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STUDIES ON INDUCED GENETIC VARIABILITY IN LIMA BEAN (*PHASEOLUS LUNATUS* L.)

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ABSTRACT

In past period the crop breeding has been performed by using the genetic variability using conventional method. But now a days such techniques are insufficient for production of new cultivars to fulfil increased food demand. In such condition induced mutation technique which is new and supplementary tool used for evolving improved mutants with desirable agronomic characters. Some desirable mutants will be used directly in selection or beneficial in breeding programme. In the present investigation lima bean seeds were mutagenized with different doses of gamma rays (GR) ranging from 200, 300 and 400Gy, varying concentration of ethyl methane sulphonate (EMS) 0.2, 0.3 and 0.4% and Sodium azide (SA) 0.02, 0.03 and 0.04%. Variations in the germination of seeds, seedling height, seedling injury, pollen fertility and survival of plant at maturity of lima bean were recorded in M_1 generation. The effects of the mutagenic treatments on quantitative traits resulting in reduction in traits such as seed germination except 200Gy (93.11%), seedling height except 200Gy (13.15cm) and 0.02%SA (13.48cm) and survival of plant at maturity but increases seedling injury except 200Gy (-1.31%) and 0.02%SA (-3.85%), and pollen sterility was observed in treated M_1 generated plants.

Key words: Genetic variability, mutagens, lima bean, seedling injury, pollen sterility

INTRODUCTION

Lima bean (*Phaseolus lunatus* L.) is bushy annual herb belongs to family Fabaceae (Leguminosae). It is locally known as pavta. It is mainly cultivated for its fresh and dry seeds. Seeds usually eaten boiled, fried in oil or baked. Lima bean sprouts, young pods, green seeds and leaves are edible and consumed as vegetables. It has been grown as a cover crop and for green manure. Cultivated lima beans has dwarf or bushy habit, with terminal and axillary inflorescence which mature within 90 - 100 days. It is cultivated mainly for its immature and dry seeds. Fresh as well as dry seeds are boiled with maize and rice and used for making special soup or stew. Juice from the leaves is used in nasal instillations against headache. In traditional Asian medicine the seeds and leaves are valued for their astringent qualities and used as a diet against fever. The vines, leaves and empty pods left after the harvest can serve as fodder, and can be made into hay or silage (Baudoin, 2006).

The nutritional value of dried raw seeds (per 100 g) is- water 11.6 g, protein 19.1 g, dietary fibre 19.4 g, carbohydrate 52.9 g, fat 1.7 g. Minerals- Ca 85 mg, Mg 190 mg, P 320 mg, Fe 5.9 mg, Zn 2.8 mg, carotene trace, thiamin 0.45 mg, riboflavin 0.13 mg, niacin 2.5 mg, vitamin B6 0.51 mg and ascorbic acid trace (Holland et al.,1991). Paul et al., (1980) reported the value of essential amino-acid (per 100 g raw lima beans) is- tryptophan 180 mg, lysine 1440 mg, methionine 280 mg, phenylalanine 1160 mg, threonine 800 mg, valine 980 mg, leucine 1560 mg and isoleucine 950 mg.

Lima bean (2n = 22) is bushy annual herb, roots thin, with glabrous or pubescent stems, leaves alternate, trifoliate, stipulet, petiole, inflorescence an axillary raceme, flowers bisexual, papilionaceous, stamens diadelphous, ovary superior, fruit pod, seeds white coloured. *Phaseolus* comprises near about 50 species. Wild as well as cultivated types of *Phaseolus lunatus* have been distinguished as var. silvester Baudet and var. lunatus, respectively. In the cultivated types first group is Sieva group

having medium-sized and flat seeds, Second- Potato group with small globular seeds, and third group is Big Lima group which have large flat seeds. Wild types from the Andes appear closest to the cultivated types.

It is well suited to humid and sub-humid tropical climates which also grow in a wide range of ecological conditions. It is found in warm temperate zones as well as in arid and semi-arid tropical regions. Optimum temperatures are 16 to 27°C, average rainfall is 500–600 mm per year. In India beans mainly cultivated in states such as U.P., M.P., A.P., Haryana, West Bengal, Karnataka, Tamil Nadu, Kerala, Telangana and Maharashtra.

MATERIALS AND METHODS

The seeds of Lima bean (*Phaseolus lunatus* L.) were obtained from local market of Manchar, Tal. Ambegaon, Dist.-Pune- 410503 (M.S.) India. Mutagenic agents such as Gamma rays (GR), ethyl methane sulphonate (EMS) and sodium azide (SA) were employed in present investigation for seed treatments. Gamma radiation from ^{60}Co source fixed in the gamma cell 200 installed at Department of Chemistry, Savitribai Phule, Pune University, Pune was used in the present work. Dry, healthy and uniform seeds of lima bean with moisture content of 10 to 12 % were treated with 200, 300 and 400 Gy. Ethyl methane sulphonate ($CH_3SO_2OC_2H_5$) molecular weight 124.16, and 8% soluble in water, manufactured by Sigma chemical Co. Ltd. USA was used for the seed treatment of lima bean. Various concentrations of EMS (0.2, 0.3 and 0.4%) were prepared in distilled water. Sodium Azide is inorganic compound. It is colour less salt, ionic compound, soluble in water and is highly toxic. Mol. Wt. is 65.0099g/mol. It is chemical mutagen and used for induction of mutations in the crop plants. Different concentrations of SA (0.02, 0.03 and 0.04%) was prepared in distilled water.

The experiments were conducted to determine the lethal dose (LD_{50}), suitable concentrations of EMS, SA and duration of seed treatment. The doses of gamma rays, 200, 300 and 400 Gy, EMS 0.2, 0.3 and 0.4% while SA 0.02, 0.03 and 0.04% were finally selected for the seed treatment and the duration fixed was four hours. Selected seeds were soaked in distilled water for 12 hours and the wet seeds were treated with different concentrations of EMS and SA for four hours. The untreated seeds served as control. For each treatment 180 seeds were used. The treated seeds washed thoroughly with tap water for one hour to leach out the residual chemicals. From each treatment 30 seeds was used for seed germination in laboratory. Three replications with 10 seeds per replication kept in petri dishes, containing seed germination paper, were used for recording seed germination, seedling height on seventh day. The remaining lot of treated seeds (150) from each treatment was used for raising M_1 generation in field. The field experiments were conducted on the research plot at Department of Botany. The soil type of the experimental field was slightly deep, fine and with good drainage. The average minimum temperature was recorded as 17.63^{0}C and maximum 32.73^{0}C with average annual rainfall 641.03mm. All the experiments were carried out in triplicate following RBD design. Each plot had 50 plants.The distance between two rows and two plants was 60 X 45cm

Observations on M_1 generation : The number of seeds showing emergence of the radical and plumule was used to calculate percent seed germination. On seventh day of sowing, 5 seedlings from control and each treatment were randomly selected for measuring the root and shoot length and the average values were recorded in table. Reduction in the mean seedling length as compared to the control was regarded as seedling injury and expressed as percentage.

% seedling injury = (Control seedling height - Treatment seedling height) / Control seedling height X 100.

Pollen sterility was determined from 5 randomly selected plants/ treatment. The pollen grains from freshly dehisced anthers were stained with 1% aceto-carmine. Pollen grains stained as uniform deep red colour were counted as fertile and others as sterile. Survival percent was calculated by scoring the number of plants attaining maturity (45days).

STATISTICAL ANALYSIS

The data were summarized as the means of three replicates with standard deviation as the measures of variability. One-way ANOVA test was performed to determine significant differences due to various treatments. Fisher's LSD (Least significant difference) was used as post hoc test to as certain significant differences among treatments at p= 0.05. Statistical analysis and graphical data presentations were carried out by using Sigma stat (ver.25).

RESULTS AND DISCUSSION

The data on seed germination percent, seedling injury, pollen fertility and survival of plant at maturity in M_1 generation of lima bean were recorded in Table-1. Seed germination in control and mutagen treatments clearly indicated that it was decreased in all the treatments except in 200Gy as compared to control. The mutagens had exerted negative effects on seed germination. The percent seed germination decreased from 79.19% to 62.38% in GR except 200Gy (93.11%), 87.72% to 65.92% in EMS and 88.24% to 67.28% in SA. The maximum (50%) decrease in percent seed germination was observed with GR treatment 400Gy (62.38%), EMS 0.4% (65.92%) and in SA 0.04% (67.28%). The results of present study have clearly shown that lima bean was sensitive to all the mutagens except 200Gy. 200Gy showed increase in seed germination percent over control. Reduction in seed germination with increasing dose/ conc. of mutagens was reported in horsegram (Bolbhat and Dhumal, 2009, Awate and Bolbhat, 2014), black turtle bean (Bolbhat et al., 2020) and in field pea (Kumar and Singh, 1996). GR and EMS are good mutagenic agents, which causes point mutations, enzyme inhibitions as well as chromosomal aberrations.

Table 1 : Effect of mutagens on growth parameters in M_1 generation of Niger

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling height (cm)	Seedling injury (%)	Pollen sterility (%)	Plant survival (45 days) (%)
Control	92.23±12.91	7.35±1.03	5.63±0.79	12.98±1.82	00.00±0.00	5.46±0.76	89.56±12.54
200Gy	93.11±7.45	7.42±0.59	5.73±0.46	13.15±1.05	-1.31±0.10	08.27±0.66	88.31±7.06
300	79.19±8.71	6.83±0.75	5.14±0.57	11.97±1.32	7.78±0.86	12.03±1.32	75.53±8.31
400	62.38±8.11	6.09±0.79	4.68±0.61	10.77±1.40	17.03±2.21	15.35±2.00	60.45±7.86
0.2 % EMS	87.72±12.28	6.95±0.97	5.08±0.71	12.03±1.68	7.32±1.02	09.77±1.37	84.27±11.80
0.3	76.03±5.32	6.21±0.43	4.58±0.32	10.79±0.76	16.87±1.18	13.56±0.95	74.69±5.23
0.4	65.92±5.27	5.33±0.43	4.19±0.34	9.52±0.76	26.66±2.13	19.44±1.56	65.37±5.23
0.02% SA	88.24±13.24	7.10±1.07	6.38±0.96	13.48±2.02	-3.85±0.58	05.72±0.86	86.04±12.91
0.03	79.51±11.13	6.52±0.91	4.92±0.69	11.44±1.60	11.86±1.66	11.49±1.61	75.16±10.52
0.04	67.28±6.06	5.72±0.51	4.31±0.39	10.03±0.90	22.73±2.05	14.16±1.27	66.41±5.98
SEM±	7.77	0.64	0.50	1.14	1.15	1.06	7.50
F-value	4.15	2.41	3.73	2.78	16.22	34.09	3.75
P-value	0.01	0.05	0.01	0.03	0.01	0.01	0.01
$LSD_{0.05}$	16.93	1.39	1.09	2.48	2.51	2.31	16.34

Data are means of three replicates ± standard deviation. Significant difference due to treatments was assessed by Fisher's LSD as a post-hoc test.

Results indicates that doses / conc. of mutagen treatments showed inhibitory effect on seedling height except in few treatments. Lowest seedling height (9.52cm) was noted in 0.4%EMS, 400Gy (10.77cm) and 0.04%SA (10.03cm). Treatments such as 200Gy (13.15cm) and 0.02%SA (13.48cm) showed increase in seedling height over control (12.98cm). Data on the effect of mutagens on seedling injury at M_1 revealed that all mutagenic treatments except few were highly injurious to the seedlings. EMS treatments had caused highest seedling injury, followed by the sodium azide and gamma radiation. The seedling injury increased with the increase in doses/ concentrations of mutagenic treatments. Maximum seedling injury (26.66%) was observed in 0.4%EMS, followed by 0.04% SA and 400Gy. Lower treatments like 200Gy (-1.31%) and 0.04%SA (-3.85%) showed negative seedling injury. The seedling injury increased with the increase in doses or concentrations of mutagenic treatments except few in lima bean. Parallel results has been reported earlier by Aney (2013) in pea, and Senapati *et al*., (2008) in blackgram.

The data recorded on pollen sterility induced by mutagens treatments indicated that there was liner increase in pollen sterility with increasing dose/ conc. of mutagens. In gamma radiation, EMS and SA treatments, the range of pollen sterility % was 8.27 to 15.35%, 9.77 to 19.44% and 5.72 to14.16%. The highest pollen sterility (19.44%) was recorded in 0.4%EMS. The pollen sterility rate increase with increase in the conc/ dose of the mutagens. These results are in agreement with earlier researchers Sagade et al., (2008) in urdbean, Barshile et al., (2006) in chickpea, Bolbhat and Bhalerao (2020) in lentil. Anaphase bridges, translocation and laggards such chromosomal abnormalities were found in the progenies obtained from treated seeds.

The results on the effects of gamma radiation, EMS and SA showed that in all the mutagenic treatments, survival % was decreased than the control. There was decrease in the survival % with increasing dose/ conc. of gamma radiation, EMS and SA. The lowest survival % at the higher treatments was noted (60.45%) in 400Gy, 0.4%EMS (65.37%) and 0.04%SA (66.41%) as compared to control (89.56%). All mutagens reduced the rate of survival at maturity, Bolbhat and Wagh (2020) in horsegram and Barshile et al., (2006) in chickpea supported the above findings.

CONCLUSION

Seed germination percent and seedling height was inhibited due to increasing doses/ concentrations of mutagens except few. All three mutagens (GR, EMS and SA) were effective in inducing pollen sterility in M_1 generation. The rate of pollen sterility percent increased while the rate of survival of plants at maturity was highly reduced with increasing dose/conc. of mutagens.

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