Volume 3, Issue 4, April 2017 ISSN NO: 2454 - 7514

North Asian International Research Journal Consortium

North Asian International Research Journal

Of

Science, Engineering and Information Technology

IRJIF IMPACT FACTOR: 3.821

NAIRJC JOURNAL PUBLICATION

North Asian International Research Journal Consortium

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ISSN NO: 2454 -7514

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INDUCED CHLOROPHYLL CHIMERAS BY CHEMICAL MUTAGENS IN GLYCINE MAX

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ABSTRACT

In present investigation effect of two chemical mutagens EMS and S.A. in M_1 generation of soybean variety JS-335 was studied. All the concentration of both the mutagens affected different biological parameters. In some varieties chlorophyll chimeras induced by these mutagens plants carrying chlorophyll chimeras exhibited does dependent variability.

Key word: ***EMS, S. A. soybean, chlorophyll chimeras, chemical mutagenesis.***

INTRODUCTION:

Gene mutations influencing the photo-synthetically active parts are among the most common spontaneous or induced alterations arising in higher plants. The chlorophyll mutation frequency is an indicator to predict the frequency of factor mutations (Gustafsson, 1951; and D'Amato et al., 1962).

Soybean [Glycine max (L.) Merr] has become the miracle crop of the 21st century. It is a triple beneficiary crop, which contains about 40% proteins, possessing high level of essential amino acids except methionine and cystine, 20% oil rich in poly unsaturated fatty acids specially omega-6 and omega-3 fatty acids, 6 to 7% total minerals, 5 to 6% crude fibre and 17 to 19% carbohydrates (Chauhan et al., 1988). .Mutation breeding is often used to development of crop plants for inducing better yield & other quantitative aspects by increasing genetic variability (Brock, 1970). The direct use of mutations is very valuable supplementary approach to plant breeding when it is desired to improve one or two easily identifiable character in otherwise well adapted variety (Sigurbjrnsson, 1970). The result of present investigation indicated that both the chemical mutagens are effective to induce a wide range of genetic variability in soybean.

MATERIAL & METHOD

Experimental plant material: Dry seed of soybean variety JS-335 were obtained from market.

The **Mutagens used:** In present investigation two chemical mutagens namely EMS and SA used**.**

METHODS:

1. **Surface Sterilization:** Healthy & uniform seed of soybean variety JS- 335 were surface sterilized with 0.1 % mercuric chloride solution for about one minute & washed thoroughly with distilled water.
2. **Pilot Experiment:** Pilot experiment was conducted for determining the sub lethal dose suitable concentration of the mutagens & duration of treatment. From pilot experiment it was established that concentration of 0.001%, 0.02% &0.03% of S.A for the duration of 4 hrs. & 0.05% , 0.10%& 0.15 % of EMS for the duration of 4 hrs.
3. **Treatment:**

A. Presoaking: Surface sterilized seeds of soybean variety JS- 335 were presoaked in distilled water for about 6hrs. (Table No.1)

B. Mutagenic Treatment: The mutagenic solution of S.A & EMS with different concentration were prepared freshly in distilled water. The pre-soaked seed were treated with mutagenic solution in conical flask with constant shaking on electric shaker for about 4hrs. (Table No.1)

The volume of chemical mutagens solution used was three time as that of seed so as to facilitate uniform condition. All the chemical mutagenic treatments were given at room temperature of 25 ± 2^0C. The different concentrations used for chemical mutagenic treatment were 0.05%, 0.10% and 0.15% for ethyl methane sulphonate (EMS) and 0.01%, 0.02% and 0.03% for sodium azide(SA).

C. Post Soaking The treated seeds were washed thoroughly under running tap water to remove excess of mutagens. Then seeds were post soaked in distilled water for 2hrs. The post soaked seeds dried in folds of filter paper.

D. Control: Seeds soaked in distilled water for 12hrs without treatment served as control.

E. Planting: The treated Seeds were planted in randomized block design with three replication in plot with a distance of 30cm between the rows and 15cm between the plants.

The resulting M_1 generation was thoroughly studied to access effect of both mutagens on leaf carrying chlorophyll chimeras.

Table: 01. Details of mutagenic treatments

Mutagen	Conc. (%)	No. of seeds treated	Presoaking time(hrs)	Mutagenic Treatment time (hrs)	Post soaking time (hrs)
SA	0.01	200	6	4	2
	0.02	200	6	4	2
	0.03	200	6	4	2
EMS	0.05	200	6	4	2
	0.10	200	6	4	2
	0.15	200	6	4	2
Control	-	200	6	-	6

RESULT AND DISCUSSION

Leaf color mutations are one kind of frequently observed mutation in both spontaneous and induced mutant populations, and used as an indicator of mutagenic effects and efficiency of various mutagens. Chlorophyll development seems to be controlled by many genes located on several chromosomes, which could be adjacent to centromere and proximal segment of chromosomes (Swaminathan, 1964). Chlorophyll mutations provide most dependable indices for the evaluation of genetic effects of mutagenic treatments and have been reported in various pulse crops by several workers including Gautam et al. (1992).

Four types of chlorophyll chimeras recorded were Albino (White), Xantha (Yellow), Chlorina (Yellow green) and Viridis (Dull green). The percentage of chlorophyll chimeras were increased with increasing concentration of mutagens in access of S.A. and in case E.M.S. were not shown particular trend. Highest frequency of chlorophyll chimeras was observed in the treatment of 0.10% EMS is 14.15%. (Table No.2). The chlorophyll chimeras might have due to alkylation of chloroplast DNA (Freese1963).

Constantin et al. (1974) reported that fast neutrons and ethyl methane sulphonate (EMS) were the most effective inducers of chlorophyll deficiencies and morphological mutants.

Table 02: Effect of mutagens on frequency of plants carrying chlorophyll chimeras in M_1 generation of soybean variety JS-335.

Treatment	Concentration (%)	Frequency of plants carrying chlorophyll chimeras %	± SE
Control	- - -	- - -	- - - -
EMS	0 . 0	6 . 9	0.33
	0 . 1 0	1 4 . 1 5	[illegible]
	0 . 1 5	1 0 . 7 1	[illegible]
SA	0 . 0	4 . 8	0.33
	0 . 0 2	8 . 0 4	[illegible]
	0 . 0 3	1 3 . 5 4	[illegible]

(SE = Standard Error)

Chlorophyll chimeras in soybean variety JS-335

Chlorina

Xantha

Albina

Viridis

CONCLUSSION:

All the mutagenic concentration induced chlorophyll chimeras in mutagenic population. The studied in present investigation revealed that both mutagens affected different biological parameters in M_1-generation of variety JS-335.

ACKNOWLEDGEMENTS

The authors are grateful to The Head, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (M.S.). for providing necessary facilities.

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