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Induced genetic variability in field pea (*Pisum sativum* var. arvense L.)

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ABSTRACT

To induce sufficient genetic variability in the existing germplasm for desirable characters such as resistance to different diseases, seed mutation techniques could offer a possible solution. Mutation breeding is a supplementary breeding method which is rapid, potential and valuable tool to create genetic variability for various quantitative and qualitative characters in crop plants. Induced mutations are produced by the use of mutagenic agents like physical mutagens and chemical mutagens. In present investigation the seeds of field pea (*Pisum sativum* var. arvense L.). were treated with various doses/ concentrations of gamma radiation (GR-300 to 500Gy), Ethyl Methane Sulphonate (EMS- 0.3 to 0.5%) and Sodium azide (SA-0.03, 0.04, 0.05%). The physiological effects on seed germination and seedling height on 7th day after sowing were investigated. Gradual reduction in seed germination, seedling height, pollen sterility and survival at maturity was recorded with increase in conc./ dose of mutagens. Almost all the mutagenic treatments caused decrease in seed germination, seedling height, seedling injury, pollen sterility and survival of plants at maturity.

Key words: Genetic variability, mutagens, field pea, seedling injury

INTRODUCTION

Field pea (*Pisum sativum* var. arvense L) is an important legume field crop. It is locally known as kala vatana. It is an important plant in human and animal nutrition because of its high protein level 23- 33%. Field pea mainly cultivated in states such as M.P, U.P, Jharkhand, Rajasthan, Assam, Bihar, Chhattisgarh and Maharastra with production of 362.9, 314.00, 40.40, 30.90, 27.70, 18.20, 10.30, 9.60 thousand tonnes respectively in 2014-15 (Anonymous, 2016). In India field pea was cultivated over an area of 0.75 million hectares with a production of about 0.93 tonnes and 880kg/ha of productivity. Among different grain legumes, field pea is one of the most important nitrogen fixing, rainfed, pulse crop of India, grown in rabbi season.

Field peas belongs to family- Leguminosae (Fabaceae), sub-family- Papilionaceae, genus- *Pisum*, species- *sativum* and var. arvense with chromosome number 2n = 14. Pea has been used as food, nutritious feed and fodder, which is advantage to the poor farmer families (Prasad et al., 2018). It play an important role in crop rotations along with other legumes as they break up cycles of disease and pest, provide nitrogen, improve diversity and activity of soil microbe, improve soil aggregation, conserve soil water, and provide economic diversity. Peas are grown as green manures as well as cover crops because it grow quickly. It also bears root nodules with rhizobium bacteria which convert atmospheric nitrogen to ammonia and contribute nitrogen to the soil (Clark, 2007 and Joshi et al.,

2015). Dry seeds are used in human diet as whole, split or ground peas. Seeds are a nutritious legume and contain 15 to 35% protein with essential amino acids like lysine and tryptophan (Elzebroek and Wind, 2008).

To induce sufficient genetic variability in the existing germplasm for desirable characters such as resistance to different diseases, seed mutation techniques could offer a possible solution. The United Nations Food and Agriculture Organization (FAO) recommended the use of mutation induction for the enhancement of genetic variability in field pea with respect to modified plant architecture, seed retention, and resistance to diseases and pests (http://www.iaea/programmes/nafa). Supplementary breeding method like mutation breeding, which is rapid, potential and valuable tool to create genetic variability for various qualitative and quantitative traits in crop plants (Govardhan and Lal, 2013).

MATERIALS AND METHODS

The seeds of field pea (*Pisum sativum* var. arvense L.) were procured from local market of Manchar, Tal. Ambegaon, Dist- Pune-410503 (M.S.) India. Ethyl methane sulphonate (EMS), Gamma rays (GR) and sodium azide (SA) were used in present investigation for seed treatments of field pea. 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 study. Healthy, dry and uniform seeds of field pea with moisture content of 12 to 14 % were treated with gamma radiation 300, 400 and 500 Gy. Ethyl methane sulphonate 8% soluble in water, manufactured by Sigma chemical Co. Ltd. USA was used for the seed treatments of field pea. Different concentrations of EMS (0.3% to 0.5%) were prepared in distilled water. Sodium Azide is inorganic compound. It is colour less salt, ionic compound, soluble in water and is highly toxic. It is chemical mutagen and used for induction of mutations in the crop plants. The concentrations of SA (0.03%, 0.04% and 0.05%) 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, 300, 400 and 500Gy, EMS 0.3, 0.4 and 0.5% while SA 0.03, 0.04 and 0.05% 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 (0.3, 0.4 and 0.5%) and SA 0.03, 0.04 and 0.05% for four hours. The untreated seeds served as control. For each treatment 330 seeds were used. The treated seeds were washed thoroughly with tap water for one hour to terminate the reaction of chemical mutagen and to leach out the residual chemicals. 30 seeds from each treatment was used for seed germination in laboratory. Three replications with 10 seeds per replication kept in petri dishes, with seed germination paper, were used for recording seed germination percentage, root and shoot length, seedling height on 7th day. The remaining lot of treated seeds (300) from each treatment was used for raising M_1 generation in field.

Present investigation was carried out at experimental field, Department of Botany, Annasaheb Awate College, Manchar, Tal. Ambegaon, Dist- Pune (410503) (M.S.). The soil type of the experimental field was slightly deep and fine. The average minimum temperature was recorded as 18.24^{0}C and maximum 33.43^{0}C with average annual rainfall 712.05mm. The crop of field pea was grown in *Rabbi* season. All the experiments were carried out in triplicate following RBD design. Each plot had 100 plants with untreated control seeds. The distance between two plants and two rows was 15 X 30 cm.

OBSERVATIONS ON M_1 GENERATION

The number of seeds showing emergence of the radical and plumule was counted from the seeds kept in petri plates lined on moist germination paper. Data was used to calculate percent seed germination. On 7th 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 height as compared to the control was regarded as seedling injury & expressed as percentage. The seedling injury was calculated as follows

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

Pollen fertility was determined from 5 randomly selected plants per treatment, along with control. The pollen grains of 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 percentage was calculated by scoring the total number of plants attaining maturity (45 days) in each treatment and expressed as percentage over the control. All the surviving M_1 plants were harvested individually and seeds of each treatment bulked together, kept separately for raising M_2 generation.

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

Results obtained in the present investigation on seed germination, seedling height, seedling injury, pollen sterility and survival of plant at maturity in M_1 generation of field pea are depicted in Table-1. Data obtained on mean seed germination percent in control and mutagen treatments indicated that the seed germination percent was decreased in all the treatments as compared to control (91.04%) showing negative effect. The seed germination percent decreased from 78.23% to 53.85% in gamma radiation, 84.54% to 59.18% in EMS and 87.11% to 61.44% in SA. Maximum reduction in germination was recorded 53.85% in 500Gy. Results indicate that, percent seed germination decreased with increasing doses or concentration of GR, EMS and SA in field pea with inhibitory effect. Similar inhibitory effect on seed germination reported earlier by Aney (2013) in pea, Bolbhat and Dhumal (2009) in horsegram. Kumar and Singh (1996) in pea reported that the effect of mutagens on seeds is expressed through delayed emergence of roots, reduction in vigour, low metabolic and enzymatic activity, losses of membrane integrity and finally loss of germinability.

Results indicated that mutagen treatments showed reduction in seedling height. Maximum decrease in seedling height (7.70cm) was noted in 500Gy, 0.5%EMS (7.73cm) and 0.05%SA (8.30cm) while in control (10.69cm). Data on the effect of GR, EMS and SA on seedling injury at M_1 shown in Table-1 revealed that all mutagenic treatments were highly injurious to the seedlings. GR treatments had caused highest seedling injury, followed by the EMS and SA. The seedling injury increased with the increase in doses or concentrations of mutagenic treatments. Highest seedling injury (27.97%) was observed in 500Gy. In the present investigation, the seedling injury increased with the increase in conc. or dose of mutagenic treatments. Similar results has been reported by Aney (2013) in pea, Bolbhat and Bhalerao (2020) in lentil. GR induce more chromosomal mutations than point mutations and it is successfully used in plant breeding programmes due to its simple application, good penetration, reproducibility and high mutation frequency and less disposal problems (Govardhan and Lal, 2013).

Data on pollen sterility is depicted in table. From the table it is clear that all mutagens were effective in inducing pollen sterility in field pea. GR was the most effective as compare to other mutagens in inducing pollen sterility. It was followed by the EMS and SA. The highest pollen sterility was recorded 500Gy (17.69%). Results obtained on pollen sterility revealed that all the mutagens used in the present investigation are effective in inducing pollen sterility in M_1 generation. The pollen sterility increase with increase in mutagen conc. or dose. These results are in agreement with earlier researchers like Aney (2013),

Table 1 : Effect of mutagen on seed germination, seedling height, seedling injury, pollen sterility and plant survival at maturity in M_1 generation of field pea

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling height (cm)	Seedling injury (%)	Pollen sterility (%)	Plant survival at Maturity (%)
Control	91.04±12.75	3.91±0.55	6.78±0.95	10.69±1.50	00.00±0.00	5.17±0.72	86.23±12.07
300Gy	78.23±6.26	3.35±0.27	6.23±0.50	9.58±0.77	10.38±0.83	9.14±0.73	70.06±5.60
400	65.42±7.20	3.04±0.33	5.94±0.65	8.98±0.99	15.99±1.76	13.55±1.49	59.32±6.53
500	53.85±7.00	2.52±0.33	5.18±0.67	7.70±1.00	27.97±3.64	17.69±2.30	44.67±5.81
0.3 % EMS	84.54±11.84	3.66±0.51	6.41±0.90	10.07±1.41	5.80±0.81	7.93±1.11	79.86±11.18
0.4	70.37±4.93	3.25±0.23	5.98±0.42	9.23±0.65	13.66±0.96	11.37±0.80	62.15±4.35
0.5	59.18±4.73	2.89±0.23	4.84±0.39	7.73±0.62	27.69±2.22	15.46±1.24	48.53±3.88
0.03%SA	87.11±13.07	3.79±0.57	6.72±1.01	10.51±1.58	01.68±0.25	06.89±1.03	83.24±12.49
0.04	73.29±10.26	3.52±0.49	6.16±0.86	9.68±1.36	9.45±1.32	09.61±1.35	66.09±9.25
0.05	61.44±5.53	3.21±0.29	5.09±0.46	8.30±0.75	22.36±2.01	13.74±1.24	52.31±4.71
SEM±	7.28	0.33	0.58	0.91	1.40	1.04	6.71
F-value	6.02	3.41	2.72	2.76	10.25	29.41	9.45
P-value	0.01	0.01	0.03	0.03	0.01	0.01	0.01
$LSD_{0.05}$	15.86	0.72	1.26	1.98	3.05	2.27	14.62

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

Kumar and Singh (1996) in pea, Bolbhat et al., (2020) in black beans, Bolbhat and Wagh (2020) in horsegram, Uttarde et.al., (2020) in sesame. At cellular level gross injury is dueto acute chromosomal aberrations. Various types of chromosomal abnormalities such as translocation, anaphase bridges and laggards were found in the progenies obtained from treated seeds (Gunkel, 1957).

The results on the effects of mutagens revealed that in all the mutagenic treatments, survival % was decreased than the control. There was linear decrease in the survival % with increasing dose/ conc. of gamma radiation, EMS, and SA. The lowest survival % at the higher treatments was noted GR (44.67%), (48.53%) in 500EMS, and 0.05%SA (52.31%) as compared to control (86.23%). All mutagens reduced the rate of survival at maturity, these results are supported by earlier researchers, Bolbhat et al., (2020) in black beans, Bolbhat and Wagh (2020) in horsegram and Bolbhat and Bhalerao (2020) in lentil.

CONCLUSION

The mutation process generates random genetic variations, resulting in mutant plants with new and useful traits. The present study revealed that the growth parameters such as germination (%), seedling height (cm), pollen sterility and survival % at maturity was inhibited due to increasing doses/ concentrations of mutagens in field pea.

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