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**Mohamed Yaseen SK**

Department of Plant Breeding and Genetics, Adhiparasakthi Agricultural College, Kalavai, Tamil Nadu, India

**Sravan Naga Parimala Kumar K**

Department of Plant Breeding and Genetics, Adhiparasakthi Agricultural College, Kalavai, Tamil Nadu, India

**Prabakaran S**

Department of Plant Breeding and Genetics, Adhiparasakthi Agricultural College, Kalavai, Tamil Nadu, India

**Suresh S**

Department of Plant Breeding and Genetics, Adhiparasakthi Agricultural College, Kalavai, Tamil Nadu, India

**Corresponding Author:****Mohamed Yaseen SK**

Department of Plant Breeding and Genetics, Adhiparasakthi Agricultural College, Kalavai, Tamil Nadu, India

## Ethyl methane sulphonate (EMS) induced mutations in M<sub>1</sub> generation of cowpea (*Vigna unguiculata* (L.) Walp)

**Mohamed Yaseen SK, Sravan Naga Parimala Kumar K, Prabakaran S and Suresh S**

**Abstract**

The study was carried out to induce genetic variability in cowpea variety Vamban 2 through chemical mutagen ethyl methane sulphonate (EMS). Totally eight treatments *viz.*, 10, 20, 30, 40, 50, 60, 70 and 80 mM were engaged. The LD<sub>50</sub> values for the mutagen were worked out based on observations recorded on seed germination, root length and shoot length under laboratory conditions. The M<sub>1</sub> generation was raised under field conditions to study different parameters such as plant height at maturity, germination of seeds, pollen fertility, number of branches per plant, number of pods per plant, number of seeds per pod, 100 seed weight and single plant yield. The results showed that the increasing concentrations of EMS had negative correlation with phenotypic expression and yield characters.

**Keywords:** Cowpea, ethyl methane sulphonate (EMS), LD<sub>50</sub> value

**Introduction**

Cowpea (*Vigna unguiculata* (L.) Walp) is an important pulse crop belongs to the family Fabaceae and subfamily of Papillnoideae. Cowpea is grown as catch crop, mulch crop, intercrop, mixed crop and green manure crop. Its quick growth, rapid ground cover and nitrogen fixing ability have made it an essential component of sustainable agriculture in marginal lands. It is an important multipurpose grain legume extensively cultivated in arid and semiarid tropics. It is good source of protein (20-25 %), which is enriched by amino acids like lysine and tryptophan. It is very nutritious with free metabolites or other toxins (Kay, 1979). Mutation breeding has become increasingly popular in recent times as an effective tool for crop improvement. In order to improve yield and other polygenic characters, mutation breeding should be effectively utilized. This crop lacks proper male sterile system commercially utilized for hybridization. The aim is to create variation in cowpea through chemical mutagens with following objectives:

- To induce genetic variability in cowpea through chemical mutagenesis
- To fix LD<sub>50</sub> value for chemical mutagens (Ethyl Methane Sulphonate)
- To analyze the sensitivity of the different morphological traits to the mutagens
- To study the extent of genetic variability in M<sub>1</sub> generation.

**Materials and Methods**

The promising cowpea variety namely VBN 2 (Vamban 2) constitutes the biological material for this study. The chemical mutagen, Ethyl Methane Sulphonate were used at different concentration to induce mutagenesis and their concentrations of EMS ranging from 10, 20, 30, 40, 50, 60, 70 and 80 mM with 10mM interval were used initially to fix LD<sub>50</sub> value.

A total of 1440 well matured healthy seeds were subjected to the mutagenic treatment. The seeds were soaked in the distilled water for five to six hours at room temperature (28±2°C) prior to treatment. Then the seeds soaked in the freshly prepared aqueous solution which about three times that of volume of seeds with corresponding concentrations of EMS *viz.*, 10, 20, 30, 40, 50, 60, 70, 80 mM for six hours at room temperature (28±2°C) with an hour of intermittent shaking. The pH of aqueous solution was 7. The untreated seeds soaked in water were used as control.

A total of 540 seeds along with the control were sown in the tray with three replications in lab condition. The seedling injury was recorded from the seedling under controlled conditions. Seven days after sowing, the germination percentage, shoot length and root length were recorded in each and every seedling. A total of 900 seeds were sown in the field along with the control in Randomized Block Design with three replications.

Based on the LD<sub>50</sub> value the treatment doses were selected for conducting field study with controls. All the M1 plants were harvested separately and biometrical observations were recorded.

## Result and Discussion

### Determination of LD<sub>50</sub> of mutagens

Probit analysis was carried out using seed germination values in VBN 2 variety to determine the Lethal Dose<sub>50</sub> (LD<sub>50</sub>). Based on probit analysis, the expected LD<sub>50</sub> observed value in VBN 2 was 50.11 mM in EMS

### Effect of mutagens in M1 generation under laboratory condition

#### Shoot length

The shoot length ranged from 25.8 to 4.3 cm in EMS treatments. In chemical mutagenic treatments, shoot length was low at 4.3 and 6.5 cm respectively. The percent reduction has been ranged from 12.4 to 83.3 in EMS treatment and the trend observed was regular with continuous reduction for mean shoot length, when the dose level increased.

#### Root length

In all the EMS treatments, the mean root length recorded was lesser than their respective control. The mean root length ranged from 3.7 to 12.4 cm treatments. The lowest mean root length was observed in seeds treated with 80 mM EMS. The percent reduction ranged from 4.8 (10 mM) to 70.2 (80 mM) in EMS treatment.

### Effect of mutagens in M1 generation under field condition

Effects of the chemical mutagen EMS on individual traits and in combination on different traits of the variety VBN 2 were studied. All the quantitative traits studied in M1 differed significantly from each other. The data of different traits recorded on the surviving plants of treated generation are presented as below.

#### Germination per cent

The response of the genotype VBN 2 for EMS treatments in relation to germination percentage was studied. The data was analysed after arc sine transformation. All the treatments revealed a dose dependent negative linear relationship between dose and germination percentage. The germination percentage ranged from 92.4 to 27.2 for EMS treatment.

**Plant height:** Maximum plant height of 56.5 cm was recorded in untreated control. The per cent reduction in plant height at maturity due to EMS treatment varied from 0.7 to 17.4 and it showed declining trend.

#### Number of branches per plant

The number of branches per plant was less in EMS than the respective controls. In EMS, the percent reduction ranged from 11.9 to 69.5. In all the treatments, it shows a declining trend with increasing doses of mutagens.

#### Number of pods per plant

The number of pods per plant for EMS VBN 2 population ranged from 7.1 to 16.9 and the percent reduction ranged from 2.9 to 18.0. In all the treatments, the mean number of pods per plant showed declining trend with increasing doses of mutagens.

### Number of seeds per pod

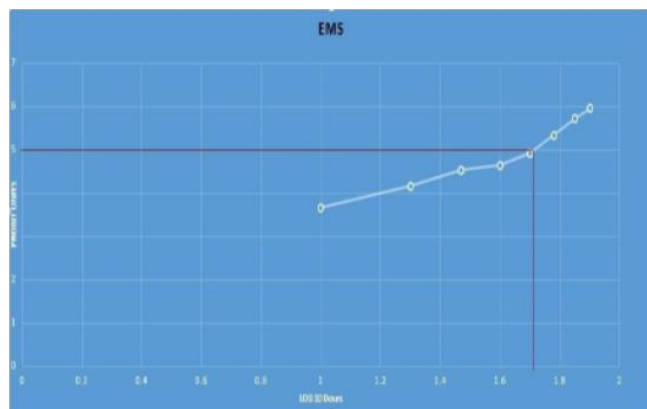
The minimum number of seeds per pod of 8.1 was recorded in 80 mM EMS treatment. The per cent reduction in number of seeds per pod due to EMS treatment varied from 8.1 to 13.8.

### Hundred seed weight

The hundred seed weight for EMS VBN 2 population ranged from 85.2 to 100.0 per cent over control.

### Single plant yield

The single plant yield for EMS VBN 2 population ranged from 55.7 to 100.0 per cent over control. In all the treatments, the mean number of plant yield showed a declining trend with increasing doses of mutagens.



Plots of log doses versus probits for calculation of LD<sub>50</sub> of EMS in cowpea

Treatments (mM)	Germination percentage	Percent over control	Percent reduction
Control	92.4	100.0	-
10	83.9	90.8	9.2
20	74.3	80.4	19.6
30	63.8	69.0	31.0
40	58.1	62.8	37.2
50	52.3	56.6	43.4
60	41.9	45.3	54.7
70	35.4	38.3	61.7
80	27.2	29.4	70.6
Mean		58.8	
SE		7.36	



Field view of M1 generation



Treating seeds with various concentration of mutagen



Effect of EMS under laboratory condition

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