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Cytogenetical and Morphological Variations in EMS treated *Glycine max* Linn. (Merr.)

V.D. Mendhulkar[1], Tejprakash Bhati[2] and Sopan N. Kharat*[3]

[1, 2, *3]Department of Botany, The Institute of Science, 15, Madame Cama Road, Mumbai, Maharashtra, India-4000 32
Corresponding Author Email: sopankharat@gmail.com

Cytogenetical and morphological investigations have been carried out in EMS treated seeds of high protein containing important crop plant, *Glycine max* Linn., a member of papilionaceae family. The healthy and uniform seeds were treated with 0.02, 0.06 and 0.1 % of potent chemical mutagen, Ethyl Methane Sulphonate for 4 hrs. and 6 hrs. treatment durations. The dose dependent fluctuations in mitotic index, the Chromosomal aberrations, and variations in biochemical content were observed in EMS treated sample system. The EMS treatment with 0.1 % concentration for 6 hrs. treatment duration was noticed as potentially adverse as it induces high level of chromosomal abnormalities (9.96 %) in comparison to all other studied treatments. The aberration types like stickiness and single and multiple bridges were of common occurrence. Other chromosomal aberrations were precocious movement, chromosomal laggards and unequal distribution of chromosomes. The mitotic index was reduced to half, (6.77%), in 0.1 % EMS with 6 hrs. treatment duration when compared to control (12.42 %). The results on protein estimation were interesting since high EMS concentration at higher treatment duration indicated slight increase in the protein content (4.12 mg/100 gms.) over control estimate (3.8 mg/100 gms.). The morphovariants with respect to change in leaf margin (0.02 % EMS, 6 hrs.), early flowering (0.1 % EMS, 4 hrs.), chlorophylls chimeras (0.1 % EMS, 6 hrs.) and variation in protein content have been observed in the treated plants.

Key words: EMS, *Glycine max*, Mitotic index and Chromosomal aberration.

Cytological analysis with respect to either mitotic or meiotic behavior is considered to be one of the most dependable indices to estimate the potency of mutagen. Therefore investigation on mitotic aberrations and there genetic consequences constitute an integral part in most of the mutation studies. Hence, the present study has been undertaken to assess the mitotic consequences and primary effect of chemical mutagens in economically important plant *Glycine max*. Total protein content in treated material also has been studied as a part of the present work because most of the protein requirement is fulfilled from plant resources and it is possible to induce changes in protein quality and quantity by mutagenic treatments.

Soybean is one of the important leguminous crops rich in proteins and oil (Schmutz et al., 2010). A number of experiments on selection and hybridization

have been conducted to obtain superior varieties but very few reports are available on mutation breeding and cytogenetical studies (Krausse and Werner, 1989; Karakaya et al., 2002; Itawa et al., 2013). The seeds treated with chemical mutagens, showing alterations in genes or break chromosomes. Soybean contains a higher percentage of proteins than many other foodstuffs. Induced mutations have been used to improve a wide variety of crops mainly legumes (Mensah et al., 2013). The main protein components are globulin and glycinine, which accounts for 80-90 % of the total protein of the seed. Other forms of globulin such as phaseolin, albumin and legumelin are also present. It is a good source of ß-amylase. Soybean is a valuable forage crop in North India. The crop is ready for harvesting at a time when other pulse crops are unavailable. It ranks high among the leguminous crop of the world.

Glycine max (2n= 40) (Hindi- bhat, bhatwar) is a small genus of twinning or sub-erect herbs, distributed throughout the tropics of Africa, Asia, and Australia. Common species of soybean i.e. *Glycine max, Glycine soja, Glycine hispida*, *Glycine pentaphylla and Glycine javanica* are recorded in India. The plant is a native of southeastern Asia. It is a annual crop with erect or climbing stem, reaching a height of 11/2 – 6 feet; densely clothed with hairs; trifoliate leaves, ovate, lanceolate, long-petiole, small flowers, inconspicuous, borne on short axillary racemes, white or purple to reddish purple in color, normally self pollinated and pods are in clusters of 3-5 with 11/2-2 inch in length. Pods are hairy, sub-torulouse, containing 2-4 seeds with long and, compressed yellow chocolate or black colored hilum. Seeds are elliptical in shape.

EMS is alkylating agent and considered as a potent mutagen as well as showing undesirable biological effects (Gustafsson, 1975; Frooeze-Gertzen et al., 1963; Nilan and Konzak, 1966; Swaminathan, 1962; Khan and Tyagi, 2010). It is most reactive and powerful chemical mutagen. It has 93 hrs of half-life period at 20^0C. EMS is a color less liquid with density 1.2, b.p.85^0C and molecular weight 124.15 and molecular formula CH_3 SO_2O C_2H_5 with functional group SO_2OH. EMS is soluble in water. Induced mutagenesis is an important tool to break the limits of variability and to create new alterations in a short period of time (Kumar and Kumar, 2007). The greater mutagenic potentiality of mutagen can be judged by inducing minimum chromosomal damage and physiological injuries and thus, the EMS effect were assessed on structural chromosomal changes and early growth parameters in *Glycine max*.

Material and Methods

Seed samples

Healthy and morphologically uniform seeds of *Glycine max* Linn. were selected and grouped in a batch of 100 and seed washed in a distilled water (with neutral pH) to remove dust particles around the surface of seeds. The seeds were presoaked for 30 minutes to improve the seed coat permeability so as to enable proper intake of mutagenic solution. The Chemical mutagen, Ethyl Methane Sulphonate (Hi-Media) was use for the present experimental work.

Preparation of Mutagen

The series of three concentrations were prepared as 0.02%, 0.06% and 0.1%. All the concentrations were made in 0.1 M Phosphate buffer to maintain the pH 7.4. The presoaked and air-dried seeds were subjected to the treatment with 0.02%, 0.06% and 0.1% EMS in two independent sets. Two sets were prepared for 4hrs and 6 hrs duration. The treatment was conducted in refrigerator at 10^0 C to create most favorable condition and to achieve maximum effect. Control was also run with each set of treatment duration. Treated seeds were washed to remove the traces of mutagenic solution.

The seeds were kept for germination in pre-sterilized petri-plates and allowed to grow till 1.5-2 cm of length. The treated material was used to study various parameters at growth stage. The Chromosomal aberrations, dose dependent fluctuations in mitotic index, changes in biochemical content and morphovariations were studied in the present experiment. For cytological studies, root tip were fixed in a Carnoy's fluid II i.e. 1:2:1 (Acetic acid: Absolute Alcohol: Chloroform). Crude protein content was estimated following Lowry's method.

Protein estimation from seed sample

The material was grinded well in mortar and pestle to make fine powder with potassium-phosphate buffer (pH 7.4) to fine slurry and then centrifuged at 5000 rpm for 15 minutes. Supernatant was used for protein quantification. Protein content was estimated according to the method of Lowry et al. (1951), as a preliminary analysis to determine the protein concentration required to be used to run gel electrophoresis. The aliquot of 0.2 to 1.0 ml standard bovine serum albumin (200 µg/mL) was pipetted into a series of test tubes and volume was made up to 1.0 mL in each case. 5 mL of alkaline copper reagent was added to all the test tube. The test tubes were allowed to stand at room temperature for 10 min followed by the addition of 0.5 mL of FC reagent. The absorbance was recorded at 660 nm after 30 min against reagent blank.

Result and Discussion

Effect of EMS treatment

Results obtained on mitotic screening revealed the occurrence of major abnormalities in metaphase stage. The aberration types like stickiness, single and multiple bridges were of common occurrence. Mitra and Bhowmik, (1996) reported stickiness as the most common abnormality in *Nigella sativa* treated with EMS. Other chromosomal aberrations observed were precocious movement, chromosomal laggards and unequal distribution of chromosomes in anaphase. (Photoplate- 1-a to e). The percent abnormalities increased with slight variation with respect increase in the EMS concentration and treatment duration. The totals aberration percentage were highest in 0.1% EMS of 4 and 6 hrs treatments i.e. (7.61% and 9.96%, respectively). The lowest aberrations (4.47% and 6.14%, respectively) were noticed in 0.02% EMS 4 and 6 hrs treatments duration. (Fig.1). the percent aberration does not vary with remarkable difference within the concentrations in both the durations. It indicates the moderate effect of studied concentrations on the plant system. There concentrations are suitable for the induction of point mutations.

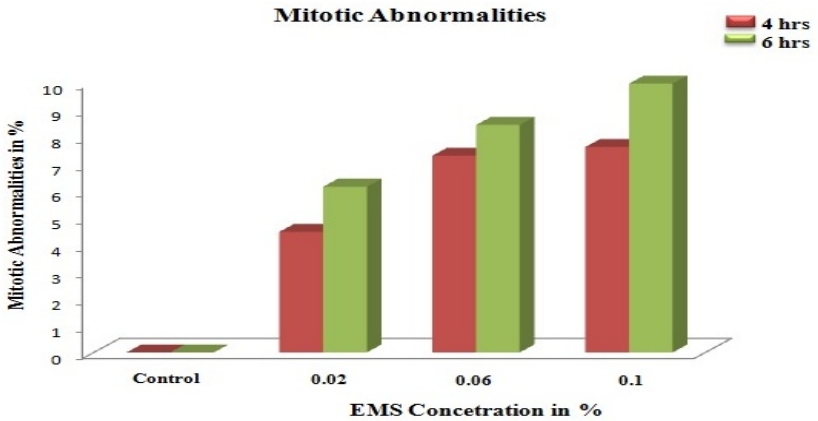

Figure 1- Mitotic Abnormalities in *Glycine max* L. treated with EMS

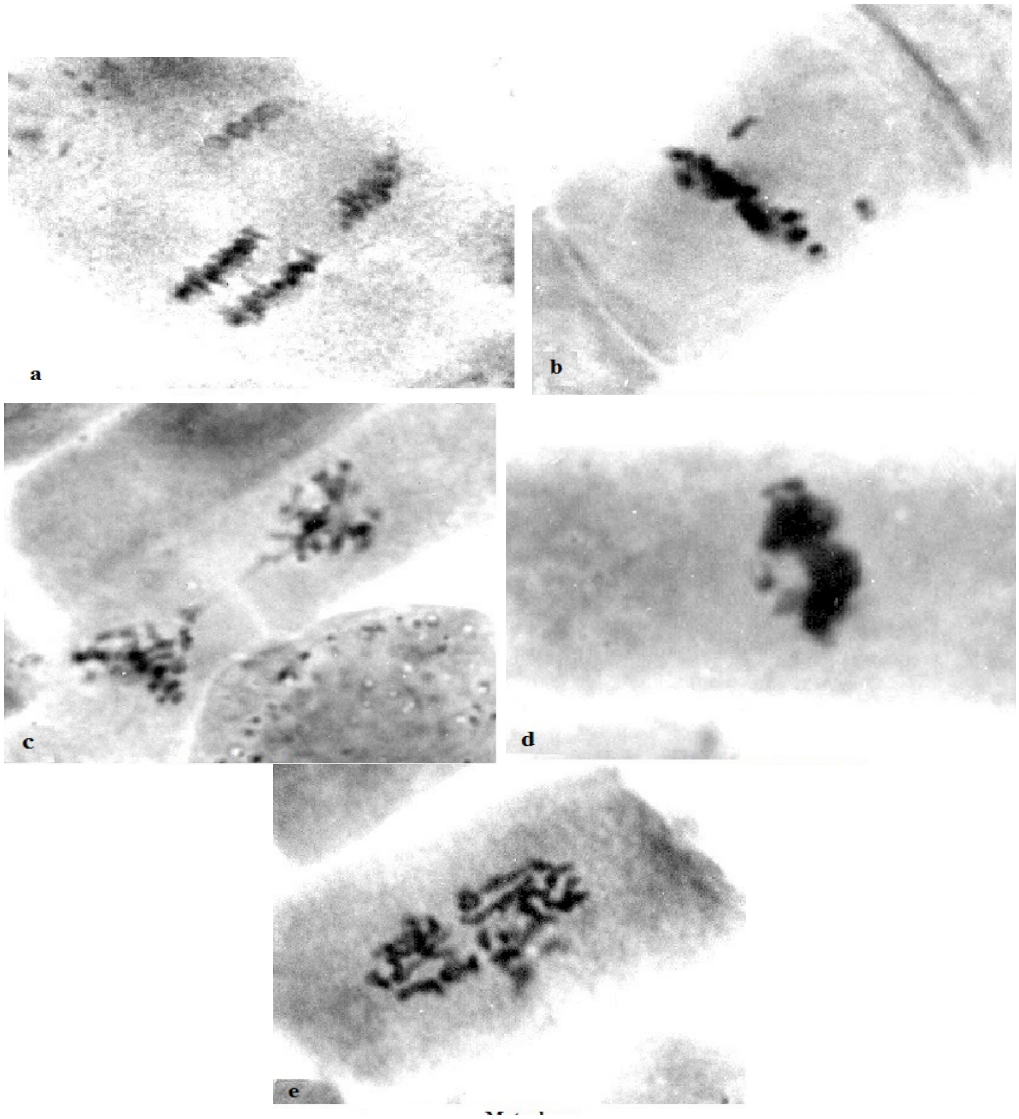

Photo plate-1- a-Bridge in Anaphase. **b-**Non oriented Chromosome in Metaphase. **c-**Bridge in Telophase. **d-**Stickiness in Metaphase.

Ignacimuthu and Babu, (1989) have studied the chromosomal aberrations induced by different mutagenic agents in mung beans. Abbasi and Anis (2002) and Mendhulkar and Gupta, (1999) have reported clastogenic effect of chemical mutagens in *Trigonella foenum graecum* Linn. Precocious movement of chromosomes is the result of inactivation of spindle mechanism. It may be due to the early terminalization, or advanced movement of the chromosomes during the anaphase (Permjit and Grover, 1985). Lagging chromosomes occurs because of improper movement of chromosomes during anaphase separation (Soheir et al., 1989). The chromosomal bridges may be due to stickiness or formation of dicentric chromosomes caused by breakage and reunion (Dempong and Maxwell, 1973). The finding in present study unearthed that the high concentration of EMS and longer treatment duration of EMS treatment induces gradual decrease in mitotic index. It is clearly highlighted that the higher concentrations

and duration's affects notably on mitotic cell division at initial stages. The difference between the individual percent mitotic indexes among treated concentrations was very less indicating lower leaf of deleterious impact on the studied plant system. A lowering in the mitotic index may be the consequence of DNA synthesis inhibition at S-phase (Sudhakar et al., 2001). In 4 hrs of EMS treatment, the lowest mitotic index was noticed (7.28%), for 0.1% EMS. The mitotic index was reduced to half i.e., 6.77 % in 0.1 % 6 hrs. EMS treatment compared to control (12.42 %) (Fig. 2).

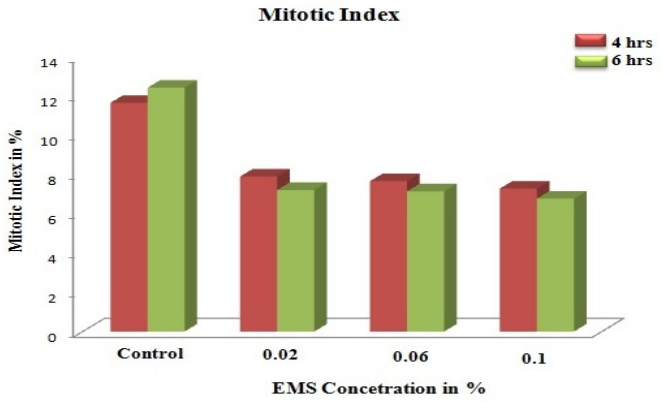

Figure 2- Mitotic Index in *Glycine max* L. treated with EMS

Protein Content in EMS Treated Seed Samples

The total protein content in treated seeds of *Glycine max* was compared with the control. It is clear that the total protein content was affected in all the EMS doses of both the treatment. The results on protein estimation were interesting since high EMS concentration at higher treatment duration indicated slight increase in the protein content (4.12 mg/100 gms.) over control estimate (3.8 mg/100 gms.) (Fig.3). These doses might elevate the rate of synthesis of protein by maximizing the activation of precursors involved in the mechanism of protein synthesis.

Morphological Variation by EMS treatment

One of the most common but prominent alterations noticed with experiment of mutagenic treatment was the modification in the shape and size of leaf. Different types of leaf variations were noted in 0.02 % EMS treatments for both the treatment durations i.e. 4 hrs & 6 hrs. (Photoplate-2- c to f). The variations were revealed to the changes in leaf margin, small leaf as well as and large leaf. Kumar and Dubey, (1998) have reported various types of leaf mutants. EMS treatment for 4 hrs duration showed an early flowering by 8 days in 0.06 % & 0.1 % compared to control. In 6 hrs EMS treatments, all the concentrations showed late flowering compared to the control. It indicates the residual effect of chemical mutagens at late growth period. According to George and Nayar (1973), earliness in flowering in linseed is due to the physiological changes caused by mutation. Early flowering mutants have been reported by Kumar and Dubey (1998), early maturing mutants have been recorded by Gaul et al. (1966) and Pawar et al. (1979) in different crop plants. Chimeral spot were observed in 0.1% EMS treatment with 6 hrs. duration. (Photoplate-2- f).

Conclusion

In the present investigation, various viable morphological variations were recorded in the EMS treated populations. Several workers have reported induction of mutations by EMS in legume plants. Patil and

Mendhulkar, (1993) reported the EMS induced mutants in *Desmodium tortuosum*. Kumar and Dubey, (1998) reported cytological and morpho-logical mutants in linseed and mitotic abnormalities are recorded by Kumar and Yadav, (2010) in *Sesamum indicum* L.

Photoplate-2- a-*Glycine max* seeds. **b-** *Glycine max* Control plant. **c-**Leaf marginal changes in 0.02% EMS with 6 hrs. **d-**Effect on chlorophyll pigmentation. **e-**Early Flowering in 0.01% EMS with 4 hrs treatment. **f-**Chimeral spot.

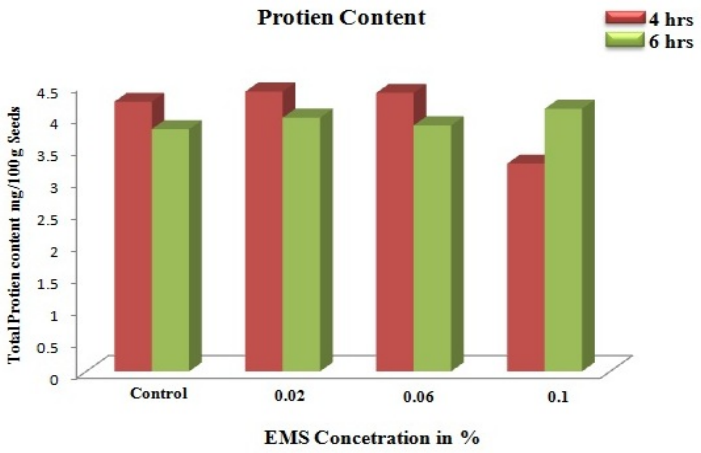

Figure 3- Protein content in *Glycine max* L. treated with EMS

In the present study the effect of EMS concentrations with varying treatment durations was analyzed in *Glycine Max*. The mutagenic solution is capable to convey chances in biochemical, cytological and morphological level by inducing variations. Chemical mutagen at some doses acts as stimulator and enhances the rate of biomolecule synthesis. The chemical mutagen is useful for the induction of desirable

characters to improve the quantitative and qualitative status of economically important plant resources. It was observed that the EMS treatment induces wider spectrum of variation in *Glycine Max* and has potential to induce structural and chromosomal changes.

Effect of EMS on the germination showed the triggering as well as delaying effect of studied concentrations and treatments. The remarkable fluctuation in total protein content in EMS treated seed of Glycine Max was noticed when compared to control. The 4 hrs EMS treatment was marked with changes in leaf margin, chimeral effect (Albina, Chlorina, Viridis and Xantha), tall variant, early and late flowering, high protein content, high mitotic index and lowest chromosomal abnormalities. The 6 hrs treatment revealed better results for said parameter compared to 4 hrs treatments. Present analysis provides an insight practically to observe varying spectrum of variability by the application of potent mutagens, EMS. Variations in the morphological, physiological and cytological characters can be effectively generated and utilized for meaning full purposes in quality improvement program of crop plants.

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