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MORPHOLOGICAL VARIATIONS INDUCED BY ETHYL METHANE SULPHONATE IN CLUSTERBEAN [*CYAMOPSIS TETRAGONOLOBA* (L.) TAUB.]

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SUMMARY

The present investigation was carried out to observe the different morphological variations induced by chemical mutagen ethyl methane sulphonate (EMS) on clusterbean commonly known as guar. Guar is an important forage crop with versatile properties. The importance of guar is mainly due to presence of guar gum produced from its seeds. EMS is a chemical potent mutagen, capable of inducing large number of mutations. Various morphological variations were produced due to treatment of EMS. Seed treated with 0.5 per cent EMS for 6 h produced more variable results as compared to 0.5 per cent, 8 h treatment. Incubation of seed in 1 per cent EMS for both 6 and 8 h was inhibitory, except to one major variation, which produced longer root and shoot as compared to the untreated seed. 1 per cent EMS resulted in more inhibitory/toxic effect on germination of guar. The concentration of 1 per cent EMS was not found to be suitable for inducing mutagenic effects.

Key words : *Cyamopsis tetragonoloba*, guar, EMS, morphological variations

During the past decades, mutation breeding has been used from four generating genetic variations and breeding of different crop varieties. Mutation breeding has become very popular in recent times for its effectiveness of developing new crop varieties (Acharya *et al.*, 2007). Mutation breeding is one of the conventional breeding methods in plant breeding. It shows relevant behaviour with various other fields like morphology, biotechnology, cytogenetic and molecular biology, etc. Induced mutations have been used in developing improved cultivars of fruits, cereals and other crops (Lee *et al.*, 2002). From various studies, it is stated that the mutations provide fruitful variation for practical plant breeding purpose. By inducing the seed with varying mutagenic agent dose, the frequency and saturation of mutations can be regulated (Menda *et al.*, 2004). Mutation methods have been successfully employed in breeding of various ornamentals, food crop varieties and other export crops (Mohamad *et al.*, 2005.)

Clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.], one of the most important annual, self-pollinated leguminous crops, is a robust annual herb with long tap roots and well developed lateral roots (Deepika and Dhingra, 2014). Guar gum produced from the seeds of guar has a high commercial value. By the use of mutagenesis, the crop of guar can be improved for maximum yield. By keeping the above facts in mind, a preliminary study was carried out to observe the effect of ethyl methane sulphonate on morphological features of guar.

MATERIALS AND METHODS

The present experiment was performed at Department of Botany & Plant Physiology in 2011. The seeds of guar HG 2-20 were collected from Forage Section, Department of Genetics & Plant Breeding. The seeds were thoroughly washed with water prior to the treatment of EMS. The seeds were incubated in EMS

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solution of different concentrations i. e. 0.5, 0.75 and 1 per cent variable durations of time i. e. 6 and 8 h. After incubation, the mutated solution was removed. Seeds were again washed with sterilized distilled water about five times. Then seeds were placed on the germination paper. Germination paper was folded and placed in beaker kept under sterile controlled condition with 16 h light and 8 h dark period. After one month of germination, morphological variations were observed. The seeds without treatment of EMS were treated as control and the others were named as treated seeds (Fig. 1).

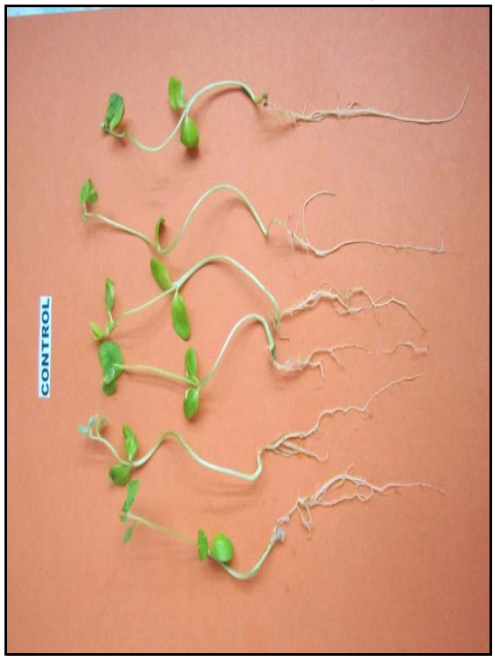

Fig. 1. Seeds germinated on germination paper without the treatment of EMS.

RESULTS AND DISCUSSION

Reduction in seed germination may be due to the effect of mutagen on meristematic tissues of the radical/plumule. The untreated seed showed normal germination. Germination was 100 per cent in untreated seed, while it showed gradation in treated seed. The decrease in seed germination at higher doses/ concentration of the mutagens may be attributed to disturbances at cellular level. Same results were reported by Kumar and Mishra (2004) in okra that germination percentage generally decreased with increasing doses of gamma rays and EMS. Similarly, reduced germination percentage with increasing doses of gamma radiation was also reported in *Pinus* (Thapa, 2004), Rye (Akgun and Tosum, 2004) and chickpea (Khan *et al*., 2005). Guar plants normally produce two cotyledonary leaves and three simple leaves followed by trifoliate leaves. EMS treatment disturbed this sequence. Mutants produced by 0.5 per cent EMS for 6 h showed two simple leaves followed by a trifoliate leaf which was again followed by a simple leaf and finally trifoliate leaves. Mutants also produced one cotyledonary leaf, then three trifoliate leaves at one node with complete inhibition of root growth (Fig. 2). The same concentration of EMS for 8 h produced different morphological variations like serrated margin, blackening of margin, reduced shoot and root growth. Treatment for 8 h produced the leaves in the same pattern as in the control (Fig. 3). Seeds treated with 0.75 per cent for 6 and 8 h did not produce so much

Fig. 2. Effect of EMS (0.5% EMS, 6 h) on changes in morphology of guar.

Fig. 3. Effect of EMS (0.5% EMS, 8 h) on changes in morphology of guar.

different results. But increasing the EMS concentration resulted in the complete inhibition of root growth at both the intervals of time. EMS induced wider spectrum of morphological mutation than gamma rays. The morphological mutations affecting maturity and flowering behaviour (42. 60%) and foliage (22.60%) occurred more frequently in clusterbean (Velu *et al.*, 2012).

The morphological variation produced by 1 per cent EMS for 6 h was the inhibition of root and shoot growth, glossy appearance, decrease in chlorophyll content, back spots on the leaf lamina and blackening on the margin. Intermittingly, one seed germinates resulting in profound root growth with formation of shoot (Fig. 4). Germination started in the seed treated with 1 per cent EMS for 8 h, but no further growth was observed, hence found to be the lethal for guar seed (Fig. 5). From the results, it was concluded that the treatment of seeds with 0.5 per cent EMS for 6 h produced more morphological variations, so this treatment could be used for further studies for producing mutant under field conditions as well as *in vitro* conditions. Among doses, lower doses of EMS and gamma rays were more effective and efficient than the higher doses. Efficient mutagens and their treatments are essential for the economic use of the mutagens as a tool for the induction of useful mutations and their direct and indirect utilization (Velu *et al.*, 2012).

Fig. 4. Effect of EMS (1.0% EMS, 6 h) on changes in morphology of guar.

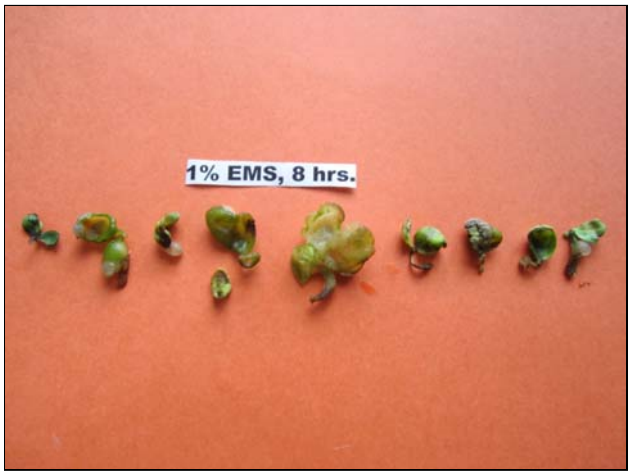

Fig. 5. Effect of EMS (1.0% EMS, 8 h) on changes in morphology of guar.

CONCLUSION

Seed germination and seedling growth were inhibited due to increasing doses/concentrations of ethyl methane sulphonate. The survival rate was highly reduced with increasing dose/concentration of mutagens. Almost for all the mutagenic treatments, the dose of 0.5 per cent for 6 h was found to be effective for altering the morphological variations. While increasing the dose up to 1 per cent was found to be lethal for seed germination and its survival.

REFERENCES

Acharya, S. N., J. E. Thomas, and S. K. Basu. 2007 : Improvement in the medicinal and nutritional properties of fenugreek (*Trigonella foenum-graecum* L.). In : *Advances in Medicinal Plant Research*, Acharya, S. N. and J. E. Thomas (eds). Research Signpost, Trivandrum, Kerala, India.

Akgun, I., and M. Tosum. 2004 : Agricultural and cytological characteristics of M_1 perennial rye (*Secale montanum* Guss.) as affected by the application of different doses of gamma rays. *Pak. J. Biol. Sci.*, **7** : 827-833.

Deepika, and H. R. Dhingra. 2014 : Effect of salinity stress on morpho-physiological, biochemical and yield characters of clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.]. *Indian J. Plant Physiol.*, **19** : 393-398.

Khan, M. R., A. S. Qureshi, A. H. Syed, and M. Ibrahim. 2005 : Genetic variability induced by gamma irradiation and its modulation with gibberellic acid in M_2 generation of chickpea (*Cicer arietinum* L.). *Pak. J. Bot.*, **37** : 285-292.

Kumar, A., and M. N. Mishra. 2004 : Effect of gamma-rays, EMS and NMU on germination, seedling vigour, pollen viability and plant survival in M_1 and M_2 generations of okra [*Abelmoschus esculentus* (L.) Moench]. *Adv. Plant Sci.*, **17** : 295-297.

Lee, Y. I., I. S. Lee, and Y. P. Lim. 2002 : Variation in sweet potato regenerates from gamma-rays irradiated embryogenic callus. *J. Plant Biotech.*, **4** : 163-170.

Menda, N., Y. Semel, D. Peled, Y. Eshed, and D. Zamir. 2004 : In silico screening of a saturated mutation library of tomato. *Plant J.*, **38** : 86.

Mohamad, O., S. Herman, B. M. Nazir, S. Shamsudin, and M. Takim. 2005 : A dosimetry study using gamma irradiation on two accessions, PHR and PHI, in mutation breeding of roselle (*Hibiscus sabdariffa* L.). In : *7th MSAB Symposium on Applied Biology, 3-4 June, Sri Kembangan.* pp. 1-10.

Thapa, C. B. 2004 : Effect of acute exposure of gamma rays on seed germination and seedling growth of *Pinus kesiya* Gord and *P. wallichiana* A. B. Jacks. *Our Nature*, **2** : 13-17.

Velu, S. L., Mullainathan, and D. Arulbalachandran. 2012 : Induced morphological variations in clusterbean [*Cyamopsis tetragonoloba* (L.) Taub]. *Int. J. Cur. Tr. Res*., **1** : 48-55.