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Evaluation of Genetic Variability in Berseem After Mutagenesis

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ABSTRACT

North India is the largest producer of the annual leguminous fodder crop *Trifolium alexandrinum*, often known as Berseem or Egyptian clover. The Trifolium genus has 290 species, 25 of which are important to agriculture. Berseem breeding is quite limited because of its small genetic base. After 110 years, only Wardan is the sole genotype that is still in use. This variability hurdle has been overcome using strategies like as the creation of polyploids, poly-crosses, and mutations, which have produced moderate gains in this important rabi crop. Three doses of EMS (0.1, 0.3, and 0.5%) were administered to dried seeds of Wardan, BB-2, and BB-3 in this investigation. The M1 generations were raised in the open air. Ten days after seeding, germination was noticed highest percentage. With 0.1 percent EMS, the maximum percentage of germination (85%) was seen, whereas with 0.5 percent EMS, the lowest rate of germination (12%) was observed. A total of 98 individual plants from various treatments were cut and threshed independently. Plant height, the number of tillers, leaves, and leaf-to-stem ratios, trifoliate, tetrafoliate, and pentafoliate leaves, and short, oblong, bright-green, somewhat hairy leaflets are all enhanced by selection. Variables include the 180–210 days it takes flower heads to mature under varied climatic circumstances, their shapes and colours, and other factors. The yields of dry matter, green fodder, and their daily production are the most important among them. The process has been successful in enhancing these traits.

Key words: *Berseem, Trifolium, Mutation*

Introduction

North India is the largest producer of the annual leguminous fodder crop *Trifolium alexandrinum*, often known as Berseem or Egyptian clover. It was introduced to India in 1904 and is now grown on roughly 2 million hectares of land (Mehta and Swaminathan, 1957). Kumar *et al.* (2003) in Haryana, it covers over 1.25 million hectares. There must be enough exploitable genetic variability in a crop species for it to be genetically enhanced. Berseem breeding is quite limited because of its small genetic base. There may be less genetic diversity in Berseem, which could make breeding output there less productive. Some breeding limitations, like incredibly tiny, fragile, and complicated floral structures, little outcrossing, self-fertility, and poor seed laying, may existbe other possible reasons for little genetic improvement in berseem.

After 110 years, only Wardan is the sole genotype that is still in use. This variability hurdle has been overcome using strategies like as the creation of polyploids, polycrosses, and mutations, which have produced moderate gains in this important rabi crop. Wardan underwent effective mutation breeding after receiving EMS treatment. How much genetic variety is produced by mutagenesis depends on a number of factors, including the crop, the mutagen used, the genotype, etc. Little has been done to break through the berseem variability barrier in fodder crops.

Chemical mutagens include sodium azide (SA), ethyl methane sulphonate (EMS), methyl methane sulphonate (MMS), diethyl sulphonate (DES), nitroso methyl urea (NMU), nitroso guanine (NG), and others. The outcome is affected by the length of the treatment and the dosage of the mutagen, which may be limited by unfavourable side effects. A thorough investigation into the potential impact of induced mutations caused by physical and chemical mutagens on crop development was launched in India in the middle of the 1950s (Swaminathan, 1957). The usage of mutagenic agents has dramatically increased during the last ten years (Khan *et al*., 2000; Koli and Ramkrishna, 2002; Avtar *et al*., 2005; Singh *et al*., 2005). The mutagen treatment administered to M1 may pass on gene and chromosomal alterations to succeeding generations.

The negative effects of mutagens include decreased germination percentage, root shoot length, plant survival, fertility, etc. An optimal dose is one that results in the greatest number of mutations with the fewest fatalities. The majority of specialists agree that the optimal dose should be in the vicinity of the LD50. The fatal dose (LD50) of a mutagen is the dose at which 50% of subjects would perish. LD50 varies depending on the mutagens used and the sorts of crops. The material contains all three forms of berseem: Wardan, BB-2, and BB-3.

During the rabi of 2021–2022, the experiment was conducted in the Experimental Farm of the Department of Plant Molecular Biology and Biotechnology, IGKV, Raipur. The key traits of these sorts are listed in the list below:

Ethyl methane sulphonate dosages of 0.1, 0.3, and 0.5 percent were applied to dry seeds of Wardan, BB-2, and BB-3 (EMS). Temperature and pH are the most important treatment factors because they control how a chemical mutagen behaves specifically. In the current study, various EMS dosages were made by dissolving the drug in phosphate buffer pH 7.4 in the appropriate proportions. 150 seeds per replication were immersed in an adequate concentration of freshly produced aqueous solution for 6 hours at 25°C with periodic stirring. In order to counteract the effects of the mutagens, the seeds were next gently washed under running water before being immersed for 10 minutes in a solution of sodium thiosulphate at a concentration of 2 percent. The seeds were planted in the field after the treatment. Plants from the M1 generation were laid out in the field in single rows of 5 m each, separated by 30 cm. The suggested set of methods were applied to create a high-quality crop. The following is a list of common field observation methods:

Germination: The appearance of coleoptile at the soil surface was thought to be a sign of germination. The percentage of seeds that sprouted after 10 days was a measure used to determine the germination rate.

Plant survival: It is taken as an indication that plants are maturing and beginning to set seed. The survival percentage was calculated as the proportion of all seedlings that germinated to the number of plants that survived to maturity.

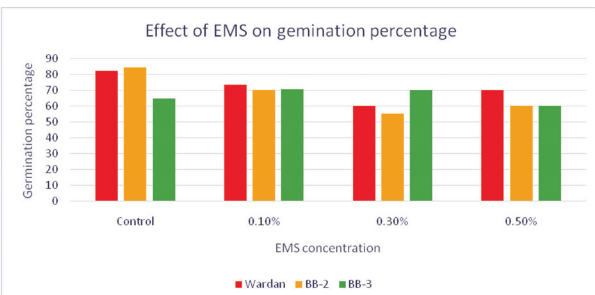
In the current investigation, different doses of the chemical mutagen EMS were applied to the seeds of the berseem cultivars Wardan, BB-2, and BB-3. For the purpose of determining the degree of genetic variability brought about by mutagenesis, the mea-

Releasingcenter	IGFRI, Jhansi	IGFRI, Jhansi	ICAR-IGFRI, Jhansi
Yearofrelease	1982	1997	2001
Features	Tolerant tobacterial disease and wilt, all India are growing region	Resistant to rootrot, stem rot and other major pest.	An autotetraploid variety which yield more fodder production.

Table 1. Effect of EMS on Germination % and plant survival

Variety	Number of seeds treated	Treatment EMS(%)	Germination	Number of plants survived	Survival%
Wardan	150	0.1%	73.33	104	94.54
	150	0.3%	60.00	87	96.66
	150	0.5%	70.00	100	95.23
	150	Control	82.00	116	94.30
BB 2	150	0.1%	70.00	108	96.22
	150	0.3%	55.33	72	91.42
	150	0.5%	60.00	86	93.33
	150	Control	84.00	121	94.42
BB 3	150	0.1%	70.66	102	92.30
	150	0.3%	70.00	96	86.74
	150	0.5%	60.00	84	95.55
	150	Control	64.66	92	96.03

surements were made on two distinct factors. Table 1 below lists the percentage of seeds that germinated and the number of plants that survived to maturity:



Graph 1. Effect of EMS on gemination percentage

According to an analysis of the data, variety Wardan produced the most germination (73.33%) at 0.1 percent EMS, while variety BB-2 produced the lowest germination (53.33%) at 0.3 percent EMS. As the mutagen dose was increased, a corresponding decrease in seed germination was observed. These findings support those that were previously published in Berseem (Anonymous, 2003, 2005, 2006; Avtar *et al.,* 2005). The reduction in seed germination was also discovered to be dose-dependent, which was corroborated by earlier findings in a number of crops (Solanki and Sharma, 1994; Yadav *et al*., 1998).

Both plant germination and survival experienced severe problems. Because mutagens affect a number of physiological processes like respiration and photosynthesis, this was also expected. The majority of the chromosomal defects caused by mutagenesis had a detrimental effect on the plant's fitness, reducing survival (Sarkar and Sharma, 1987). According to Wellen Sick's 1965 research, seed germination, survival, and fertility all suffer when exposed to various mutagens. The pattern in plant survival indicates that plant survival declined as mutagen doses rose. We gathered and threshed 98 distinct plants from various treatments. Based on phenotype, future generations will select superior plants. Similar findings were noted by Singh *et al.* (2005)

Table 2. Effect of EMS on Plant height in Berseem

Variety	Col. conc.	45 DAS	60 DAS	75 DAS	95 DAS
Wardan	Control	20.21	28.45	43.31	60.20
	0.10%	22.47	28.34	45.45	63.87
	0.30%	22.87	29.84	44.89	64.10
	0.50%	21.30	29.11	46.24	62.91
BB-2	Control	20.21	28.45	43.31	60.20
	0.10%	19.22	24.31	45.72	27.43
	0.30%	22.45	22.41	42.82	25.31
	0.50%	21.42	25.61	47.63	26.51
BB-3	Control	20.21	28.45	43.31	60.20
	0.10%	22.11	23.42	41.54	60.13
	0.30%	26.82	25.11	43.41	61.21
	0.50%	28.76	27.43	44.11	62.11

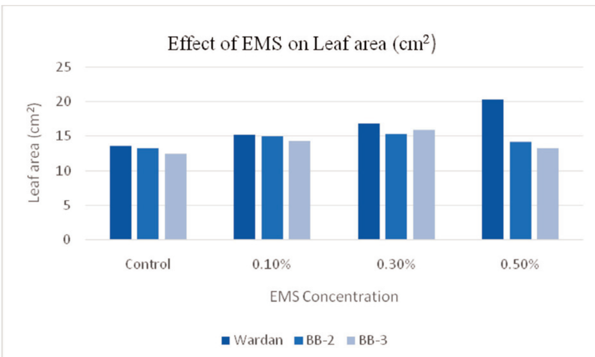
and Mehta and Swaminathan (1975) as well as Manju *et al*. (1983).

Plant growth characters

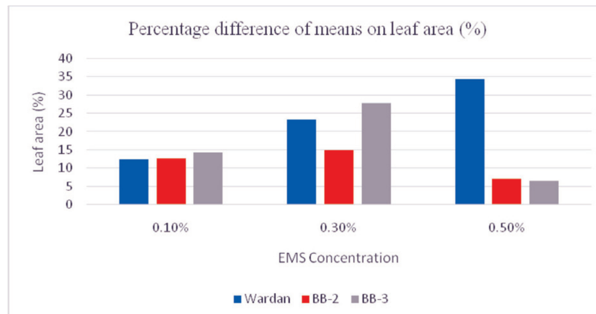
Plant height (cm): Ten guarded random plants were taken from each plot at cutting to determine the Plant height.

Leaf size and area: For the investigation of leaf size and area, periodic measurements of the top, middle, and bottom leaflets of each suspect plant were made at 45 DAS, 60 DAS, 75 DAS, and 95 DAS, or the first, second, third, and fourth, respectively. The averages were used to compute the percentage mean variant.

The highest average leaf area (cm^2) in wardan at 95 DAS was 13.61 (cm^2) for the control plant, 15.22 (cm^2) for 0.10% concentration, 16.77 (cm^2) for 0.30% concentration, and 22.30 (cm^2) for 0.50% concentration. In the BB-2 variety, the average leaf area (cm^2) for 0.10% concentration was 14.98 (cm^2), for 0.30% concentration it was 15.36 (cm^2), for 0.50% concentration it was 14.22 (cm^2), and for diploid plants it was 13.29 (cm^2). In the BB-3 variety, the average leaf area (cm^2) for 0.10% concentration was 14.24 (cm^2), for 0.30% concentration it was 15.92 (cm^2), for 0.50%



Graph 3. Effect of EMS on leaf area (cm^2)



Graph 4. Percentage difference of means on leaf area (%) of Wardan, BB-2 and BB-3

Table 3. Average values on leaf area (cm^2) of Wardan, BB-2 and BB-3

Variety	EMS Conc.	45 DAS	60 DAS	75 DAS	95 DAS
Wardan	0.10%	8.21	10.14	13.74	15.22
	0.30%	9.27	11.48	14.69	16.77
	0.50%	11.47	12.34	17.21	22.30
	Control	7.11	9.43	11.37	13.61
BB-2	0.10%	6.24	9.24	13.19	14.98
	0.30%	7.96	11.48	13.44	15.36
	0.50%	8.24	10.29	13.10	14.22
	Control	6.10	9.36	12.45	13.29
BB-3	0.10%	7.14	9.69	12.21	14.24
	0.30%	8.43	10.92	13.46	15.92
	0.50%	8.11	9.67	11.39	13.29
	Control	6.96	8.64	10.45	12.47

Table 4. Percentage difference of means on leaf area (%) of Wardan, BB-2 and BB-3

Variety	Col.conc.	45DAS	60DAS	75DAS	95DAS
Wardan	0.10%	16.47	7.52	20.84	12.48
	0.30%	30.33	21.73	29.19	23.27
	0.50%	62.32	30.85	54.36	34.32
BB-2	0.10%	2.30	-1.28	5.94	12.57
	0.30%	31.49	22.65	7.59	14.82
	0.50%	35.08	9.94	7.22	7.15
BB-3	0.10%	2.59	12.15	16.84	14.19
	0.30%	22.12	26.39	28.80	27.66
	0.50%	16.52	11.92	9.00	6.59

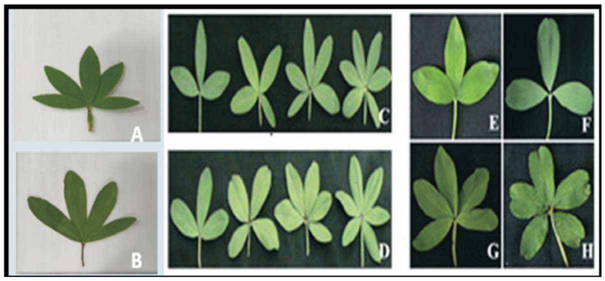


Fig. 1. Multifoliation in leaf

A: Tetraploid pentafoliate leaf
B: diploid tetraploid leaf
C: Different types of diploid leaves
D: Different types of tetraploid leaves
E: Diploid trifoliate leaf
F: Tetraploid trifoliate leaf
H: Tetraploid pentafoliate leaf

concentration and 12.47 (cm^2) for diploid plant.

Screening Based on stomatal parameters

To confirm the precipitating polyploidy of the stomatal examination, the phenotypically selected plants underwent screening. The examination covered stomatal length (m), stomatal breadth (m), and stomatal frequency for each treatment. For each of the three types, Wardan, BB-2, and BB-3, averages had been taken, and % variance of method had been calculated. Confirmed polyploids from each of the treatments (0.10%, 0.30% & 0.50%) were tested with an autotetraploid check variety, namely BB-3, and their stomatal properties compared to their wild diploid counterparts.

Stomatal length of putative tetraploid in Wardan variety the mean value was 45.32 and width it was 26.29. In BB-2 variety Stomatal length of putative tetraploid the mean value was 45.65 and width it was 28.64. In BB-3 variety Stomatal length of putative tetraploid the mean value was 46.81 and width it was 25.61.

Conclusion

When compared to the normal gene pool, genetic variety brought about by mutations is more effective. Due to India's severe fodder shortage, it is crucial to apply various strategies, including mutant breeding, to increase the supply of fodder in order to achieve animal food security. Plant height, tillers per plant, leaves per plant, leaf: stem ratio, trifoliate/ tetrafoliate/pentafoliate leaves, short leaflets, oblong, bright green, and somewhat hairy are only a few of the phenotypic characteristics that are improved through selection. Diverse climatic variables affect flower head forms and colours, as well as the 180–210-day maturation period. The most significant of these are their daily productivity, dry matter yield, and green fodder yield. The process has helped these personalities improve to a respectable degree.

Estimates dropped linearly with an increase in dose of the chemical mutation, according to parameters captured in the M1 generation. When the dose was at its peak, the most harm was done. Simple selection based on the criteria of leaf: stem ratio, plant height, tillers per plant, and quantity of seed sets was recommended for further improvement.

Conflict of Interest - None

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