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Impact Of Chemical Mutagens On Cluster Bean (*Cyamopsis Tetragonoloba* (L.) TAUB.)

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Abstract

In the present study the variety of cluster bean namely Ankur Rani was used to induce genetic variability. For these study two chemical mutagens, namely EMS of different concentration such as 0.05%,0.10%,0.15% and SA of different concentration such as 0.01%, 0.02% and 0.03% were used. The seeds of variety Ankur Rani of Cluster bean were obtained from Local market of Aurangabad (Maharashtra).These seeds were subjected to mutagenesis by using chemical mutagens. M1 generation was raised and effect of these mutagens on different parameters like germination percentage, seedling height, leaf morphological changes, chlorophyll chimeras, pollen sterility, and plant survival percentage. Both the mutagens had shown an inhibitory effect on seed germination. The 0.15% EMS showed highest germination percentage. The 0.03% SA showed lowest germination percentage. Seedling height was found to increase as concentration of both the mutagens. But exceptionally it is found to be decreased in 0.03% SA concentration. In all the treatments the leaves of plants exhibited variations Bi and tri furcation of leaf lamina and Distorted leaf lamina. Survival of plant at maturity was found to be decreased at higher concentration of mutagens. The highest percentage was shown by EMS (0.05%). The lowest percentage was shown by SA (0.03%).

Keywords: EMS, SA, Ankur rani, Cluster bean.

Introduction:

Cluster bean (*Cyamopsis tetragonoloba* (L.)Taub.) (2n=14) is a drought tolerant annual legume that was introduced for feed, green fodder, vegetable, green manuring and grain purposes. The endosperm of cluster bean contains galactomannan gum, which has several diversified industrial uses. India has huge amount as foreign exchange from the export of its gum and its derivatives. Cluster bean left after the extraction of the gum contains about 45% proteins and forms a valuable proteinaceous raw material (Bhosale and Kothekar, 2010). It is most important crop in India and about 80% of world production occurs in India.

Mutation breeding technique has generated several mutants, which are being used as new cultivars (Gottschalk and Wolff, 1983). Mutation breeding plays significant role in plant genomic research (Kharkwal *et al.,* 2004).

Materials and methods:

The experimental plant material used in present investigation comprised a variety of cluster bean (*Cyamopsis tetragonoloba (L.)TAUB.)* namely Ankur Rani. Seeds of this variety were obtained from local market of Aurangabad, (Maharashtra) India.

Two chemical mutagens namely 1) Ethyl methane sulphonate (EMS) ($CH_3SO_2OC_2H_5$) manufactured by Sisco Research Laboratory, Mumbai and 2)Sodium azide (SA) (NaN3) manufactured by Spectrochem, Pvt Ltd. Mumbai was used in the present investigation to induce mutations in selected plant material.

Healthy and well dried seeds with uniform size were surface sterilized with 0.1% mercuric chloride solution for about one minute and washed thoroughly with distilled water. The seeds were presoaked in distilled water for 6 hours. The mutagenic solutions were prepared freshly in aqueous medium at room temperature of 25±2°C prior to treatment. Presoaked seeds were immersed in the mutagenic solution and conical flasks were kept on electric shaker. The treatment was given for 6 hours with intermittent shaking. The volume of the chemical mutagenic solution used was three times as that of seeds so as to facilitate uniform conditions. The different concentrations used for chemical mutagenic treatment were 0.05%EMS, 0.10% EMS, 0.15%EMS and 0.1%SA, 0.2%SA, 0.3% SA.Immediately after the completion of treatment, the seeds were washed thoroughly under running tap water to remove excess of mutagens.

All the mutagen treated seeds were immersed in distilled water for 2 hours. The post soaked seeds were dried in folds of filter paper. Seeds soaked in distilled water for 12 hours served as control.200 seeds were used for each treatment. 50 seeds from each treatment were kept on moist blotting paper in petriplates to record germination percentage. The remaining 150 seeds of each treatment were sown in the field following randomized block design (RBD) with two replications each consisting of 75 seeds along with control for raising the M_1 generation. The seeds were sown at a distance of 15 cm between the plants and 60cm between the rows. The field experiments were carried out at the experimental field of Botany Department, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad.

The plants emerged during M1 generation were carefully observed and observation were recorded for Germination percentage, Seedling height, Leaf morphological changes, Chlorophyll deficient sectors/chimeras, Pollen sterility, Survival of plants at maturity.

Results:

M_1 generation was screened for various morphological parameters and results were recorded for following parameters.

1) Seed germination percentage: (Table 1)

In cluster bean maximum number of seeds germinated on third day after sowing in variety Ankur Rani. It is clear from the data germination percentage that all the concentration of mutagens hade inhibitory effect on germination. In control the germination percentage was 94%. The decreasing trend of seed germination percentage could be recorded with a decreased concentration of EMS. The germination percentage was found to be decreased with increased concentration of SA. The

values ranged from 92-96% in case of EMS and 92-88% in case of SA. The highest germination was noticed in 0.15% EMS Treatment. The lowest germination was noticed in 0.03% SA treatment.

Table 1: Effect of mutagens on seed germination percentage in M1 generation of cluster bean in variety Ankur Rani.

Treatment	Concentration (%)	Seed germination (%)
Control	--	94%
EMS	0.05%	92%
	0.10%	94%
	0.15%	96%
SA	0.01%	92%
	0.02%	92%
	0.03%	88%

2) Seedling height: (Table 2)

Data collected on seedling height revealed that all the treatments had an inhibitory effect on seedling height. Increased seedling height was recorded as compared to control in all EMS and SA treatments. The value ranges from 6.2,6.1, 6.9 in case of EMS and 6.6,6.3,6.2 in case of SA. The 0.15% dose of EMS showed highest value 6.9, whereas the 0.01% dose of SA showed highest value i.e. 6.6.

Table 2: Effect of mutagens on seedling height in M1generation of cluster bean of variety Ankur Rani

Mutagen	Treatment concentration %	Seedling height (cm)
Control	--	5.6
EMS	0.05%	6.2
	0.10%	6.1
	0.15%	6.9
SA	0.01%	6.6
	0.02%	6.3
	0.03%	6.2

3) Leaf morphological changes: (Table 3)

Various types of leaf morphological changes were recorded in M1 generation comparison with leaves in control. Variations in leaf size, shape, number of leaflets, margin and length of petiole were detected in all mutagenic treatments. Several plants exhibited large and unequal leaflets.

Unifoliolate, bifoliolate and tetrafoliolate leaves were seen in several treated plants.EMS treatments showed 9.10%,12.60% and 13.15% and SA treatment showed 10.12%, 11.50% and 13.70% frequency of plants carrying leaf morphological changes in variety Ankur Rani. Highest induction of leaf abnormalities was observed at 0.03% SA treatment.

4) Chlorophyll chimeras: (Table 4)

Chlorophyll chimeras were observed in all treatments. The percentage of chlorophyll chimeras increased with increasing concentration of mutagens in both of EMS and SA. All the chimeras were found to be affecting the leaflets partially and at the margins. Maximum frequency of chlorophyll chimeras was observed in the 0.03% treatment of SA. Lowest frequency of chlorophyll chimeras was observed in the 0.05% treatment of EMS.

Table 3: Effect of mutagens on leaf morphological changes in M1 generation of Cluster bean Variety Ankur Rani

Treatment	Concentration (%)	Leaf morphological changes (%)
Control	--	--
EMS	0.05%	9.10
	0.10%	12.60
	0.15%	13.15
SA	0.01%	10.12
	0.02%	11.50
	0.03%	13.70

Table 4: Effect of mutagens of frequency of plants carrying chlorophyll Chimeras in M1 generation of cluster bean Variety Ankur Rani

Treatment	Concentration (%)	Frequency of plants carrying chlorophyll chimeras (%)
Control	--	---
EMS	0.05	1.33
	0.10	2.75
	0.15	3.33
SA	0.01	1.70
	0.02	2.55
	0.03	3.70

5) Pollen sterility: (Table 5)

Pollen sterilityincreased with increasing concentration of mutagens. The range of pollen sterility occurs from 08%-18% in EMS and 10%-16% in SA. The maximum pollen sterility (18%) seen at 0.15% EMS.

Table 5: The effect of mutagens on pollen sterility in M1 generation of Cluster bean variety Ankur Rani

Treatment	Concentration (%)	Pollen sterility (%)
Control	--	05
EMS	0.05%	08
	0.10%	10
	0.15%	18
SA	0.01%	10
	0.02%	14
	0.03%	16

6) Survival of plants at maturity: (Table 6)

Survival of a plant in each treatments and their respective control were recorded in the fields at the time of maturity. The plant survival decreased as concentration of the mutagens increased .The survival ranged from 90.50% to 88.70% in EMS and 87.10% to 81% in SA.

Table 6: The effect of mutagens on survival of plants at maturity in M1 generation of Cluster bean variety Ankur Rani

Treatment	Concentration (%)	Survival of plant at maturity (%)
Control	--	93
EMS	0.05%	90.50
	0.10%	89.60
	0.15%	88.70
SA	0.01%	87.10
	0.02%	82.76
	0.03%	81

Discussion:

In the present study the variety of cluster bean namely Ankur Rani was used to induce genetic variability. For these study two chemical mutagens, namely EMS of different concentration such as 0.05%,0.10%,0.15% and SA of different concentration such as 0.01%, 0.02% and 0.03% were used. The M1 generation was raised and effect of these mutagens on different parameters like germination percentage, seedling height, leaf morphological changes, chlorophyll chimeras, pollen sterility, and plants survival percentage were studied.Both the mutagens had shown an inhibitory effect on seed germination. The 0.15% EMS showed highest germination percentage. The 0.03% SA showed lowest germination percentage,similar results were reported by Mahamune and Khothekar (2012); Kashid (2019). Seedling height was found to increase as concentration of both the mutagens. But exceptionally it is found to be decreased in 0.03% SA concentration, similar findings was reported by Shaikh et al., (2019). In all the treatments the leaves of plants exhibited variations Bi and tri furcation of leaf lamina and Distorted leaf lamina. Pollen sterilityincreased with increasing concentration of mutagens, A quite dissimilar result was reported by Shaikh et al., (2019). Survival of plant at maturity was found to be decreased at higher concentration of mutagens. The highest percentage was shown by EMS (0.05%). The lowest percentage was shown by SA (0.03%).

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