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SHORT COMMUNICATIONS

EVALUATION OF GENETIC VARIABILITY IN BERSEEM AFTER MUTAGENESIS

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SUMMARY

Trifolium alexandrinum, commonly known as Berseem or Egyptian clover, is the annual leguminous forage crop grown over wide areas in north India. The genus *Trifolium* comprises 290 species out of which 25 are of agricultural importance. Breeding of berseem is highly restricted due to narrow genetic base. Mescavi is the only genotype which is being used for the last 110 years. To break this variability barrier, some approaches like induction of polyploids, polycrosses and mutations have been followed to harvest some gains in this important crop of **rabi** season. In this experiment, three doses of EMS (0.1, 0.3 and 0.5%) were applied to dry seeds of HB 1, HB 2 and Mescavi. M_1 generations were raised in the field. Germination was recorded 10 days after sowing. Maximum germination (85%) was observed with 0.1 per cent EMS, whereas minimum germination (12%) was observed with EMS 0.5 per cent. A total of 98 single plants in different treatments survived and were harvested and threshed individually. Superior plants based on phenotype will be selected in the subsequent generations. Various phenotypic characters are improved through selection viz., plant height, tillers per plant, leaves per plant, leaf : stem ratio, trifoliate/tetrafoliate/ pentafoliate leaves, small leaflets, oblong, bright green and slightly hairy. Flower head shapes and colour, days to maturity which spread from 180-210 days which are spread over varying climatic conditions. Green fodder yield, dry matter yield and their per day production are the most important among them. The procedure has proved beneficial in improving these characters to a reasonable extent.

Key words : Berseem, *Trifolium*, mutation

Trifolium alexandrinum, commonly known as Berseem or Egyptian clover, is the annual leguminous forage crop grown over wide areas in north India. It was introduced in India in 1904 (Mehta and Swaminathan, 1975) and now it is cultivated on an area of around 2 million hectares (Kumar *et al.*, 2003). In Haryana, it occupies around 1.25 lac hectares. Presence of sufficient exploitable genetic variability is pre-requisite for genetic improvement of any crop species. Breeding of berseem is highly restricted due to narrow genetic base. Quantum of breeding output in berseem is less which may be due to presence of lesser exploitable genetic variability. Certain breeding limitations like very tiny, delicate and complex flower structure, lesser extent of out crossing, self fertility and low seed setting may be other possible reasons for little genetic improvement in berseem. Mescavi is the only genotype which is being used for the last 110 years. To break this variability barrier, some approaches like induction of polyploids, polycrosses and mutations have been followed to harvest some gains in this important crop of *rabi* season. Mutation breeding was used successfully where one variety HB 2 was released after selection from Mescavi treated with gamma rays @ 70 kR. Genetic variability creation through mutagenesis depends on a number of factors like crop, mutagen used, genotype, etc. Very little work has been done in forage crops to break the variability barrier in berseem. A number of physical and chemical mutagens known for their mutagenesis capabilities have been used in different crops. Physical mutagens include treatment of seeds with X-ray, gamma rays. Chemical mutagens are ethyl methane sulphonate (EMS), methyl methane sulphonate

(MMS), diethyl sulphonate (DES), sodium azide (SA), nitroso methyl urea (NMU), nitroso guandine (NG), etc. Mutagen concentration (dose) and duration of treatment also impact the outcome, which may be restricted by undesirable effects resulting from the treatment. A systematic study for evaluating the potential of induced mutations by physical and chemical mutagens for crop improvement in India was initiated in mid-fifties (Swaminathan, 1957). The use of mutagenic chemicals has been increased significantly during the past decade (Khan *et al.*, 2000; Koli and Ramkrishna, 2002; Avtar *et* al., 2005; Singh *et al.*, 2005). Gene and chromosome mutations arising from the mutagen treatment may be transferred from M_1 to the subsequent generations. Deleterious effects of mutagens may be in the form of reduction in germination percentage, root shoot length, plant survival, fertility, etc. An optimum dose is the one which causes maximum mutations and minimum killings. It is generally agreed that a dose close to LD_{50} should be optimum. LD_{50} is that dose of a mutagen, which would kill 50 per cent individuals. LD_{50} varies with crop species and with mutagens used.

Material includes three varieties of berseem viz., Mescavi, HB 1 and HB 2 released by Forage Section, CCSHAU, Hisar. The trial was conducted during **rabi** 2012-13 at Forage Research Farm, CCSHAU, Hisar. The main characteristics of these varieties are given below :

Three doses of ethyl methane sulphonate (EMS) (0.1, 0.3 and 0.5%) applied to dry seeds of Mescavi, HB 1 and HB 2. The specificity of action of chemical mutagen depends upon particular condition of treatment, the most important of which are temperature and pH. In the present study, different doses of EMS were prepared by dissolving appropriate quantities of the chemical in phosphate buffer pH 7.4. A total of 100 seeds in each replication were soaked in freshly prepared aqueous solution of the respective concentration for 6 h at 25±1°C with constant intermittent stirring at hourly intervals. After this, the seeds were dipped in 2 per cent aqueous solution of sodium thiosulphate for 10 min to stop the action of the mutagens and then washed thoroughly with running tap water. The seeds were sown in the field immediately after treatment. M_1 generation was planted in the field in single rows of 5 m each with 30 cm spacing. The recommended package of practices was followed to raise a good crop. General techniques used for field observations are given below :

- **Germination :** The emergence of coleoptile at the soil surface was taken as an indication of germination. The number of seeds germinated after 10 days was taken as a parameter to determine germination percentage.
- **Plant survival :** It is taken as plants reaching maturity and produce seeds. The survival percentage was calculated as proportion of plants surviving till maturity out of the total number of seedlings germinated.

• Released variety in 1978 • Semi-erect plant, fast growth • Good regeneration • Slightly elongated head shape	• Released variety in 2005 • Fast growing and good regeneration capacity • Fodder is very nutritious and palatable.	• Released variety in 2013 • Longer duration • Better regeneration • Resistant to stem rot disease, which is the major problem in Haryana

In the present study, the seeds of berseem varieties Mescavi, HB 1 and HB 2 were treated with different doses of chemical mutagen EMS. The observations were recorded on two different parameters to check the extent of genetic variability induced through mutagenesis. The germination per cent and number of plants survived up to maturity are given in Table 1 below :

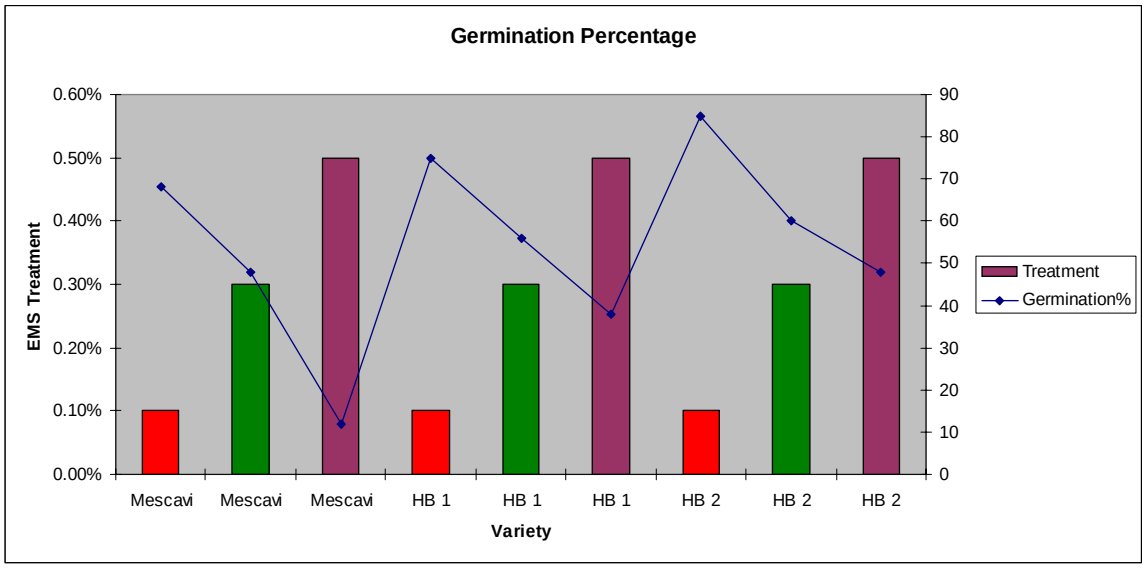
A perusal of the observations reveals that maximum germination (85%) was observed with 0.1 per

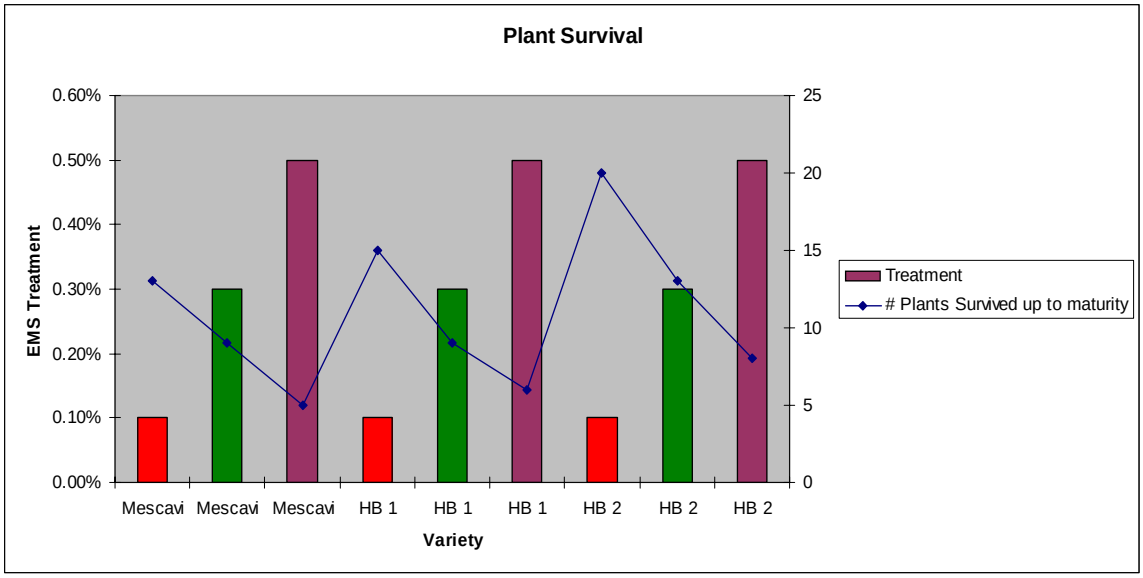
TABLE 1
Germination % and plant survival after treatment

Variety	Treatment EMS (%)	Germination (%)	No. of plants survived up to maturity
Mescavi	0.1%	68	13
	0.3%	48	9
	0.5%	12	5
HB 1	0.1%	75	15
	0.3%	56	9
	0.5%	38	6
HB 2	0.1%	85	20
	0.3%	60	13
	0.5%	48	8

cent EMS in variety HB 2, whereas minimum germination (12%) was observed with EMS 0.5 per cent in variety Mescavi. As the dose of the mutagen was increased, a corresponding decrease in seed germination was observed. These findings are in agreement with those reported earlier in berseem (Anonymous, 2003, 2005, 2006; Avtar *et al.*, 2005). Moreover, the reduction in seed germination was also observed to be dose dependent which was confirmed by earlier reports in various crops (Solanki and Sharma, 1994; Yadav *et al.*, 1998).

Like germination, plant survival was also reduced drastically. This was also expected as mutagens affect various physiological processes i. e. photosynthesis and





respiration. Most of the chromosomal aberrations caused due to mutagenesis adversely affected the fitness of the plant reducing survival (Sarkar and Sharma, 1987). Wellensick (1965) found positive correlation between seed germination, survival and fertility all of which decrease after treatment with different mutagens. The trend of plant survival indicated that increasing doses of mutagen caused reduction in plant survival. A total of 98 single plants in different treatments survived and were harvested and threshed individually. Superior plants based on phenotype will be selected in the subsequent generations. Similar findings were also reported by Manju *et al*. (1983) in horsegram and Mehta and Swaminathan (1975) and Singh *et al.* (2005) in berseem.

CONCLUSIONS

Genetic variation induced by mutations is more efficient as compared to natural gene pool. Due to huge fodder deficit in India, it is important to use different tools such as mutation breeding for enhancing the fodder production for a goal towards animal food security. Various phenotypic characters are improved through selection viz., plant height, tillers per plant, leaves per plant, leaf : stem ratio, trifoliate/tetrafoliate/pentafoliate leaves, small leaflets, oblong, bright green and slightly hairy. Flower head shapes and colour, days to maturity which spread from 180-210 days are spread over varying climatic conditions. Green fodder yield, dry matter yield and their per day production are the most important among them. The procedure has proved beneficial in improving these characters to a reasonable extent.

Parameters recorded in M_1 generation showed that estimates decreased linearly with an increase in dose of the chemical mutant. Maximum damage was caused when the dose was highest. For further improvement, simple selection based upon the characteristics viz., leaf : stem ratio, plant height, tillers per plant, boll size and number of seed sets was suggested.

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