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Induction of variability in gladiolus (*Gladiolus grandiflorus* L.) by chemical mutagens

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ABSTRACT

The present study was conducted to see the effect of chemical mutagens on vegetative growth, flowering, corm and cormels attributes of gladiolus var. Rani Sahiba. Chemical dosages of DES and EMS ranging from 0.1 % to 0.5 % were used. Lower doses of DES *viz.*, 0.2 % proved better as compared to EMS and control in terms of minimum days required for spike initiation and first floret opening with maximum spike length, rachis length, floret diameter, number of spikes per plant and weight of cormels per plant. Moreover, one mutant was isolated and selected on the basis of colour with desirable characteristics from this treatment. Significantly higher number of leaves, number of florets per spike and vase life was recorded in plants received treatment of 0.3 % EMS. Maximum plant height was found better in 0.1 % DES while, maximum number of cormels per plant was recorded in 0.4 % DES.

Key words: *Chemical, DES, EMS, Mutation, Mutant*

Introduction

Floriculture is a rigorous and lucrative art and science that involves cultivating flowers to greatness. The Indian floriculture industry has indeed attained the status of a huge opportunity sector, owing to improved living standards, burgeoning environmentalism, ordinarily shifting consumer orientation for flowers based on ethnicity, shape, size, shelf life, and other factors, demand for new ornamentals and a flourishing landscape industry. Gladiolus is one of the most important commercially valuable cut flowers in both the national and international markets. As the demand for gladiolus is rising, it is essential to focus on genetic improvement. Any crop's potential to improve is ascertained by the natural variability available to it. Among all breeding techniques of crop improvement, mutation breeding is very effective specially in flower crops. Using various mutagens, mutation breeding has been effectively applied to a variety of crops. Despite the development of tools for new variety generation, induced mutagenesis and mutant induction by the use of physical and chemical mutagens remains an important aspect of ornamental breeding. Chemical mutagenesis is an effective and simple method for obtaining valuable starting material for plant breeding using ethyl methyl sulphonate, diethyl sulphate, dimethyl sulphate, azide and N-nitroso compounds *etc*. which are able to induce a high frequency of non-lethal point DNA mutations and generate novel genetic diversity in various crops. Among chemical mutagens, ethyl methyl sulphonate (EMS) and diethyl sulphonate (DES), an alkylating agents, are the most

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frequently used in various flower crops and other crops, as they can cause a high frequency of nucleotide substitution variation and has a potential to generate many new mutants with desirable characters. The keeping importance of crop and method of crop improvement in mind, present investigation was conducted and the emphasis was laid on finding out desirable variation by chemical mutagen.

Materials and Methods

The present experiment was carried out at Floriculture Research Farm, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, during winter season of year 2020-21. The experiment was laid out in randomized block design (RBD) and replicated thrice. Gladiolus corms were treated with different concentrations of chemical mutagens *viz.,* DES (0.1 %, 0.2 %, 0.3 %, 0.4 % and 0.5 %) and EMS (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) by immersing in the chemical solutions for 4 hours. After the treatments, these corms were washed in running tap water for 20 minutes. In control, corms were soaked in water for 4 hours.

Results and Discussion

Vegetative growth parameters

The data on different vegetative characters are presented in Table 1. The data revealed that significantly minimum days required for sprouting (5.79) was observed in T_{11} (control) while, maximum days required for sprouting (7.55) was noted in 0.5 % DES (T_5). Early sprouting at lower doses of mutagenic treatments might be because of the lower concentration of mutagens that help enzymes to set free and cause stimulation in growth and the stimulatory effect of chemical mutagens at lower concentrations recorded for early sprouting agreed completely with the findings of Pooja Kaintura *et al.* (2016) and Yadav *et al.,* (2018) in tuberose. Number of sprouts per corms was recorded highest (1.59) at 0.1 % DES while, lowest number of sprouts (1.10) was recorded in T_6 (0.1 % EMS). Increase in number of sprouts per corm might be due to the enhancement in activities of gibberellins and auxins and disappearance of inhibitors with treatments of mutagens. It is also known that amylolytic activity is shown by germinating corms due to pre-treatment with chemical mutagens (Moustafa *et al.,* 2018). These findings are in conformity with Pooja Kaintura *et al.* (2016) in tuberose, Sudha Patil *et al.* (2017) and Ayesha Manzoor *et al.* (2018) in gladiolus. In case of sprouting percentage, it was recorded that significantly maximum sprouting (98.67 %) was observed in T_{-11} (control) whereas, minimum sprouting percentage (71.00) was observed at 0.1 % EMS. It might also be due to the damage of cell material and other cell constituents at molecular level leading to breaks, physiological injuries and ultimately stopping the metabolic activity of the cells resulting in the reduction of sprouting percentage (Kumar *et al.,* 2013). Similar findings were reported by El-Tayeb (2014) in

Table 1. Effect of chemical mutagens on vegetative growth parameters of gladiolus var. Rani Sahiba

Treatments	Number of days required for sprouting	Number of sprouts per corms	Sprouting (%)	Percentage of survival	Plant height (cm)	Number of leaves per plant	Length of leaf (cm)	Width of leaf (cm)
T_1: DES @ 0.1 %	5.92	1.59	90.83	77.50	72.13	8.88	46.42	2.89
T_2: DES @ 0.2 %	5.86	1.34	91.67	85.83	67.45	7.30	47.01	3.10
T_3: DES @ 0.3 %	5.97	1.30	88.50	84.83	65.19	7.07	50.90	2.95
T_4: DES @ 0.4 %	6.13	1.25	83.67	80.83	64.34	6.85	48.06	2.86
T_5: DES @ 0.5 %	7.55	1.20	82.50	75.83	61.16	6.85	45.19	2.81
T_6: EMS @ 0.1 %	6.30	1.10	71.00	64.83	64.46	6.27	45.78	2.73
T_7: EMS @ 0.2 %	6.24	1.24	82.00	77.50	67.41	6.41	49.27	2.84
T_8: EMS @ 0.3 %	6.05	1.30	90.00	83.33	68.51	7.00	47.18	2.91
T_9: EMS @ 0.4 %	5.96	1.28	89.17	83.33	63.01	6.92	46.88	2.89
T_{10}: EMS @ 0.5 %	6.15	1.26	83.33	79.17	54.73	6.90	46.14	2.80
T_{11}: Control	5.79	1.33	98.67	96.83	64.01	6.92	46.91	2.88
S.Em. ±	0.25	0.08	4.29	4.23	2.86	0.37	2.70	0.25
C.D. at 5 %	0.74	0.23	12.65	12.47	8.43	1.09	NS	NS
C.V.%	7.05	10.53	8.59	9.05	7.65	9.10	9.90	14.89

gladiolus and Kayalvizhi *et al.* (2016) in tuberose. Similarly maximum survival of plants was also observed in T_{11} (control) *i.e.* 96.83 % while, minimum survival (64.83 %) was noted in 0.1 % EMS. The decrease in percentage survival of plants at higher doses of the mutagens is attributed to disturbances at cellular level including chromosomal damages (Priyanka Tirkey and Devi Singh, 2019). These findings are in conformity with Dilta (2003) in chrysanthemum and Amanda Berenschot *et al.* (2008) in petunia. Significantly maximum plant height was attained (72.13 cm) by plants of T_1 (DES @ 0.1 %) but plants remained dwarf (54.73 cm) in T_{10}(0.5 % EMS). The increase in the growth at higher doses of DES might be because of the reason that alkylating agents are unstable, where certain chemical mutagens produce single base substitutions with different mutation spectra because of which broad variation occured (Abdullah *et al.,* 2009). These findings are in conformity with Schiva *et al.* (1984) in gerbera and Dilta (2003) in chrysanthemum. Maximum number of leaves per plant (8.88) were observed in T_1 (DES @ 0.1 %) while least number of leaves per plant (6.27) was produced in 0.1 % EMS. Increase in number of leaves with increase in doses upto certain level, refers to the ability of mutagens to overcome the apical dominance and hence, stimulate the lateral buds to develop into new leafy shoots (Vainola, 2000). These findings are in conformity with Yasser and Nashar (2012) in calendula and Ghani *et al.* (2013) in gerbera.

Flowering parameters and isolated mutant

Table 2 shows the findings on distinct flowering characteristics. Minimum days for spike initiation (50.06) and first floret opening (58.25) were observed in T_2 (0.2 % DES) while maximum days to spike initiation (66.99) and first floret opening (76.65) were recorded by plants grown in T_{11} (control). Increase in doses upto certain level induced early spike initiation, which probably due to absorption of more nutrients at lower doses and thereby higher amount of photosynthesis occurs which ultimately resulted in early sprouting, good growth and hence, early spike emergence and flowering (Cantor and Korosfoy 2002). Early flowering at lower doses of chemical mutagens may be because of physiological changes which occur in plant and delayed flowering occur at higher doses due to inhibitory effect, it might also be due to reduction in rate of physiological processes which assists in synthesis of flower inducing substances (Moustafa *et al.*, 2018). These findings are in conformity with Nashar and Asrar (2016) in calendula, Pooja Kaintura *et al.* (2016) in tuberose. Length of spike and rachis length were observed significantly maximum in T_2 (0.20 % EMS) *i.e.* 94.17cm and 47.01 cm, respectively while, minimum spike length was observed in T_1 (0.1 % DES), *i.e.* 71.35 cm and rachis length was noted down minimum (35.92 cm) in T_5 (DES @ 0.5 %). Moreover, according to results obtained on vegetative parameters, it was observed that lower concentrations of both the mutagens had

Table 2. Effect of chemical mutagens on flowering parameters of gladiolus var. Rani Sahiba

Treatments	Number of days required for spike initiation	Number of days required for first floret opening	Rachis length (cm)	Number of florets per spike	Floret diameter (cm)	Number of spikes per plant	Vase life (days)
T_1: DES @ 0.1 %	58.75	68.17	38.87	10.07	7.28	1.20	6.20
T_2: DES @ 0.2 %	50.06	58.25	47.01	11.53	8.41	1.36	7.27
T_3: DES @ 0.3 %	57.40	67.66	43.86	10.82	8.25	1.22	6.00
T_4: DES @ 0.4 %	59.93	68.81	37.83	10.39	7.45	1.12	5.47
T_5: DES @ 0.5 %	60.56	70.34	35.92	9.36	7.39	1.10	5.87
T_6: EMS @ 0.1 %	59.82	68.73	37.44	10.78	7.17	1.06	5.93
T_7: EMS @ 0.2 %	59.67	67.53	43.53	11.15	7.59	1.18	6.13
T_8: EMS @ 0.3 %	56.65	65.03	44.81	12.75	8.20	1.28	7.60
T_9: EMS @ 0.4 %	58.82	67.08	39.11	10.76	7.44	1.20	6.47
T_{10}: EMS @ 0.5 %	59.81	68.45	37.21	10.29	7.39	1.14	6.27
T_{11}: Control	66.99	76.65	37.56	9.65	7.32	1.18	6.27
S.Em. ±	2.52	2.81	1.97	0.51	0.33	0.06	0.33
C.D. at 5 %	7.44	8.28	5.82	1.50	NS	0.16	0.98
C.V.%	7.41	7.16	8.49	8.22	7.47	8.06	9.09

positive effect while detrimental effect was observed in plants treated with higher concentrations. These findings are in line with the findings of Archana Bhajantri and Patil (2013) in gladiolus, Singh *et al.* (2015) in tuberose. Number of florets per sike was observed significantly maximum (12.75) in T_8 (EMS @ 0.3 %) while minimum number of florets per spike (9.36) was recorded in corms treated with 0.5 % DES (T_5). Mutagenic treatment led to auxin destruction, irregular auxin synthesis, failure of assimilation, mechanisms or inhibition of mitotic and chromosomal changes or damage with association of secondary physiological damage which in turn reduced number of florets per spike and these findings are in line with the findings of Priyanka Tirkey and Devi Singh (2019) in gladiolus and Ghormade *et al.* (2020) in chrysanthemum. Moreover, significantly maximum number of spikes per plant (1.36) was recorded in corms under 0.2 % DES (T_2) while minimum number of spikes per plant was produced in T_6 (0.1 % EMS), *i.e.* 1.06. Increase in number of spikes per plant was observed with increase in dosage of DES and EMS but decreased from 0.3 % DES and 0.4 % EMS, respectively. Wide variations observed in terms of production due to chemical mutagens. The alkylating agents are highly unstable which are responsible for sudden changes which produce single base substitutions with different mutation spectra due to which broad variation occurs as compared to control (Khan *et al.*, 2009). These findings are in line with the findings of Sudha Patil *et al.* (2017) in gladiolus and Samatadze *et al.* (2019) in calendula. Vase life was recorded significantly maximum (7.60 days) in spikes produced in T_8 (EMS @ 0.3 %) while, minimum vase life (5.47 days) was observed in T_4 (DES @ 0.4%).

Mutagenesis is a useful tool for genetic improvement of plant varieties and ornamentals where important traits have been changed without disturbing the whole genotype and may show significant effects on improving shelf life and vase life (Khan *et al.*, 2009). The results are in conformity with Sedaghathoor *et al.* (2017) in tulips and Dhawani Patel *et al.* (2018) in gladiolus. One desirable mutant of gladiolus var. Rani Sahiba was isolated from 0.2 % DES mutagenic treatment. (Fig.1). Change in

Fig. 1. Isolated mutant from T_2: (0.2% DES) mutagenic treatment

T_{11}: Control (Original flower of var. Rani Sahiba)

Mutant of var. Rani Sahiba (White markings on florets)

Table 3. Effect of chemical mutagens on corm and cormels parameters of gladiolus var. Rani Sahiba

Treatments	Number of corms per plant	Diameter of corm per plant (cm)	Weight of corms per plant (g)	Weight of cormels per plant (g)
T_1: DES @ 0.1 %	1.27	6.29	71.22	8.55
T_2: DES @ 0.2 %	1.40	5.90	64.34	9.65
T_3: DES @ 0.3 %	1.52	5.75	59.04	8.48
T_4: DES @ 0.4 %	1.77	5.85	73.66	7.58
T_5: DES @ 0.5 %	1.27	5.73	63.22	3.69
T_6: EMS @ 0.1 %	1.30	5.50	54.21	6.09
T_7: EMS @ 0.2 %	1.43	5.88	64.18	6.57
T_8: EMS @ 0.3 %	1.40	6.70	72.14	7.05
T_9: EMS @ 0.4 %	1.33	5.89	71.73	8.66
T_{10}: EMS @ 0.5 %	1.13	5.76	62.35	5.84
T_{11}: Control	1.70	7.07	83.07	7.09
S.Em. ±	0.10	0.27	5.25	0.42
C.D. at 5 %	0.31	0.81	15.48	1.24
C.V.%	12.79	7.83	13.53	10.13

flower colour might be due to quantitative and/or qualitative changes in the colour pigments caused by mutations in their biosynthetic pathways or induced at independent loci that control flower colour (Ruminska and Zalewska, 2005).

Corm and Cormel parameters

The data on different vegetative characters are presented in Table 3. Significantly maximum number of corms per plant (1.77) was produced by corms treated with 0.4 % DES (T_4) while, minimum number of corms per plant (1.13) was obtained in T_{10} (EMS @ 0.5 %). Diameter of corm (7.07 cm) and weight of corms per plant (83.07 g) were recorded maximum in T_{11} (control) while minimum corm diameter (5.50 cm) and corm weight (54.21 g) were observed in T_6 (EMS @ 0.1 %). Moreover, weight of cormels per plant (9.65 g) was recorded significantly maximum in T_2 (0.2 % DES) while minimum weight of cormels per plant (3.69 g) was observed in $T\text{-}_5$ (0.5 % DES). These parameters increased may be due to increase in the enzyme level which activates metabolism of cells responsible for translocation of source to sink (Rajadurai, 2001). Similar results were found byEl-Tayeb (2014) in Gladiolus, Pooja Kaintura *et al.* (2016) in tuberose and Dhawani Patel *et al.* (2018) in gladiolus.

Conclusion

Different concentrations of chemical mutagens like diethyl sulphonate and ethyl methyl sulphonate could significantly influence the various vegetative, flowering and corm and cormels parameters of Gladiolus var. Rani Sahiba. Among all the treatments, 0.2 % DES (T_2) recorded significantly minimum number of days required for spike initiation and first floret opening along with maximum length of spike, rachis length, floret diameter, number of spikes per plant and weight of cormels per plant. Moreover, one mutant was also isolated from this treatment. On the basis of the results obtained from the investigation, it can be concluded that 0.2 % DES recorded desirable and better results over other treatments.

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