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Effect of mutagen ethyl methane sulfonate on growth characters of tuberose (*Polianthes tuberosa* L.) cv. Prajwal

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Abstract
The present study was carried out at experimental orchard of Department of Horticulture Chaudhary Charan Singh Haryana Agricultural University, the treatments included five concentrations of EMS (0, 0.25, 0.5, 0.75 and 1.0%) and with three dipping durations (5minutes, 4 hours and 8 hours) resulting in 15 treatments laid out in RBD. The results showed that with treatment of bulbs with EMS the number of days taken to initiation of sprouting, complete sprouting, days to spike emergence decreased at lower concentration of EMS (0.25%) but at higher doses they delayed the initiation parameters. The lower doses (0.25% EMS) also increased plant height, number of leaves, decreased length of leaves while higher doses increased length of leaves and reduced number of leaves and plant height. The dipping duration of 8 hours was found effective to increase plant height, number of leaves and reduce length of leaves. The interaction effect was found highest in treatment combination 0.25% EMS with 8 hours dipping duration for growth parameters. The interaction effect was non-significant for days to complete sprouting, spike emergence and plant height.

Keywords: Tuberose, ethyl methane sulfonate, dipping duration, growth parameters

1. Introduction
Tuberose (*Polianthes tuberosa* L.), a perennial garden plant belonging to the family asparagaceae / agavaceae native to Mexico, is one of the most important flowers used for both cut and loose flower purpose. It is extensively cultivated in many subtropical and tropical parts of the world including India. It is native of Mexico. It is a bulbous perennial plant producing long spikes, bearing waxy white fragrant flowers, which impregnate the atmosphere with their sweet fragrance. It is a crop, which flowers profusely. Due to the long keeping quality of spikes [1] they are in great demand for making floral arrangement and bouquets.
The spikes as a whole in double types (with more than three rows of corolla segments) can be used as cut flowers, whereas, the florets of single varieties (with one row of corolla segments) are used for making garlands, veni, gajra, bangles, etc. and also for essential oil extraction. The flower yields a very valuable floral concrete (0.08-0.11%) upon solvent extraction [2]. The essential oil extracted from floral concrete of tuberose is used in the preparation of various high value perfumes and cosmetics. Tuberose (*Polianthes tuberosa* L.) is a plant with low genetic diversity and cannot be used for cross breeding. Therefore, the use of modern breeding techniques such as mutation and gene transfer are usually considered.
The agents used for induction of mutation are known as mutagens. The mutagen type comprises of Physical mutagens (X-rays, gamma rays, alpha particles and Ultraviolet radiations), DNA reactive chemicals (Nitrous acid, Dimethyl sulphate (DMS), Ethyl methane sulfonate (EMS) Ethyl ethane sulfonate (EES)), Base analogs (5-bromourcail and 5-chlorourcail), Intercalating agents (Acridine orange, ethidium bromide and proflavine) Metals (arsenic, cadmium, chromium, nickel) and Biological agents (virus, transposonand becteria). EMS among chemical mutagens is the most potent mutagen [3]. It acts as a selective mutagen and induces lethal mutations in lower frequency as compared to gamma rays. It produces random mutations in genetic material by nucleotide substitution, particularly by guanine alkylation. This typically produces only point-mutations. It can induce mutations at the rate of $5x10^{-4}$ to $5x10^{-2}$ per gene without substantial killing. The alkyl group of chemical mutagens reacts with DNA, which change the nucleotide sequence and cause a point mutation [4].

EMS alkylates are guanine bases and lead to mispairing alkylated G pairs with T instead of C, resulting in primarily G/C to A/T transitions [5]. Therefore the present investigation was carried out to the effect of EMS on growth characters of tuberose cv. Prajwal.

2. Materials and methods

The present investigation was carried out at Experimental Orchard of the Department of Horticulture, CCS Haryana Agricultural University, Hisar during 2016-17. The experimental material was bulbs of tuberose cultivar Prajwal. The plot sizes were 1.2 m X 1.2 m, and the spacing was 30X30 accommodating 16 plants each. The RDF (recommended dose of fertilizer *i.e*. 50 t/ha farm yard manure, 400 kg/ha urea, 375 kg/ha SSP and 110 kg/ha MOP) was applied in whole experimental plots before laying out plots, as recommended in package and practices of fruit flowers and vegetables, CCSHAU, Hisar [6]. The experiment was laid out in RBD with 15 treatment combinations of mutagen dose (0, 0.25, 0.50, 0.75, 1% EMS) and dipping duration (5 minutes, 4 hours and 8 hours) having three replications each which were compared to control (0% EMS with 5minuters dipping). The solution of EMS was prepared by dissolving required amount of EMS in 2 litre water and bulbs were dipped in the solution using gunny bags. Bulbs were taken out as per the dipping duration and dipped in 1% Sodium thiosulfate solution to restrict the activity of EMS, then the bulbs were taken out, dried in shade and sown in field. Days taken for initiation of sprouting were counted from the day of sowing to the day when sprouting was first observed in bulbs. Days taken for complete sprouting were calculated from the day of sowing to the day when the sixteen plants had sprouted. The plant height was measured with the help of meter scale from base of plant to the tip of the last floret and represented in centimeters (cm) and average was recorded. Length of leaves was measured when all the leaves were fully developed and no new leaf was emerging. Five leaves at representative node were measured and average length was recorded for all the five representative plants in each replication. The number of leaves was counted when all the leaves were fully developed and no new leaf was emerging. The number of leaves was counted and average was worked out for all the five selected plants in a replication. For days taken to spike emergence the number of days was counted from the day of sowing to the day when spike emergence was first seen in plant. The number of days taken to spike emergence was recorded for the five selected plants in a replication. In case where more than one spike was present, the average of the number of days taken to spike emergence was recorded. The experiment was designed in randomized block design (RBD) and replicated thrice. The data observed were subjected to statistical analysis [7]. The data were transferred from where ever required before suitability of ANOVA analyzed in statistical package SAS version 7.0.

3. Results and discussion

The treatment of tuberose bulbs with EMS significantly delayed the days taken for sprouting of bulbs as compared to control at higher doses of EMS, while lower dose decreased the number of days taken for sprouting of bulbs. The maximum delay in days taken for sprouting of corms was recorded in treatment EMS 1.0% (29.27 days). The minimum number of days taken for sprouting of bulbs was observed in bulbs treated with EMS 0.25% (27.01 days). The dipping was not found significant for number of days taken for sprouting of bulbs. The best combination of dose and dipping duration was observed for 0.25% concentration with 8 hours dipping (26.32 days), which was statistically at par with control with 8 hour dipping (26.85 days) and 0.25% EMS concentration with 4 hours dipping (27.05 days) treatment combinations. The range of days taken for sprouting of bulbs depicts that EMS treatment delayed sprouting. This might be because of the fact that above optimum level EMS could have formed certain toxic substances, which caused death of cells or delayed cell division, which in turn caused delayed sprouting at higher doses as well as number of days taken for complete sprouting. The results of present study are in conformity with the findings of [8] in Duch iris, [9] in gladiolus and [10] in gladiolus, who reported that lower doses of EMS enhanced sprouting, while higher doses delayed it.

Table 1: Effect of different concentration of EMS and dipping duration in EMS solution on number of days taken for initiation of sprouting in tuberose cv. Prajwal.

Concentration of EMS (%)	Dipping duration			Mean
	5 min	4 hours	8 hours	
0	27.92	27.92	26.85	27.56
0.25	27.67	27.05	26.32	27.01
0.50	28.04	28.37	28.74	28.33
0.75	28.15	28.58	29.24	28.66
1.0	28.79	29.16	29.87	29.27
Mean	28.11	28.28	29.24	
C.D. (P = .05)	Concentration (C)	Dipping time (T)	Concentration x dipping time	
	0.43	NS	0.74	

The data in Table 2 depicted that the treatment of tuberose bulbs with EMS significantly delayed the days taken for complete sprouting of bulbs as compared to control at higher doses of EMS, while lower dose decreased the number of days taken for complete sprouting of bulb. The maximum delay in number of days taken for complete sprouting of bulbs was recorded in treatment 1.0% EMS (41.22 days), which was found statistically at par with EMS 0.75% (38.78 days) and 0.50% EMS (38.44 days). The minimum number of days taken for complete sprouting of bulbs was observed in bulbs treated with 0.25% EMS (36.22 days), which was found at par with control (37.11 days), EMS 0.50% (38.44 days) and EMS 0.75% (38.28 days). The effect of dipping and interaction effect was found non-significant. The range of days taken for sprouting of bulbs depicts that EMS delayed sprouting.

Table 2: Effect of different concentration of EMS and dipping duration in EMS solution on number of days taken for complete sprouting in tuberose cv. Prajwal.

Concentration of EMS (%)	Dipping duration			Mean
	5 min	4 hours	8 hours	
0	37.67	37.00	36.67	37.11
0.25	36.67	36.00	36.00	36.22
0.50	37.67	38.67	39.00	38.44
0.75	38.00	38.00	40.33	38.78
1.0	38.67	41.33	43.67	41.22
Mean	37.73	38.2	39.13	
C.D. (P = .05)	Concentration (C)	Dipping time (T)	Concentration x dipping time	
	2.90	NS	NS	

Data presented in table 3 indicates that maximum plant height was recorded in treatment EMS 0.25% (85.62 cm) and the minimum plant height was observed in bulbs treated with EMS 1.0% (82.3 cm), which was found at par with EMS 0.75% (82.61 cm). The maximum height was recorded for 5 minutes dipping (84.73 cm) and the minimum plant height was observed for 8 hours dipping (83.06 cm). The interaction effects were found non-significant. The perusal of range of plant height depicts that EMS reduced the plant height. The control and EMS 0.25% concentration treatments had almost similar trend of days taken for complete sprouting and plant height. Stimulatory effects of lower concentrations of chemical mutagens have been reported by various workers [11] in tobacco and [12] in onion. On the other hand, increase in the dose of chemical mutagens caused damaging effects on biological activities of plants which might be due to inactivation of cells because of mitotic disturbances or chromosomal aberrations at higher doses of EMS, leading to poor growth of the plants [12]. The higher concentration of EMS results in dwarfing of the plant. The higher concentration of EMS results in dwarfing of the plant. The mutation might be attributed to a drop in auxin level [13], inhibition of auxin synthesis [14], chromosomal aberrations [15] or due to the decline of assimilation mechanism [16]. The mutagens particularly EMS affects the auxin synthesis in plants.

Table 3: Effect of different concentration of EMS and dipping duration in EMS solution on plant height (cm) in tuberose cv. Prajwal.

Concentration of EMS (%)	Dipping duration			Mean
	5 min	4 hours	8 hours	
0	85.04	85.17	84.47	85.01
0.25	86.61	85.69	84.55	85.62
0.50	84.82	84.21	83.18	84.07
0.75	83.2	82.83	81.81	82.61
1.0	83.04	82.74	81.11	82.30
Mean	84.73	84.21	83.06	
C.D. (P = .05)	Concentration (C)	Dipping time (T)	Concentration x dipping time	
	0.48	0.37	NS	

The data in Table 4 indicates that the treatment of tuberose bulbs with EMS significantly increased the length of leaves as compared to control at higher doses of EMS. The maximum increase in length of leaves was recorded in treatment EMS 1.0% (33.37 cm). The minimum length of leaves was observed in control (30.97 cm). The maximum in leaf length was found in dipping duration of 4 hours (33.22 cm) and the minimum length of leaf was observed with 5 minutes dipping (31.32 cm). The maximum leaf length was observed with EMS 1.0% treatment for 4 hours dipping (34.19 cm), which was statistically at par with 0.75% concentration with 4 hour dipping (33.85 cm) and the minimum length of leaves, was observed in control with 5 minutes dipping (28.8 cm). A decrease in the length of leaves was obtained with the increase in dose of mutagen has been reported by [17] in tuberose and [18] in *Dendrobium senile.* Mostafa *et al.* [19] also reported in *Celosia argentea* that the plants treated with higher concentrations of dimethyl sulphate (3000 and 4000 ppm) resulted in higher number of leaves in both VM_1 and VM_2 generations.

Table 4: Effect of different concentration of EMS and dipping duration in EMS solution on length of leaves (cm) in tuberose cv. Prajwal.

Concentration of EMS (%)	Dipping duration			Mean
	5 min	4 hours	8 hours	
0	28.8	31.42	32.7	30.97
0.25	30.21	33.09	31.47	31.59
0.50	32.12	33.55	31.78	32.48
0.75	32.45	33.85	31.99	32.76
1.0	33.03	34.19	32.88	33.37
Mean	31.32	33.22	32.16	
C.D. (P = .05)	Concentration (C)	Dipping time (T)	Concentration x dipping time	
	0.32	0.25	0.54	

The data in Table 5 conceals that the treatment of tuberose bulbs with EMS significantly decreased the number of leaves per clump as compared to control at higher doses of EMS. The maximum increase in number of leaves per clump was

recorded in treatment EMS 0.25% (48.60). The minimum number of leaves per clump was observed in treatment EMS 1.0% (36.44). The maximum number of leaves per clump was reported in dipping duration of 4 hours (42.70) and the minimum number of leaves per clump was observed with 5 minutes dipping (40.74). The best combination of dose and dipping duration was observed with 0.25% concentration with 8 hours dipping (53.04). The minimum number of leaves per clump was observed in control with EMS 1.0% with 8 hours dipping (35.03) treatment combination. The range of the number of leaves per clump depicts that EMS increased the number of leaves per clump up to 0.25% concentration and then decreased. The results of this study are supported by [20] in gladiolus, who reported the decrease in number of leaves at higher doses of EMS, which might be due to activation of physiological substances present in corms at lower doses, while higher doses retard cell division by arresting mitotic cell division and causing ill effects on auxins. Moderate doses of EMS showed stimulatory effect on growth of some vegetative characters. It might also be due to the increased activity of enzymes involved in biosynthesis of hormones like gibberllins, cytokinins, *etc*. in cell at lower doses of mutagen [21] studied in gladiolus. Decrease in the number of leaves per plant with increase in dose of mutagen has been also reported by [17] in tuberose and by [18] in *Dendrobium senile* and [19] in C*elosia argentea.*

Table 5: Effect of different concentration of EMS and dipping duration in EMS solution on number of leaves in tuberose cv. Prajwal.

Concentration of EMS (%)	Dipping duration			Mean
	5 min	4 hours	8 hours	
0	38.50	42.13	43.81	41.48
0.25	44.06	48.70	53.04	48.60
0.50	44.38	47.89	42.23	44.83
0.75	39.05	38.16	36.87	38.03
1.0	37.70	36.60	35.03	36.44
Mean	40.74	42.70	42.20	
C.D. (P = .05)	Concentration (C)	Dipping time (T)	Concentration x dipping time	
	0.45	0.35	0.78	

Treatment of tuberose bulbs with EMS significantly delayed the days taken for spike initiation as compared to control at higher doses of EMS.

The maximum delay in spike initiation was recorded in treatment EMS 1.0% (104.81 days), which was found statistically at par with EMS 0.75% (104.19 days). The minimum number of days taken for spike initiation was observed in bulbs treated with EMS 0.25% (101.45 days). The effect of dipping duration and interaction effects with different dipping EMS concentrations were found non-significant. The perusal of range of days taken for spike initiation depicts that EMS delayed spike initiation. The delay in spike initiation due to EMS treatment has been reported earlier by [22] in *Dianthus* they reported that days taken to spike initiation increased with the increase in doses of EMS. Similar result was also observed by [23] in gladiolus who observed that delay in flowering might be due to disturbances in biochemical pathway, which assisted in synthesis of flower inducing substances. This might be because of that the above optimum level of EMS could have formed certain toxic substances, which caused death of cells or delayed cell division, which in turn caused delayed sprouting at higher doses as well as number of days taken for complete sprouting. The results of present study are in conformity with the findings of [8, 9, 10] in gladiolus, who reported that lower doses of EMS enhanced sprouting, while higher doses delayed it.

Table 6: Effect of different concentration of EMS and dipping duration in EMS solution on number of days taken for spike emergence in tuberose cv. Prajwal

Concentration of EMS (%)	Dipping duration			Mean
	5 min	4 hours	8 hours	
0	103.30	102.36	102.28	102.64
0.25	102.60	101.35	100.42	101.45
0.50	102.30	102.43	103.20	102.64
0.75	103.58	104.28	104.72	104.19
1.0	103.36	104.60	105.48	104.81
Mean	103.23	103	103.22	
C.D. (P = .05)	Concentration (C)	Dipping time (T)	Concentration x dipping time	
	1.05	NS	NS	

4. Conclusion

Various mutagens are present for induction of mutation but chemical mutagens such as EMS, MMS, DES, colchicine etc are preferred due to ease of treatment and effectiveness. EMS is better over other mutagens due to its high penetration power and lower frequency of inducing lethal mutations. Treatment of bulb at higher dose of EMS and longer dipping duration have inhibitory Effect on growth parameters while lower dose 0.25% with higher dipping duration had stimulatory effect on growth parameters. This effect might be due to destructive effect of EMS at higher dose and increased absorption of EMS with increase in dipping duration, while lower doses have been reported to have stimulatory effects and help in auxin synthesis. Therefore 0.25% EMS concentration and dipping duration of 8 hours could be suggested for enhancement of growth characters of tuberose such as reduction of days taken to initiation of sprouting, complete sprouting, increased plant height, more number of leaves, reduced length of leaves and early spike emergence.

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