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STUDIES ON INDUCED MUTATIONS IN MOTH BEAN [VIGNA ACONITIFOLIA (JACQ.) MARECHAL.]

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ABSTRACT:

Induced mutagenesis has bean used to create desired genetic variability, the base of crop improvement. Induced genetic variability in crop plants is a important resource from which plant breeders can select and combine different desired characteristics to produce best crop plants. The desirable characters which have been bred through induced mutations. In present investigation the seeds of moth bean treated with various doses/ concentrations of mutagens such as gamma rays (GR), ethyl methane sulphonate (EMS) and sodium azide (SA) and physiological effects on seed germination as well as seedling height on 7th day after sowing were investigated. Gradual reduction in seed germination and seedling height was recorded with increase in concentration /dose of mutagens. Majority of the mutagenic treatments caused decrease in seed germination, seedling height and survival of plants at maturity, but lower treatments showed stimulatory effect.

Keywords : Mutations, moth bean, mutagens, seedling height, seedling injury.

INTRODUCTION

Moth bean [*Vigna aconitifolia* (Jacq.) Marechal], is also called matki, belongs to family Fabaceae. It is important minor, rainfed pulse crop. Moth bean is drought tolerant and having good nitrogen fixing ability. With protein supplement in human diet, it has medicinal value. It also provides concentrated feed for cattle and domestic animals. It is grow in *kharif* season as main crop, or as a mixed crop with tur, bajra. It is cultivated for its immature pods as well as mature seeds, which is consumed by people all around the world, as it possesses high nutritional value and is a potential source of protein. (Mathur, 2006). The pods, protein rich seeds and sprouts of moth bean are normally consumed in all over India (Singh and Ansari, 2018).

It is a native crop of dry & hot habitats of north as well as western regions of India. This crop is used as a source of food, feed, fodder, green manuring and green pasture. Green pods are delicious source of vegetables. Being a pulse, it is a cheap source of vegetable protein for balancing nutritional deficiency. The crop was characterized for primitive plant type, grain yield (300-400 kg/ha.), long maturity (90-95 days) having poor survival value. It was generally used for fodder purposes and conserving soil and soil moisture (Indian Council of Agricultural Research). Moth bean is grown in arid and semi-arid areas throughout the tropics, sub tropics and even warm temperate areas of different countries. In India it is cultivated mainly in the arid and semi-arid zones that too on marginal lands. It has been reported to be extremely drought tolerant and highly salt sensitive pulse crop (Sharma and Kakani, 2002). It is cultivated generally under rainfed conditions, in states such as Rajasthan, Haryana, Gujarat, Maharashtra and Punjab (Kumar and Singh, 2002).

The seeds of moth bean contain (per 100 g edible portion) water 9.7g, protein 22.9 g, fat 1.6g, carbohydrate 61.5g, Ca 150mg, Mg 381mg, P 489mg, Fe 10.9mg, Zn 1.9mg, vitamin A 32 IU, thiamine 0.56 mg, riboflavin 0.09mg, niacin 2.8mg, vitamin B6 0.37mg, folate 649µg and ascorbic acid 4.0mg. The essential amino-acid composition per 100g edible portion is: tryptophan 147mg, lysine 1248mg, methionine 220mg, phenylalanine 1028mg, valine 734mg, leucine 1541mg and isoleucine 1138mg. The principal fatty acids are per 100g edible portion: linoleic acid 485mg, palmitic acid 313mg, linolenic acid 265mg, oleic acid 129mg and stearic acid 51mg (USDA, 2005). Moth bean consists of trypsin inhibitor; tannins phytic acid; and antioxidant activity (Mandal, 2000). Moth bean is known for higher proportion of albumin and glutamine fractions of protein. It is also a good source of lysine and leucine amino acids. Dry seeds of moth bean are used for a number of delicious confectionery items (papad and numkins) which are commonly used as daily snacks. These industries are coming up in a big way exporting such commercial edible products and generating employment in agro based industries. It is an important legume crop but under exploited. The induction of physical or chemical mutations is the quickest way to produce new varieties.

MATERIALS AND METHODS

Seed material : The seeds of Moth bean [*Vigna aconitifolia* (Jacq.) Marechal] were obtained from local market of Manchar, Tal. Ambegaon, Dist Pune-410503 (M.S.) India. Gamma rays (GR), ethyl methane sulphonate (EMS) and sodium azide (SA) were used in present investigation for the treatments of moth bean seeds. Gamma radiation from ^{60}Co source fixed in the gamma cell 200 installed at Chemistry Department, Savitribai Phule Pune University, Pune was used in the present work. Dry, uniform and healthy seeds of moth bean with moisture content of 10-12 % were treated with 200, 300 and 400 Gy. Ethyl methane sulphonate (Sigma chemical Co. Ltd. USA) was used for the seed treatments of moth bean. Different concentrations of EMS (0.2 0.3 to 0.4%) were prepared in distilled water. Sodium Azide (SA) with different concentrations (0.02%, 0.03% and 0.04%) was prepared in distilled water and used for the seed treatment.

Treatment details : The pilot 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 400Gy, 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 10 hours and the wet seeds were treated with different concentrations of EMS (0.2, 0.3 and 0.4%) and SA 0.02, 0.03 and 0.04% for four hours. The untreated seeds served as control. For each treatment 180 seeds were used.

The seeds treated with different concentrations of SA and EMS were washed thoroughly under tap water for one hour. It terminate the reaction of chemical mutagen. A total of 30 seeds from each treatment was used for seed germination in laboratory. Three replications with 10 seeds / replication kept in petri dishes, having seed germination paper, were used for recording seed germination percent, seedling height, on 7^{th} day. The remaining lot of treated seeds (150) from each treatment was used for raising M_1 generation in field.

Experimental site : Present investigation was carried out at experimental field, Department of Botany. The soil type of the experimental field was slightly deep and calcareous with good drainage. The average minimum temperature was recorded as 18.65^0C and maximum 34.63^0C with average annual rainfall 671.09mm.

Experimental design for field experiments: The field experiments were conducted on the experimental field at Department of Botany. The crop of moth bean was grown in *Kharif* season under uniform conditions. All the experiments were carried out in triplicate followed RBD design. Each plot with single treatment had 50 plants.

The distance between two rows and two plants was 60 X 45 cm and two adjacent plots was one meter. A total of 10 treatment combinations in M_1 generation with untreated seeds were used as control.

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 with moist germination paper, data was used to calculate percent seed germination. On 7^{th} day of sowing, 10 seedlings from control and each treatment were selected randomly for measuring the root and shoot length and the average values were recorded. Reduction in the seedling height as compared to the control was regarded as seedling injury. The seedling injury was calculated as follows

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

Survival percentage was calculated by scoring the total number of plants attaining maturity (45 DAS) in each treatment and expressed as percentage over the control. All the surviving M_1 plants were harvested individually and bulked seeds of plant from each treatment were 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 IBM SPPS (ver.25).

RESULTS AND DISCUSSION:

Results obtained in the present investigation on seed germination, seedling injury and survival of plant at maturity in M_1 generation of moth bean are given in Table-1. Data obtained on mean percent seed germination in control and mutagen treatments clearly indicated that it was decreased in all the treatments except 0.2%EMS and 0.02%SA as compared to control. It has clearly indicated that the mutagens had exerted negative effects on seed germination. Seed germination percent was decreased with the increase in doses/ conc. of the mutagens. The percent seed germination decreased from 84.71% to 56.03% in gamma radiation, 89.31% to 61.40% in EMS and 88.12% to 58.77% in SA. The maximum (50%) decrease in percent seed germination was observed with gamma radiation treatment 400Gy (56.03%), EMS 0.4% (61.40%) and in SA 0.04% (58.77%). Thus 400Gy treatment was very effective in reducing percent seed germination in moth bean to almost 50%. This clearly indicates that the mutagens have exerted an inhibitory effect on seed germination. Similar inhibitory effect on seed germination reported earlier by, Bolbhat and Dhumal (2009) in horsegram, Bolbhat et al., (2020) in black beans and Sagade (2008) in urdbean. The reduction in germination may be due to genetic as well as physiological processes inhibited by the mutagens resulting in cell maturity.

Data presented in table indicated that doses of GR, conc. of EMS and SA treatments showed inhibitory effect on seedling height except lower treatments. Maximum decrease in seedling height (7.88cm) was noted in 400Gy, 0.4%EMS (8.48cm) and 0.04%SA (8.71cm). Data on the effect of mutagens on seedling injury at M_1 shown in table revealed that all mutagenic treatments were highly injurious to the seedlings. The seedling injury increased with the increase in doses/ conc. of mutagenic treatments. But lower treatments such as 200Gy (-03.30%), 0.2%EMS (-01.82%) and 0.02%SA (-07.11%) showed negative seedling injury. Maximum seedling injury (31.66%) was observed in 400Gy.

In the present investigation it was observed that the seedling injury increased with the increase in conc. or dose of mutagenic treatments in moth bean except in lower treatments. Similar increase in seedling injury with increased dose/conc. of mutagens has been reported by Sharma et al., (2005) in urdbean, Bolbhat et al., (2020) in black beans. The results on the effects of GR, EMS and SA revealed that in all the mutagenic treatments, survival % was decreased except 0.2%EMS (85.05%) and 0.02%SA (86.47%) over the control (84.37%). There was decrease in the survival % with increasing conc. /dose of mutagens. The lowest survival % at the higher treatments was noted (51.92%) in 400Gy, 0.4%EMS (57.64%) and 0.04%SA (53.09%)

Table 1 : Mutagenic effect on seed germination, seedling height, seedling injury, and plant survival at maturity in M_1 generation of moth bean.

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling height (cm)	Seedling injury (%)	Plant survival at Maturity (%)
Control	87.19±12.21	5.41±0.76	6.12±0.86	11.53±1.61	00.00±0.00	84.37±11.81
200Gy	84.71±6.78	5.60±0.45	6.31±0.50	11.91±0.95	-03.30±0.26	80.21±6.42
300	70.28±7.73	4.62±0.51	5.23±0.58	9.85±1.08	14.57±1.60	65.45±7.20
400	56.03±7.28	3.31±0.43	4.57±0.59	7.88±1.02	31.66±4.12	51.92±6.75
0.2 %EMS	89.31±12.50	5.50±0.77	6.24±0.87	11.74±1.64	-01.82±0.25	85.05±11.91
0.3	74.46±5.21	5.09±0.36	5.78±0.40	10.87±0.76	05.72±0.40	70.14±4.91
0.4	61.40±4.91	3.81±0.30	4.67±0.37	8.48±0.68	26.45±2.12	57.64±4.61
0.02%SA	88.12±13.22	5.62±0.84	6.73±1.01	12.35±1.85	-07.11±1.07	86.47±12.97
0.03	73.64±10.31	5.25±0.74	5.98±0.84	11.23±1.57	02.60±0.36	68.52±9.59
0.04	58.77±5.29	4.02±0.36	4.69±0.42	8.71±0.78	24.46±2.20	53.09±4.78
SEM±	7.41	0.48	0.56	1.03	1.42	7.06
F-value	5.86	6.15	3.97	4.84	19.16	7.11
P-value	0.01	0.01	0.01	0.01	0.01	0.01
LSD $_{0.05}$	1.61	1.05	1.22	2.24	3.09	15.38

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

as compared to control (84.37%). All mutagens reduced the rate of survival at maturity Datir et al., (2007) in horsegram, Bolbhat et al., (2020) in black beans supported the above findings.

CONCLUSION

Seed germination percent and seedling height was inhibited due to increasing doses/ concentrations of mutagens except lower mutagenic treatments. All three mutagens (GR, EMS and SA) were effective in inducing seedling injury in M_1 generation. The rate of seedling injury percent increased but lower treatments showed decrease in seedling injury over control. The rate of survival of plants at maturity was highly reduced except lower treatments with increasing dose/conc. of mutagens.

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