

## EFFECT OF ETHYL METHANE SULPHONATE ON CALLUS GROWTH OF CLUSTERBEAN [*CYAMOPSIS TETRAGONOLOBA* (L.) TAUB.]

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### SUMMARY

The present study was carried out to study the effect on ethyl methane sulphonate on callus growth of clusterbean. The experiment was put in process at department of botany and Plant Physiology in 2011. Seeds of clusterbean HG 2-20 were injected with the various doses of EMS (0.5%, 0.75% and 1.0%) for different duration of time (6 hr and 8 hr). The mutated seed were cultured on germination media for obtaining explants i.e. cotyledonary node. It was then grown on callusing media. Fresh and dry weight of the callus was recorded. From results, it was found that 0.75 per cent EMS concentration was more effective in callus growth, while the 1 per cent EMS was found to be lethal dose.

**Key words :** Callus, guar, *Cyamopsis tetragonoloba*, EMS

Clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.], commonly known as guar, is one of the most important summer annual legumes which were introduced into USA from India in 1993 (Khanzada *et al.*, 2003). It is a robust annual herb with long tap root and well developed laterals. It is cultivated throughout India for its edible pod and as fodder crop (Deepika and Dhingra, 2014). However, it is unable to meet the required demand (Dube *et al.*, 2011). Studies have shown that application of tissue culture technique following mutagen treatment increases mutagenesis frequency expands the spectrum of trait variation and results in more target mutants. Among chemical mutagens, the alkylating agent like EMS is a potent inducer of specific and predictable mutations (Talebi *et al.*, 2012). It can induce high frequency of gene mutation and low frequency of chromosomal aberrations. Improvement of crops through plant tissue culture can be achieved by growing plants in a laboratory through callus initiation and further regeneration of shoots. Once callus is formed, it can be manipulated by genetic transformation to produce desired effects (Deepika *et al.*, 2014). Therefore, the present study was carried out to study the effect of mutagen on callus growth and its viability.

### MATERIALS AND METHODS

#### Sterilization of Glassware/Plasticware

Glasswares and plasticwares were thoroughly washed with liquid detergent. The glasswares were dried in an oven at 160°C for 2 h and plasticwares at 60°C for 2 h and rinsed with double distilled water before use.

#### Preparation and Storage of Stock Solutions

Stock solutions of major and minor salts, chelating agent (Fe-EDTA), vitamins and growth regulators were prepared (mg/ml) according to their solubility separately and stored in the refrigerator at 4°C. Wherever required amber coloured bottles were used for storage to eliminate the light effect. All the stock solutions were used within one month of their preparation.

#### Preparation, Sterilization and Storage of Culture Media

Murashige and Skoog's (MS, 1962) medium was used as basal medium, 3 per cent sucrose, myo-

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inositol (100 mg,w/v) and 0.8% agar throughout the course of present investigation. All the components of the medium were mixed and final volume was made by adding double distilled water and then pH was adjusted to 5.8 (before adding agar) with the help of 1 N NaOH/ 1 N HCl followed by addition of agar. After melting the agar, the medium was dispensed into culture flasks/tubes. These were plugged with non-absorbent cotton. The medium was sterilized in an autoclave at a pressure of 1.08 kg/cm<sup>2</sup> (Temp. 121°C) for 15 min. The autoclaved medium was stored at 26±2°C and used within 3-4 days of its preparation.

### Explants and their Preparation

Seeds (Explant) were dipped in different doses of EMS, 0.55, 0.75% and 1.0% for 6 and 8 h. After incubation, mutagen solutions were removed and seeds were surface sterilized with 70 per cent alcohol for 1 min and then with 0.1 per cent mercuric chloride solution for 5 min. The explants were then rinsed thoroughly three to four times in sterile distilled water on the hood of laminar flow to remove all traces of mercury. These sterilized explants were inoculated on medium containing 3 per cent sucrose and 0.8 per cent agar under aseptic conditions. Inoculated flasks were kept in culture room at 25±1°C temperature, under the photoperiod of 16 h light and 8 h darkness. Seeds were germinated and various further explants were collected for callus induction. Explants used were cotyledonary leaf, leaf lamina, hypocotyls, etc. Growth of callus in terms of fresh and dry weight was recorded.

### RESULTS AND DISCUSSION

The influence of ethylmethane sulphonate on the callus growth in terms of its weight has been tested. The callus was obtained by using cotyledonary node as explants. Among various combination of growth hormones tried for induction of callus; the callus obtained from cotyledonary node segments on MS medium with 2,4-D (2mg/l) and BAP (1 mg/l) was found to be compact and large in amount. Combining tissue culture techniques with chemical mutagen has been found to increase the frequency of variation in most of the plants. (Hassan, *et al.*, 2014).in the present study, different treatment of EMS was given to guar for different time period. The treatment of EMS at 0.5% for 6 hr resulted in increase in callus fresh weight from 2.425g (Control) to 2.524g (Fig.1)

and dry weight from 0.834g to 0.875g. (Fig.2). Likewise treatment 0.5% for 8 hr, 0.75% for 6 hr, 0.75% for 8 hr resulted in increase in fresh weight 2.562, 2.892, 2.862 and dry weight 0.896, 0.924, 0.902 respectively (Fig.1 and 2). Further increasing the concentration led to serious decline in the fresh as well as dry weight of callus. Colour of callus also changed with change in the EMS concentration colour changed from greenish, greenish yellow and finally brownish black at 1.0 per cent EMS concentration for 8 h. There was also variation in the callus growth in duration of treatment within the same dose of concentration. At 0.75 per cent EMS concentration, 6 h treatment was found to be more effective in enhancing the callus growth, while 8 h treatment led to decrease in the fresh as well as dry weight. In an experiment by Svetleva and Crino (2005), it was found that the times of the mutagenic treatments

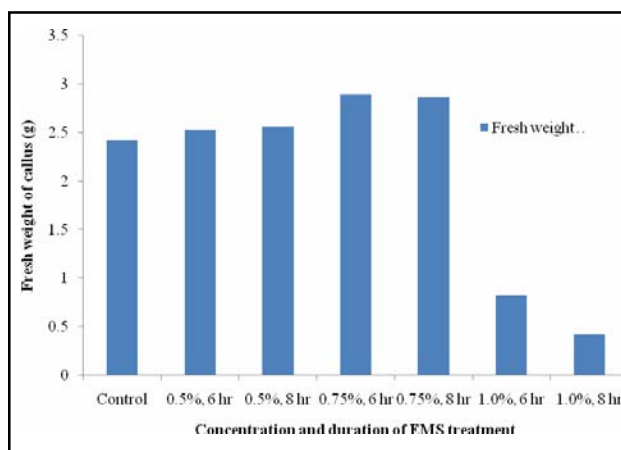


Fig. 1. Effect of ethyl methane sulphonate on fresh weight of callus of Cluster bean.

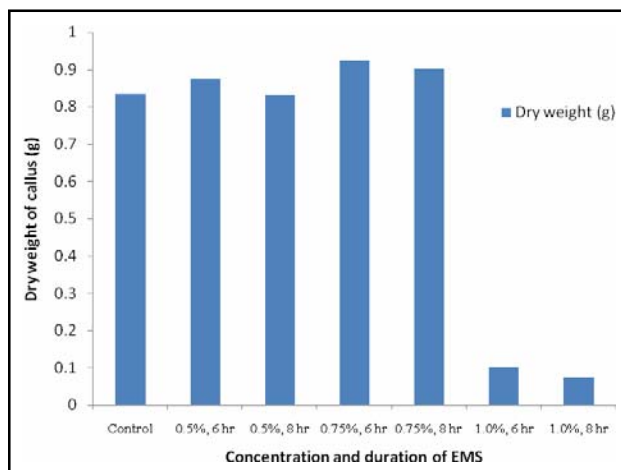


Fig. 2. Effect of ethyl methane sulphonate on dry weight (mg/g) of callus of Cluster bean.

influenced callus growth, calli from 30-min treatment with both mutagens (EMS and ENU) showing the highest weights. In both the cases, the 90-min mutagen application caused a too relevant effect either on callus browning or growth inhibition. In general, ENU showed a stronger effect than EMS. The effect of sub-cultures on callus growth was higher than mutagenic treatments. The decrease in the fresh and dry weight of callus may be due to changes induced by EMS resulted into change of pair of (Adenine :: Thymine) to (Guanine :: Cytosine). which results into morphological variation and other effects.

### CONCLUSION

Based on the performed investigation, we can conclude that callus growth in terms of fresh and dry weight strongly influenced by concentration and duration of EMS. Fresh and dry weight of callus obtained from cotyledonary node treated for 0.75% for 6 hr was found to be maximum.

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