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Effect of chemical mutagen ethylmethane sulfonate (EMS) on the reproductive parameters of clusterbean plants (Cyamopsis tetragonoloba linn.)

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Article

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Abstract

Clusterbean plants were raised from seeds soaked in 0.5% EMS or water for 6 h under saline conditions. Observations of the percentage of seed germination, time required to achieve final germination, morphological variation in leaf arrangement and shape, number of floral clusters plants, number of flowers/plant, pollen viability and germination, number of pods/plant, number of seeds/plant and weight of 100 seeds were recorded. The results indicated that EMS-treated seeds exhibited a decrease in and delay in seed germination, plant height, *in vitro* pollen germination and tube length under non saline conditions. EMS treatment produced mutants with profuse vegetative growth under 8 and 12dSm^{-1} salinity. It also induced changes in the shape of the stigma from capitate to subapical and crescent shapeshaped under saline as well as nonsaline conditions. In some cases, these plants were also covered by trichomes. The number of pods and seeds per plant and the test weight of 100 seeds decreased in the plants raised from EMS-soaked seeds under nonsaline conditions. The number of flowers and pods and the seed yield per plant and the test weight of 100 seeds were greater for the mutants growing under 12dSm^{-1} salinity than for their corresponding counterparts from untreated seeds.

Introduction

Soil salinity is one of the major abiotic stresses that restricts the distribution and productivity of crop plants. Hardly any climatic zone is free from salinization, yet the general perception is focused on arid and semiarid regions. Soil salinity is a common problem in Haryana, affecting more than 320,000 hectares of land, or approximately 10% of the state's cultivated area. The problem has worsened in recent years, with the saline area growing by 35–80% in the last 20 years. The most recent FAO's Global Map of Salt-Affected Soils (GSASmap) covers 118 countries with measurements at two intervals estimating salinity at 424 million hectares of topsoil (0–30 cm) and 833 million hectares of subsoil (30–100 cm) (FAO, 2021a). Soil salinization, as a resource and ecological problem that currently exists on a large scale in all countries worldwide, is one of the main types of land desertification and soil degradation (Ding, 2011). According to a more recent report, more than 6% of the world’s land (19.5% of irrigated land plus 2.1% of dry land agriculture) is affected by salinity. This loss of farmable land is in direct conflict with the needs of the growing world population, which is projected to increase by 1.5 billion by 2030. Several mechanical and biochemical methods have been devised to reclaim saline soils; these methods are expensive and not readily feasible. The unique physicochemical and biological properties of saline soils have a variety of negative impacts. These include reductions in soil fertility and productivity levels and reductions in crop yields and harvests (Wang, Z. 2021:. Wu, Y, 2018), waste agricultural resources, destabilize the ecological environment, and cause other secondary hazards (Li, J, 2014). An alternative option is to have plant species with the trait of salinity tolerance. Salinity tolerance is a polygenic trait, and conventional breeding methods have failed to provide the desired results (Epstein et al. 1980). Moreover, taxa belonging to Fabaceae lack genetic variability among the genotypes. To increase this variability, *in vitro* tissue culture techniques offer a viable alternative, and the

resulting somaclonal variations have been exploited for salt tolerance improvement (Kirti et al. 1991; Lutts et al. 1999). However, difficulties in plant regeneration from selected cell lines, particularly from leguminous taxa, have resulted in the popularization of somaclones. Another option is to induce variability through mutagens. Improving plant stress tolerance is a powerful measure for improving plant yield. Moreover, there are many studies on improving resistance through EMS mutagenesis (Thangwana et al., 2021; Li et al., 2022). Mutation breeding has recently become increasingly popular as an effective tool for crop improvement, especially for crops with narrow genetic bases (Acharya et al. 2007), and an effective means of supplementing existing germplasms for cultivar improvement. Some leguminous crops whose prevalence has improved through mutation include soybeans, string beans, French beans (Sigurbjornsson and Micke 1974) and fenugreek and peas (Petropoulos 2002). Ethyl methanesulfonate (EMS) is one of the most commonly used alkylating agents and can induce chemical modification of nucleotides through the introduction of an active alkyl group, which can cause base changes and nucleotide mutations (Sabetta et al., 2011; Arisha et al., 2014; Gillmor and Lukowitz, 2020). Among chemical mutagens, ethyl methane sulfonate (EMS) is a potent mutagen that induces a high proportion of point mutations (Hajra 1979) with a low frequency of chromosomal aberrations (Van Harten 1998). Mutants with high yields and increased ascorbic acid contents have been generated by EMS treatment of *Capsicum annuum* (Pillai and Abraham 1996). Similarly, Basu et al. (2008) succeeded in developing useful mutants in Fenugreek exhibiting early seed maturity coupled with high seed yield, seed quality and determinate growth habit by EMS treatment. EMS is a type of nontransgenic chemical mutagen, and EMS mutagenesis is an important way to obtain mutations and discover new genes in plants. Special protocols have been established for many plant species (Unan et al., 2021). The traits affected by EMS mainly include yield, quality, and resistance (Wang H et al., 2016). EMS has been successfully applied to a large number of plants to increase morphological diversity and promote the improvement of ideal traits (Lethin et al., 2020). EMS-mediated improvements in the quality of peanut seeds through increases in protein and oil contents have also been reported (Wang et al. 2007). Luan et al. (2007) succeeded in raising NaCl-tolerant sweet potato lines from EMS-mutated calli. Similarly, Mallikarjun et al. (2008) reared sugarcane mutants treated with EMS for 2.5 h against salt tolerance via *in vitro* selection and regeneration under NaCl stress. Keeping in mind these encouraging achievements, the present study was therefore undertaken to explore the feasibility of improving salinity tolerance in guars by soaking in EMS, which is a drought-tolerant multipurpose crop grown for feed, green fodder, vegetables, green manuring and grain purpose.

Materials and Methods

Guar (*Cyamopsis tetragonoloba* cv. HG2 20) seeds obtained from the Forage Section, Department of Genetics and Plant Breeding, CCS HAU Hisar were used for raising the crop. Preliminary studies revealed that soaking seeds in 0.5% EMS for 6 h delayed germination but induced optimal germination under nonsaline conditions beyond which the soaking started to adversely affect root growth. Therefore, seed soaking was performed in 0.5% EMS for 6 h, after which the EMS was drained, and the plants were thoroughly washed with sterile water for no less than 30 min. Water-soaked (control) and EMS-treated

plants were raised in pots under different salinity conditions, and the number of days required for completion of germination was recorded. Plant morphological characteristics were recorded before the onset of flowering. During flowering, the number of floral clusters/plant, flower/plant pollen diameter, pollen viability (Hauser and Morrison 1964), *in vitro* pollen germination and tube growth, shape of the stigma and number of ovules/pistils were recorded. For *in vitro* germination, semisolid medium consisting of 35% sucrose, 100 ppm boric acid, 300 ppm calcium nitrate and 0.8% agar was used. Pollen plants inoculated on the above media in petriplates were incubated at a temperature of $^{30°C}$ for 4 h in the dark. At maturity, yield-related parameters were recorded.

Results and Discussion

Soaking of the guar seeds in 0.5% EMS for 6 h reduced germination compared with that of the control. Seed germination was also delayed (Table 1) under both saline and nonsaline conditions. Although salinity decreased the germination of EMS-treated seeds, germination was greater at 4 dS m^{-1} salinity than at other salinity levels under nonsaline conditions. A similar reduction and delay in EMS-treated seeds has been reported (Gohal et al. 1970). Sharma (1965) also reported decreased germination in sorghum seeds soaked in 0.5% EMS for 6 h. A reduction in seed germination may be due to the effect of mutagenesis on the meristematic tissues of the radical/plumule. An increase in the germination percentage was recorded in the 0.05% and 0.10% EMS treatments. Moreover, there is no reduction in germination due to mutagenic treatments (Mahamune, 2021). An increase in germination after mutagenic treatment may be attributed to physiological changes. Interestingly, EMS treatment significantly reduced plant height under nonsaline conditions and 4dS m^{-1} salinity, but some plants (mutants) raised under higher salinities of 8 and 12 dS m^{-1} salinity had plant heights and leaf areas comparable to those of the control, and all these plants were fertile (Fig. 1). The seedling height decreased as the mutagenic concentration increased. The maximum decrease in seedling height was observed in the 0.15% treatment (Mahamune, 2021). (Guar plants normally produce two cotyledonary leaves, 3 simple leaves and three trifoliate leaves. EMS treatment disrupted this sequence. Some of the mutants produced two simple leaves followed by a trifoliate leaf, which was again followed by a simple leaf and finally, trifoliate leaves. Under saline conditions, a similar sequence of leaf production was evident. In one of the mutants raised under 12dS m^{-1} salinity, four simple leaves were produced before the trifoliate leaves were produced. Another mutant even produced a tetrafoliate leaf. In addition, thickening, broadening, shortening and crinkling of leaves were also evident. These morphological variations in leaves are indicators of effective mutagen treatment. EMS-mediated changes in the leaf arrangement and shape have been reported in *Capsicum annuum* (Jabeen and Mirza 2004).

EMS or salinity had no significant effect on the initiation of flowering, but flowering was completed early in EMS-treated plants raised under nonsaline conditions and at relatively low salinity (4dSm^{-1}). Guar plants generally produce 3–5 clusters of flowers, while treating seeds with EMS reduced the number of clusters to 2–3 under nonsaline and saline conditions. The number of floral buds and flowers per plant decreased drastically in the plants raised from the EMS-treated plants. It decreased with increasing level

of salinity in plants raised from treated and untreated seeds; the reduction was greater in the latter cases. The percentage of floral buds transformed into flowers was greater in plants raised from untreated seeds under saline conditions than in those raised from EMS-treated seeds under both saline and nonsaline conditions. The pollen grains were 37.44 µm in diameter and 95.76% viable. Neither of these parameters was significantly affected by salinity or EMS treatment. However, Sanders et al. (1999) observed nondehiscing, late dehiscing and pollenless mutants of *Arabidopsis thaliana* following EMS treatment. *In vitro* pollen germination and tube growth decreased in EMS-treated plants raised under saline or nonsaline conditions; the reduction was more pronounced in the pollen grains of plants from EMS-treated seeds than in those from untreated seeds (Table 2). Interestingly, EMS treatment induced changes in the shape of sigma phase. It changed. The shape of the plants ranged from capitate to subapical, crescent shaped and well-developed (Fig. 2). These strains were functionally defective because they did not support pollen germination; hence, a reduction in yield in both saline and nonsaline conditions was evident. Komaki et al. (1988) also observed aberrant structures of stigmas in Fl82 and Fl89 mutants of *Arabidopsis thaliana*. The stigma had two clumps of papillae and horn-shaped projections.

The number of pods and seeds per plant and the test weight of 100 seeds decreased in the plants raised from the EMS-treated plants compared with those raised from the untreated plants under nonsaline conditions. Salinity adversely affected these parameters; the difference was more pronounced in the untreated plants than in the treated plants. Seed yield/plant decreased with salinity in untreated seeds. Seed treatment with EMS led to a significant reduction in seed yield under nonsaline conditions, while an increase in yield was evident in plants treated with 12dSm^{-1} compared with untreated plants (Table 3). The percent pod set remained unchanged up to 8 dSm^{-1} salinity in plants raised from untreated seeds and then decreased significantly at 12dSm^{-1}. The percent pod set was relatively greater in the plants raised from EMS-treated seeds than in those raised from untreated seeds, and the value was greatest at 12dSm^{-1}. The percentage of pollen sterility demonstrated a steady increase sterile plants steadily increased within each treatment with increasing concentrations of the mutagen. The maximum sterility was observed at the highest concentration. (Mahamune, 2021). Pod length decreased in plants raised from EMS-treated seeds under both saline and nonsaline conditions, and the difference was greater in the former than in the latter. Seed set and seed and pod characteristics were not significantly affected by EMS treatment, which was also shown by Gohal et al. (1970). However, in Luhua 11 cv. of peanut, EMS produced both longer and shorter pod mutants (Wang et al. 2007). It also increased the protein and oil content of the seeds. Francois et al. (1990) reported that the seed yield of two cvs. of guar was not affected by salinity levels up to 8.8 dSm^{-1}, and each unit increase in salinity above this reduced yield was 17%. These authors further showed that a reduction in the number of pods/plant and seed weight are the primary factors contributing to reduced yield. However, such yield reductions in cowpea resulted from a decrease in the number of pods (West and Francois 1982).

It is thus evident that EMS treatment inhibited plant growth and yield parameters under nonsaline conditions. However, this treatment improved the plant height and leaf area of plants raised under 8 and

12 days of Sm^{-1} salinity. The number of flowers and pods and the seed yield per plant and the test weight of 100 seeds were greater for the mutants growing under 12dSm^{-1} salinity than for their corresponding counterparts from untreated seeds. These increases in yield parameters under saline conditions were definitely lower than those under the nonsaline untreated control, but EMS treatment offers a new avenue for harvesting relatively better yields under relatively high salinity conditions.

Declarations

There are no conflicts of interest. Additionally, there was no funding from any source.

Author Contribution

Author had done research work on guar during her Ph.D.

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Tables

Tables 1 to 3 are available in the Supplementary Files section.

Figures

Figure 1

Effect of soaking in 0.5% EMS for 6 h on the growth of plants raised under 4dSm^{-1} (B), 8dSm^{-1} (E) and 12dSm^{-1} (F) salinity conditions. A represents the nonsaline control.

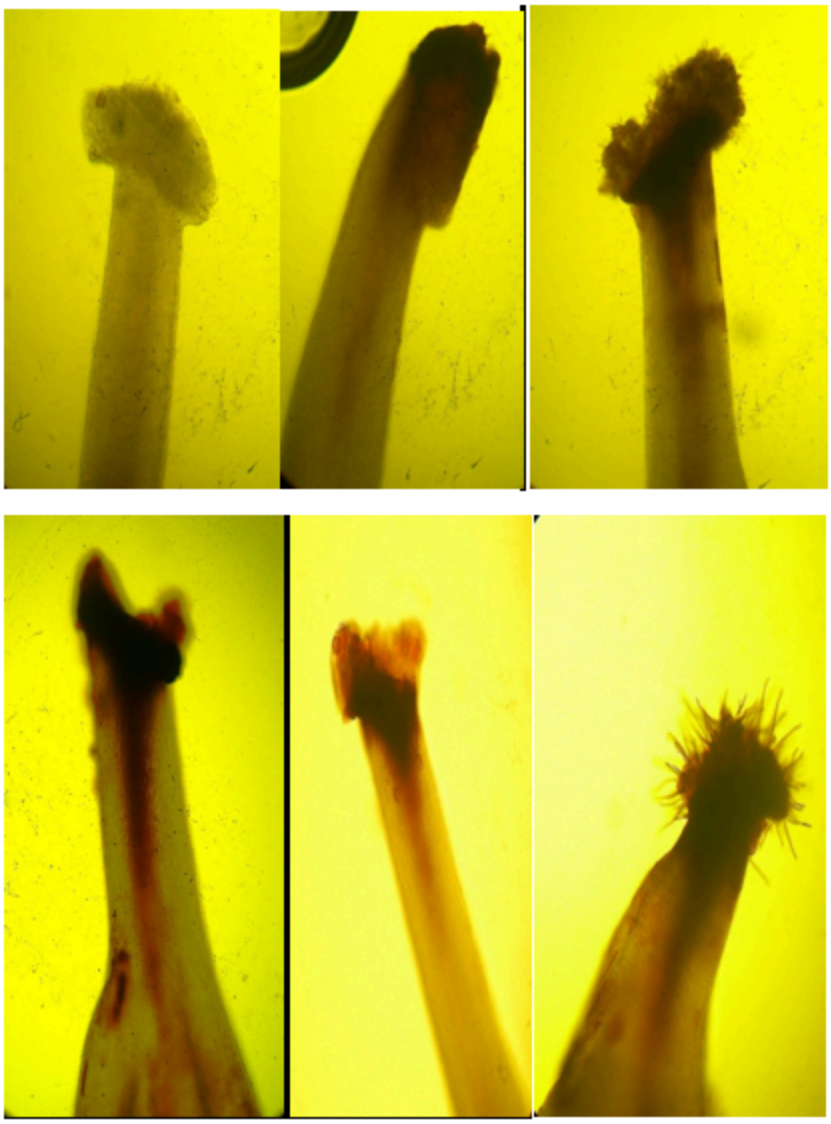

Figure 2

Effect of seed soaking in 0.5% EMS for 6 h on the shape of the stigma in guars

Supplementary Files

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