their response to mutagens. The response to seed treatment with various mutagens provides valuable information for mutation breeding because it simplifies the planning of experiments aimed at increasing mutation frequency. Further, crop genetic improvement is dependent on the amount of genetic variability in the population. However, the available genetic variability within the cultivated gene pool has been exploited to the maximum level of productivity in french bean (*Phaseolus vulgaris* L.), necessitating the creation of additional variations through mutations. In general, mutation breeding has contributed significantly to the improvement of self-pollinated crops such as cowpea [8], black gram [27], soybean [18,19] etc. Although there are several techniques used for mutation breeding, gamma ray is one of the most promising physical mutagens which have been used for inducing biotic and abiotic stress tolerance in seed propagating crops [21,10]. To date, various new crop cultivars have been developed in anthurium [20], coriander [22], mungbean [23] and tomatoes [25], using gamma rays treatments. Furthermore, the ionizing radiation method has also played an important role in the development of superior plants with economically important traits [10]. Also, chlorophyll mutations may be useful in understanding the various physiological functions, biochemical reactions, and pathological invasion when determining the effectiveness of mutagens.

Although mutagens cause changes in nucleotide sequence of DNA and the mode of action of each mutagen is unique, the accompanying undesirable effects like lethality or sterility may decrease its efficiency. Thus, in order to exploit induced mutagenesis for crop improvement, mutagens must be effective and efficient in order to produce the desired effects [2,17]. Therefore, the present study has been aimed at understanding the mutagen sensitivity of local named landraces of french bean belonging to Jammu and Kashmir province of India, including similarities or differences between landraces with regard to radio and chemo sensitivity, mutability, relationship between mutagen sensitivity and mutability using gamma-rays and ethyl methane sulphonate (EMS).

Materials and Methods

Genetic materials

The genetic materials used in the present study were two named landraces of french bean, Bhaderwah Local (BL) and Poonch Local (PL). The orthodox seed samples of these two landraces were exposed to 50, 100, 150, 200 and 250 Gy gamma rays using 5500 Ci 60Co Gamma-chamber 4000 at Bhabha Atomic Research Centre (BARC) in India with dose rate of 120. The same genetic materials were also treated with 0.00 (control), 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0% solution of ethyl methane sulphonate (EMS) that were prepared in phosphate buffer solution at pH 7.0 for 6 hrs with intermittent shaking at 25ºC. After the chemical treatment, the seeds were gently washed under running tap water for half an hour. The seed material treated with mutagens along with 100 untreated control seeds (soaked in phosphate buffer only) were sown immediately in the germinating trays and field without any post-treatment. The observations such as root length, shoot length and number of leaves of 7- days old seedlings grown at 25 ± 1ºC temperature and 97% relative humidity under laboratory conditions were used to assess the dose response. The LD_{50} was calculated for root length, shoot length, number of leaves and germination percentage. Then, LD_{50} with respect to biological parameters was determined using the regression equation of Y (dose) on X (damage) as Y= a+ bx. The seeds from each M_1 plant were harvested separately. Further, M_1 plant progenies were grown in order to score the chlorophyll mutations in M_2 generation. The frequency of chlorophyll mutations was calculated as percentage of families segregating for any type of chlorophyll mutation (M_2 family basis) as well as percentage of chlorophyll mutants in the population of a particular treatment (M_2 mutant basis). The mutation frequency is one of the most dependable indices for evaluating the genetic effects of mutagenic treatments [11]. It was calculated as percentage of mutated M_2 progenies for both chlorophyll as well as morphological mutations of each treatment. The genetic materials were grown in bulk populations and the mutant frequency was estimated by dividing the total number of mutants confirmed by the total number of M_2 plants in the bulk population [7].

Results

The present study revealed that germination percentage, root and shoot length along with number of leaves decreased progressively with increasing doses of gamma rays and EMS in both BL and PL landraces. The study indicated significant correlation coefficients of biological parameters with the doses of mutagens (Figure 1a, 1b and 2a, 2b). The dose dependent relationship was observed for different biological parameters in M_1 generation of two landraces with both physical mutagen (gamma rays) and chemical mutagen (ethyl methane sulphonate). Overall, the biological parameter doses and traits in percentage were inversely proportional to each other. However, reduction in 50% germination percentage occurred at 248.058 Gy gamma rays in BL and 234.167 Gy in PL along with 0.626% in BL and 0.573% in PL with ethyl methane sulphonate (Table 1). The reduction in number of leaves to an extent of 50% was caused by 9.573 Gy in BL and 7.278 Gy in PL with 0.005% and 0.004%, respectively. LD_{50} for shoot length was higher in PL at 8.06 Gy dose as compared to BL at 7.19 Gy whereas for EMS, LD_{50} was at par in both the landraces viz. 0.004% and 0.005% for BL and PL, respectively. Reduction in root length to an extent of 50% was caused by 2.569 Gy in BL and 2.479 in PL whereas for EMS, both the landraces had same LD_{50} (0.002%). At biologically comparable doses for different M_1 parameters, PL was relatively more radio and chemo-sensitive than BL (Table 2), except for shoot length in both the landraces. However, in case of chemo-sensitivity, parallelism was found in both BL and PL for root length. Moreover, BL was more sensitive to both radio and chemo-sensitivity for germination %, number of leaves and root length than PL. Mutability within landraces Chlorophyll mutant percentage varied from 0.09 to 0.29% in BL with EMS. Among the different chlorophyll mutants, chlorina mutants were found to be much higher in percentage followed by viridis and xantha mutants (Table 3). However, chlorophyll mutants ranged from 0.08% to 0.31% in PL with EMS. Xantha mutants were much higher in % followed by viridis and chlorina. Among the two landraces, BL showed maximum number of chlorophyll mutants as compared to PL. Moreover, the rate of production of chlorophyll mutants was 7.07% higher in BL as compared to PL with EMS. Chlorophyll mutation rate increased with an increase in the dose of mutagens up to a certain level, beyond which it decreased (Table 3). Further, the number of chlorophyll mutants varied from 59 to 184 in BL with gamma rays. Among the chlorophyll mutants, chlorina mutants (227) were maximum followed by xantha (190) and viridis (69). Among the different doses, LD_{50} (250 Gy) exhibited maximum number of chlorophyll mutants. However, in PL, maximum number of chlorophyll mutants was exhibited by 250 Gy followed by 275 Gy and 225 Gy along with 100 Gy and 150 Gy which showed least number of mutants (45). Higher dose than LD50 produced lesser number of chlorophyll mutants. It increased from lower dose 100 Gy to 250 Gy. Among the different chlorophyll mutants obtained by both gamma rays and EMS, maximum number of xantha mutants were generated followed by chlorina and viridis. BL produced 29% higher chlorophyll mutants as compared to PL. These chlorophyll mutants could be used as genetic markers and can be employed in the study of photosynthesis, biogenesis of chloroplast and plastids. Mean performance of induced mutants in M_2 generation. An induced mutant at 250 Gy of BL showed higher seed weight, pod length, early maturity and seed yield in comparison to untreated material *i.e.* control along with complete resistance against *Colletotrichum lindemuthianum* (Table 4). Similarly, 250 Gy, an induced mutant line of PL in M_2 generation also exhibited moderate resistance against anthracnose along with higher seed yield in comparison to untreated seeds of PL. This line may be utilised as genetic stock for use as donor parent for the introgression of anthracnose resistant trait. An induced mutant line (0.50%) of PL also manifested moderate resistance against anthracnose in M_2 generation along with higher seed yield and yield attributing traits as compared to control.

Figure 1a: Effects of gamma rays on different M1 biological parameters in Bhaderwah local (BL).

Biological Parameters	Bhaderwah Local	Poonch Local	Bhaderwah Local	Poonch Local
	Physical (Gamma rays (Gy)		Chemical (EMS %)	
Germination %	248.058	234.167	0.626	0.573
No. of Leaves	09.573	07.278	0.005	0.004
Shoot Length(cm)	07.198	08.060	0.004	0.005
Root Length(cm)	02.569	02.479	0.002	0.002

Table 1: LD_{50} values of landraces of *Phaselous vulgaris* L. for different biological parameters in M_1 generation.

Biological Parameters	Bhaderwah Local	Poonch Local	Bhaderwah Local	Poonch Local
	Physical mutagen- Gamma rays (Gy)		Chemical mutagen- (EMS %)	
Germination %	BL>PL		BL>PL	
No. of Leaves	BL>PL		BL> PL	
Shoot Length(cm)	PL>BL		PL>BL	
Root Length(cm)	BL>PL		BL=PL	

Table 2: Relative mutagen sensitivity of Rajmash landraces.

BL- Bhaderwah Local and PL- Poonch Local.

Landraces	NPP	NPG	Albina	Chlorina	Xantha	Viridis	Total no of mutants	Overall frequency (%)
LR-1 (BL)	500	489	-	-	-	-	-	-
0.4% (SL)	500	470	-	15 (0.03%)	12 (0.03%)	18 (0.04%)	45	0.09
0.5% LD_{50}	500	450	-	54 (0.12%)	35 (0.08%)	40 (0.08%)	129	0.29
0.6%(SH)	500	424	-	36 (0.08%)	40 (0.09%)	40 (0.09%)	116	0.27
Total	-	-	-	105	87	98	290	0.22
(PL)	500	483	-	-	-	-	-	-
0.4% (SL)	425	420	-	10 (0.02%)	13 (0.03%)	14 (0.03%)	37	0.08
0.5% LD_{50}	450	416	-	31(0.08%)	58 (0.14%)	41(0.10%)	130	0.31
0.6%(SH)	450	392	-	11(0.03%)	40 (0.10%)	10(0.03%)	61	0.16
Total	-	-	-	52	111	65	228	0.18
Grand Total	-	-	-	157	198	163	518	
LR-1 (BL)	500	495	-	-	-	-	-	-
100 Gy	556	500	1	22 (0.04%)	25 (0.05%)	11 (0.02%)	59.0	0.12 %
150Gy	475	450	-	53 (0.12%)	40 (0.08%)	14 (0.04%)	107	0.24 %
200Gy	600	559	-	70 (0.13%)	55 (0.10%)	12 (0.02%)	137	0.25%
250Gy (LD_{50})	550	502	02	82 (0.16) %	70 (0.14%)	32 (0.06%	184	0.37%
Total	-	-	03	227	190	69	487	0.75%
LR-2 (PL)	500	490	-	-	-	-	-	-
100 Gy	400	355	-	12 (0.03%)	15 (0.04%)	18 (0.05%)	45	0.13%
150Gy	550	400	-	15 (0.04%)	10 (0.03%)	20 (0.6%)	45	0.13%
225Gy	500	335	-	25 (0.07%)	22 (0.07%)	05 (0.01%)	52	0.16%
275 Gy	456	300	-	10 (0.03%)	25 (0.08)	34 (0.11%)	69	0.23%
250 Gy (LD_{50})	600	522	-	56 (0.10%)	72 (0.14%)	38 (0.07%)	166	0.32%
Total	-	-		118	144	115	377	0.19

Table 3: Chlorophyll mutants' frequency in M_2 population of *Phaseolus vulgaris* L.

LR/Mutagens	Dose	100 Seed wt (g)	Length of Pod (cm)	Days to flowering	Days to maturity	Yield/Plant (g)	Anthracnose Reaction
	BL-Control	8.12	6.02	83	125	4.03	S
Bhaderwah Local Gamma rays	100 Gy BL	15.05	7.24	72.80	114.33	4.72	MR
	150 Gy BL	15.24	7.08	76.00	115.19	6.61	MS
	200 Gy BL	11.78	7.13	74.00	113.08	7.87	R
	250 Gy BL	18.78	7.50	70.20	118.90	10.49	R
	PL-Control	7.06	5.64	100	128	6.65	S
Poonch Local	100 Gy PL	12.60	6.70	96.66	116.00	7.68	S
	150 Gy PL	14.19	6.80	78.55	118.00	8.21	MR
	200 Gy PL	7.95	7.45	67.50	118.66	5.57	HS
	225 Gy PL	12.34	6.66	76.00	107.08	4.95	MR
	250Gy PL	15.41	7.32	68.33	114.16	8.90	MR
EMS	0.50% PL	12.11	6.70	75.77	123.41	8.17	MR
	0.60% PL	13.87	7.01	72.25	125.31	3.44	HS

Table 4: Mean performance of M_2 mutants for agro-morphological traits and reaction against anthracnose disease.

MR- Moderately Resistant, R- Resistant, S- Susceptible, MS- Moderately Susceptible and HS- Highly Susceptible, Gy- Gray.

Discussion and Conclusion

The different biological parameters showed a dose-dependent relationship in the M_1 population of two landraces exposed to both physical (gamma rays) and chemical (EMS) mutagens. Mostly, the biological parameter doses and traits in % were inversely proportional to each other. The increasing doses of mutagens caused a progressive increase in the biological damage measured in terms of reduction in germination, number of leaves, root and shoot length (Figure 1a, 1b and 2a, 2b). Notably, [28] have reported negative correlation between all plant growth traits among common bean cultivars. The application of gamma rays affected plant survival rate considerably than the percentage of germination. Similar results have also been reported by [13,14]. The retardation in the root length was more pronounced than that found in the shoots (Table 1). The root system appeared to be relatively more sensitive to mutagens. This can possibly be due to an inhibition of division in root cells by mutagens, which exerts less effect on the elongation of shoot cells. The shoot growth is reported mainly due to the cell elongation while root growth is more dependent on cell division. [16] also found greater delay in rooting than that of shooting in Kalanchoe diagremmntiana leaves following irradiation with gamma rays. [1] found a positive relationship between seed size and mutagen sensitivity in Phaseolus species. Further, the reduction in 50% germination percentage occurred at 248.058 Gy gamma rays in BL and 234.167 Gy in PL whereas 0.626% and 0.573% reductions occurred in BL and PL, respectively with EMS. It is stated that reduction in the percentage of germination caused by high doses of radiations applied may result in reduction in the amount of internal growth regulators depending on the mutagens (Kiong., *et al*. 2008). Ellyfa., *et al*. (2007) also reported that the LD_{50} increased with increasing gamma radiation. At biologically comparable doses for different M_1 parameters, PL was relatively more radio and chemosensitive than BL (Table 2) except for shoot length, however, parallelism was observed in both the landraces under EMS. Generally, a parallelism occurs between the landraces for trait root length. Some of the radio sensitive mutants have also been found to possess increased sensitivity towards alkylating chemicals [4]. LD_{50} value for BL was determined as 248.058 Gy and 234.167 Gy for PL (Table 1). In case of EMS, the concentration of 0.20% was found to be the best. However, the LD_{50} value obtained in this study was found to be different from that indicated by them. This comparison also revealed that genotypes respond differently to the mutation dose and dosescaling studies must be performed for large-scale studies. The relationship in root length was more pronounced than that found in shoot length (Table 1). The root system appeared to be relatively more sensitive to mutagens. This can possibly be due to an inhibition of division in root cells by mutagens, which exert less effect on the elongation of shoot cells. [29] stated that 75Gy-175 Gy application is sufficient for lethality and mutation rates in two Cuban bean genotypes. [26] reported that LD_{50} value was found at 100 Gy for seed germination in *Phaseolus lunatus*. [10] also observed that the dose of 150 Gy was found to be most efficient among the different doses of gamma rays with few exceptions. The chlorophyll mutation frequency was 1.97% more induced by mutagen gamma rays than ethyl methane sulphonate (EMS). However, frequency of induced chlorophyll mutants was more in BL than PL in both the mutagens *viz*., gamma rays and EMS (Table 3). In general, gamma rays were more effective than EMS. Moreover, the frequency of induced chlorophyll mutants was 66.69% more by gamma rays than EMS. Similar results were also reported by [12,13,28]. Furthermore, chlorophyll mutation rate increased with an increase in the dose of mutagen up to LD_{50} in both the landraces for both the mutagens, beyond which it decreased (Table 3). The phenomenon has been attributed to intra somatic selection, reduction in the number of M_2 plants produced by high sterile M_1 plants and other processes of gamete as well as zygote elimination. Similar results were also reported by [6,24]. [8] have also reported that frequency of chlorophyll mutations was dose dependent and increased with increasing dose of each mutagen. Thus, chlorophyll mutations could be exploited for identifying the threshold dose of a mutagen that would increase the probability of retrieving economically useful mutations in ensuing generations. An induced mutant of 250 Gy of BL showed higher seed weight, pod length, early maturity and seed yield in comparison to control along with complete resistance against C. lindemuthianum (Table 4). It may have occurred due to beneficial mutation at dose 250 Gy of gamma rays (LD_{50}). This mutant line may be used as genetic stock for trait introgressions as well as for further screening towards development of an improved variety. An induced mutant line (0.50%) of PL also manifested moderate resistance against anthracnose in M_2 generation along with higher seed yield and yield attributing traits as compared to control. In conclusion, these mutant lines could be utilised as genetic stocks for french bean improvement programme in future crop research.

Conflict of Interest

The authors declare that there is no conflict of interest.

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Author Contributions

SK, MS-conceived the study, conducted experiments and prepared original draft NM, AJS, SJ-formal analysis and helped in final editing JPS, MS-helped in field observations. All authors read and approved the final version of manuscript.

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