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Effect of induced mutagenesis on different characters of gladiolus (*Gladiolus grandifloras* L.)

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Abstract

A field experiment was conducted at Department of Horticulture, Sam Higginbottom University of Agriculture, Technology & Science, Prayagraj, India during the year 2016-17. The experiment was laid out in randomized block design on traits of gladiolus traits of gladiolus 'White Prosperity', bulbs were induced by gamma rays, Dry Ethyl Methane Sulphonate (DEMS) and soaked Ethyl Methane Sulphonate (SEMS). The treatments consisted of 2 Kr, 4 Kr, 6 Kr, 8 Kr, 10 Kr and 12 Kr of gamma rays; 0.25%, @ 0.5%, @ 0.75%, @ 1.0% and @ 1.25% of DES; 0.25%, @ 0.5%, @ 0.75%, @ 1.0% and @ 1.25% of EMS and control (untreated).. Different concentrations of EMS and different level of gamma radiations had a little effect on sprouting and survival percentage but it significantly delayed the emergence of sprouts. About one third decreases in plant height along with reduction in number of leaves per plant, leaf area, length and width, diameter and number of cormles per corm was observed in treated plants. In general, the treated population had manifested reduced expression than the control (untreated population) for most of the characters. Higher the dose of mutagens, lower was the expressivity of the traits. After the observation it found that maximum plants height was observed in control while minimum height observed in the treatment with Gamma Rays 12Kr while maximum number of leaves and number of shoot per corm were recorded in treatments with Gamma Rays 2Kr and SOAKED EMS @ 0.25% respectively. The gladiolus plants could not survive in treatments of gamma rays and its combination with EMS. On the basis of above observations, it may be concluded that 0.25 to 0.50 per cent dry and soaked EMS doses are suitable for inducing mutation in gladiolus.

Keywords: Gladiolus, gamma rays mutagenesis, dry ethyl methane Sulphonate (DEMS) and soaked ethyl methane Sulphonate (SEMS)

Introduction

Gladiolus (*Gladiolus Grandifloras* L.) belong to family to Iridaceae. It is native to South Africa the queen of the bulbous ornamentals, is the leading geophytes grown worldwide for cut flower trade and garden displays. Among the commercial Gladiolus is one of the most important flower in India because of its majestic spike containing attractive elegant and delicate florets of various shades sequential opening of flower for longer duration and good keeping qualities of cut flowers. Gladiolus needs attention to word genetics improvement as the demand of flowers is increasing continuously since garden varieties of today came from diverse genetic percentage that are hetroplids ranging from $2n= 30$ to 180 and hipoanuploids so that we production by seedin this case no meaning maintain the varietal identy but for evaluation of new forms. Due to its heterozyjosity in genetics constitution this makes it promising test material for introduction mutagenesis were only one or a few characters are to be improved upon without changing the entire genotype offers promising pose bilities. gladiolus grown vegetativey to perfection and so mutation breeding offers great potentialities as the mutated part can be conveniently perpetuated by vagatative means resulting in the devolopement of new from.

Mutation is a natural process which creates changes in DNA sequences. The genetic variation created is useful because it helps population to survive and change over time. Similarly, experimental mutagenesis permits to increase possibilities of creation of variability with high ornamentation (Cantor *et al.*, 2002). Induced mutation with gamma radiation and chemical mutagens in ornamental plants have been used for genetic changes, high flower yield, disease resistance, early maturity, etc. Gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cell and tissues (Abdullah *et al.*, 2009)^[1]. Ethyl methane sulphonate is a very potent mutagen which adds its alkyl group to bases in DNA, causes a mistake and produces point mutations. The effects of gamma rays on gladiolus have been studied by several workers but very few varieties have been developed through gamma radiation. Hence, in the present investigation, emphasis was laid on finding out variation caused by gamma radiation in morphological characters including colour variation in succeeding generation.

Materials and Methods

The research work was carried out under the Department of Horticulture, Allahabad School of Agriculture, Sam Higginbottom University of Agriculture, Technology and Sciences Prayagraj, during the year 2016-2017. Therefore, the present study has been carried out to study the effect of EMS and gamma rays on various morphological characters of gladiolus with the objective of creating genetic variations in the subjected plant material. The main objectives of the studies are to develop new varieties through mutations using chemical and physical mutagens and to study the effect of induced mutagenesis on different characters of gladiolus crop. The corms of uniform size (2.5 cm to 4.0 cm) used for treatment with one physical mutagen (gamma ray) and two chemical mutagens *viz.*, Dry Ethyl Methane Sulphonate (DEMS) and soaked Ethyl Methane Sulphonate (SEMS) were used in the study. Gamma irradiation and treated with gamma rays in the gamma chamber with six different irradiation level (2 Kr, 4 Kr, 6 Kr, 8 Kr, 10 Kr and 12 Kr) and then planted in the beds with a spacing of row to row 30 cm and corm to corm 20 cm during November to March 2016. Ethyl Methane Sulphonate (CH₃ SO₂ OC₂ H₂) procured from M/s. Sigma-Aldrich Company, U.S.A was used. It has a Molecular weight-124.16, boiling point of 80/100 mm Hg and Density of D⁴²⁵ = 1.203 g/ml). It was stored in dry air at 0 °C to maintain its purity. Prior to use, it was removed from refrigerator and placed in a desiccator with calcium chloride until it reached room temperature. The Gladiolus (*Gladiolus Grandifloras* L.) with white prosperity variety was grown with each comprising of treatments replicated thrice. Source of variables were Gamma irradiation level (2 Kr, 4 Kr, 6 Kr, 8 Kr, 10 Kr and 12 Kr); Dry Ethyl Methane Sulphonate (DEMS) (@ 0.25%, @ 0.5%, @ 0.75%, @ 1.0% and @ 1.25%); soaked Ethyl Methane Sulphonate (SEMS) (@ 0.25%, @ 0.5%, @ 0.75%, @ 1.0% and @ 1.25%); and combination of them with control treatment taken as fifty one treatments. The treated corms were planted in the well prepared beds of size 1 x 1 m² at a spacing of 25 x 25 cm and 5-7 cm deep in soil in Randomized Block Design (RBD) in replicates. Before planting the corms were dipped in 0.2 percent bavistin for 30 minutes and air dried in shade. Standard package of practices were followed accordingly. For collection of morphological data, 16 plants in a lot per replication were observed and average was calculated. Various growth parameters such as duration of sprouting, sprouting percentage, plant height, floral characters, length of spike, corm weight, diameter, etc. were calculated and analyzed statistically at different period of time.

Results and Discussion

The EMS treatment and gamma radiation significantly affected the various morphological characters of gladiolus *Gladiolus grandifloras* L. (white prosperity). The results obtained from the experiment are discussed below:

Effect on vegetative parameters of gladiolus

The data in Table 1 revealed that the treatment of corms with gamma rays, SEMS and DEMS significantly delayed the days taken for sprouting of corms as compared to control (13.86 days). The maximum delay was recorded in T₆ (24.00 days) which was at par with T₅ (23.33 days), T₄ (22.21 days) and T₃ (21.69 days). The minimum days taken for sprouting of corms were observed in corms in control (13.86 days) followed by T₁₂ (14.78 days), and T₁₃ (15.33 days). The treatment of

corms with gamma rays, SEMS and DEMS decreased the sprouting percentage of corms as compared to control. The results in the present study are in conformity with the findings of Rather *et al.* (2002)^[15], Srivastava *et al.* (2007)^[20], Kumar *et al.* (2012)^[12] and Berenschot *et al.* (2008)^[2]. Khan and Tyagi (2009)^[9] attributed the effects of mutagens on the meristematic tissues. The decrease in sprouting at higher doses of the mutagens may also be attributed to disturbances at cellular level including chromosomal damages or due to the effect of both.

Maximum sprouting percent (98.83% in 2016-17) recorded in T₀ (control) and minimum sprouting percent (75.90 in 2016-17) in were observed with T₆ (Gamma rays 12kr).

Maximum Plant height (98.88 cm in 2016-17) recorded in T₀ (control) and minimum Plant height (91.98 cm in 2016-17) were observed with T₆ (Gamma Rays 12Kr) at 120 DAP. Similar findings were reported by Datta (1990a) in ornamentals. The reduction in plant height could also be attributed to the inhibition of growth due to low rate of cell division, decreased amylase activity and increased peroxidase activity. Besides this, production of diffusible growth retarding substances, inhibitory action of enzymes concerned with the initial growth processes, delay in the onset of first meiosis (Natarajan *et al.*, 1982 in red gram), change in the specificity of enzymes, inhibition of DNA synthesis (Mickaelson, 1968) and reduction of IAA have also been attributed as reasons for the retardation of plant height.

Maximum number of leaves (10.23 in 2016-17) recorded in T₁ (Gamma Rays 2Kr) and minimum number of leaves (8.15 in 2016-17) were observed with T₁₆ (Soaked EMS @ 1.25%). Brock (1965, 1967) in clover and *Arabidopsis thaliana* proposed that in random mutations of characters with definite selection history, each character had varying levels of response for a common mutagen and its doses. The shift in mean values was also found to vary between characters in response to different mutagens. Similar results were obtained by Patil (2014) in gladiolus.

Maximum number of shoot/corm (2.07 and 2.11 in 2016-17 and 2017-2018 respectively) were recorded in T₁₂ (SOAKED EMS @ 0.25%) and minimum number of shoot/corm (1.21 cm and 1.23 cm in 2016-17 and 2017-2018 respectively) recorded in T₀ (control).

Effect on flowering characters

According to their data significantly earliest spike initiation 79.50 days were recorded in T₄ (Gamma Rays 8Kr) for the years 2016-17 and followed by 81.00 days in T₁₂ (Soaked EMS @ 0.25%) for the years 2016-17 which were at par with each other. However, maximum number of days taken for spike initiation 93.50 days were recorded in T₀ (Recommended dose of nutrients through chemical fertilizers) for the years 2016-17. Maximum no. of spike per plant 1.49 were recorded in T₆ (Gamma Rays 12Kr) and T₁₆ (Soaked EMS @ 1.25%) for the years 2016-17 and followed by 1.48 in T₅ (Gamma Rays 10Kr) and T₁₅ (Soaked EMS @ 1.00%) for the years 2016-17 which were at par with each other. However, minimum number of spike per plant 1.43 was recorded in T₀ (Recommended dose of nutrients through chemical fertilizers) for the years 2016-17. The increase or decrease in the number of spikes per plant was significant. However, the decrease in number of spikes may be due to deleterious effects of EMS at higher concentration. These are in conformity with results of Bhajantri and Patil (2013)^[3] in gladiolus and Roychowdhury and Tah (2011)^[17] in Dianthus.

Table 1: Effect of chemical and physical mutagens on vegetative parameters of gladiolus

Treatments	Days taken for sprouting of corms	Sprouting percentage	Plant height (cm)	No. of leaves per plant	No. of shoots per corm
T ₀ - Control	13.86	98.83	98.88	8.27	1.21
T ₁ -Gamma Rays 2Kr	19.66	97.80	96.82	10.23	2.11
T ₂ -Gamma Rays 4Kr	20.42	96.90	95.98	9.73	2.02
T ₃ -Gamma Rays 6Kr	21.69	94.90	94.64	9.30	1.95
T ₄ -Gamma Rays 8Kr	22.21	90.00	94.45	8.86	1.87
T ₅ -Gamma Rays 10Kr	23.33	83.83	93.10	8.37	1.76
T ₆ -Gamma Rays 12Kr	24.00	75.90	91.98	8.09	1.68
T ₇ -DRY EMS @ 0.25%	16.85	97.88	97.88	10.00	2.12
T ₈ -DRY EMS @ 0.50%	17.21	96.93	96.97	9.77	2.03
T ₉ -DRY EMS @ 0.75%	18.78	95.90	96.15	9.53	1.95
T ₁₀ -DRY EMS @ 1.00%	19.72	90.46	95.85	8.90	1.88
T ₁₁ -DRY EMS @ 1.25%	20.69	82.73	94.30	8.45	1.85
T ₁₂ -SOAKED EMS @ 0.25%	14.78	97.91	97.88	9.85	2.29
T ₁₃ -SOAKED EMS @ 0.50%	15.33	97.12	96.97	9.37	2.08
T ₁₄ -SOAKED EMS @ 0.75%	16.59	95.32	96.15	8.97	2.04
T ₁₅ -SOAKED EMS @ 1.00%	17.08	90.50	94.86	8.56	1.98
T ₁₆ -SOAKED EMS @ 1.25%	19.67	84.45	94.25	8.15	1.85
S.E. ±	1.25	3.01	1.79	2.13	0.46
C.D. (P=0.05)	3.59	8.60	7.84	3.37	4.98

Table 2.a: Effect of chemical and physical mutagens on flower parameters of gladiolus

Treatments	Days taken for initiation of spike	No. of spike/plant	Length of spike (cm)	Days to opening of first florets	Diameter of the florets (cm)	Number of florets per spike
T ₀ - Control	93.50	1.43	75.25	99.50	12.57	11.11
T ₁ -Gamma Rays 2Kr	84.50	1.45	79.73	86.50	10.25	13.95
T ₂ -Gamma Rays 4Kr	84.75	1.46	78.53	89.00	9.84	13.16
T ₃ -Gamma Rays 6Kr	85.50	1.47	76.47	90.50	9.35	12.67
T ₄ -Gamma Rays 8Kr	79.50	1.47	77.13	91.50	8.76	12.23
T ₅ -Gamma Rays 10Kr	90.00	1.48	76.78	94.00	7.96	11.83
T ₆ -Gamma Rays 12Kr	90.50	1.49	78.40	97.50	7.25	11.31
T ₇ -DRY EMS @ 0.25%	85.50	1.44	80.60	89.50	10.22	13.99
T ₈ -DRY EMS @ 0.50%	87.50	1.45	78.60	90.50	9.45	13.57
T ₉ -DRY EMS @ 0.75%	88.50	1.45	77.93	91.25	9.10	13.12
T ₁₀ -DRY EMS @ 1.00%	89.50	1.46	76.60	91.95	8.58	12.64
T ₁₁ -DRY EMS @ 1.25%	89.75	1.47	76.47	92.20	7.89	11.86
T ₁₂ -SOAKED EMS @ 0.25%	81.00	1.46	81.73	84.25	10.29	14.12
T ₁₃ -SOAKED EMS @ 0.50%	83.00	1.46	78.47	85.00	9.67	13.76
T ₁₄ -SOAKED EMS @ 0.75%	89.50	1.47	76.73	91.50	9.18	13.08
T ₁₅ -SOAKED EMS @ 1.00%	92.50	1.48	76.33	94.50	8.76	12.68
T ₁₆ -SOAKED EMS @ 1.25%	94.50	1.49	75.87	97.50	7.97	11.96
S.E. ±	6.05	0.88	5.27	4.21	1.02	3.15
C.D. (P=0.05)	15.91	3.67	11.45	9.54	2.32	4.54

Table 2.b: Effect of chemical and physical mutagens on vegetative parameters of gladiolus

Treatments	Days to first florets durability	No. of corms per plant	Diameter of corm (cm)	No. of cormels per plant	Vase life (days)
T ₀ - Control	8.20	1.34	4.17	12.55	5.13
T ₁ -Gamma Rays 2Kr	9.20	1.50	4.16	16.18	7.81
T ₂ -Gamma Rays 4Kr	10.40	1.49	4.14	15.90	8.03
T ₃ -Gamma Rays 6Kr	9.60	1.48	4.13	15.45	7.12
T ₄ -Gamma Rays 8Kr	10.00	1.47	4.11	14.56	9.16
T ₅ -Gamma Rays 10Kr	9.40	1.45	4.09	14.12	7.88
T ₆ -Gamma Rays 12Kr	9.60	1.43	4.08	13.12	5.90
T ₇ -DRY EMS @ 0.25%	10.60	1.48	4.15	16.88	9.36
T ₈ -DRY EMS @ 0.50%	10.20	1.47	4.14	15.17	8.20
T ₉ -DRY EMS @ 0.75%	9.40	1.46	4.13	14.75	7.89
T ₁₀ -DRY EMS @ 1.00%	8.40	1.46	4.12	13.95	7.04
T ₁₁ -DRY EMS @ 1.25%	8.28	1.45	4.10	13.43	6.46
T ₁₂ -SOAKED EMS @ 0.25%	11.40	1.51	4.15	18.21	10.77
T ₁₃ -SOAKED EMS @ 0.50%	10.60	1.50	4.14	17.78	9.95
T ₁₄ -SOAKED EMS @ 0.75%	10.40	1.48	4.12	17.23	9.49
T ₁₅ -SOAKED EMS @ 1.00%	9.20	1.46	4.10	16.86	8.44
T ₁₆ -SOAKED EMS @ 1.25%	9.00	1.45	4.08	16.12	7.21
S.E. ±	0.79	1.28	3.01	1.79	1.54
C.D. (P=0.05)	1.11**	3.67	8.60	7.84	4.17**

The length of spike also increased with increase in concentration of EMS treatment. Maximum length of spike (cm) 81.73 cm were recorded in T₁₂ (Soaked EMS @ 0.25%) for the years 2016-17 and followed by 80.60 cm in T₇ (Dry EMS @ 0.25%) for the years 2016-17 which were at par with each other. However, minimum length of spike (cm) 75.25 cm was recorded in T₀ (Recommended dose of nutrients through chemical fertilizers) for the years 2016-17.

It is evident from the data given in Table 2b that treatment of gladiolus corms with EMS delayed the number of days taken for opening of basal floret in treatment 0.2 per cent EMS. Earliest opening of first florets 84.25 days were recorded in T₁₂ (Soaked EMS @ 0.25%) for the years 2016-17 and followed by 85.00 days in T₁₃ (Soaked EMS @ 0.50%) for the years 2016-17 which were at par with each other. However, maximum number of days taken for opening of first florets 99.50 days, were recorded in T₀ (Recommended dose of nutrients through chemical fertilizers) for the years 2016-17. Maximum diameter of the florets (cm) 12.57 cm was recorded in T₀ (Recommended dose of nutrients through chemical fertilizers) for the years 2016-17 and followed by 10.29 cm in T₁₂ (Soaked EMS @ 0.25%) for the years 2016-17 which were at par with each other. However, minimum diameter of the florets (cm) 7.25 cm was recorded in T₆ (Gamma Rays 12Kr) for the years 2016-17. Maximum number of florets per spike 14.12 were recorded in T₁₂ (Soaked EMS @ 0.25%) for the years 2016-17 and followed by 13.99 in T₇ (Dry EMS @ 0.25%) for the years 2016-17 which were at par with each other. However, minimum number of florets per spike 11.11 was recorded in T₀ (Recommended dose of nutrients through chemical fertilizers) for the years 2016-17. Similar stimulated effect on number of florets per spike was observed by Bhajantri and Patil (2013) [3]. Maximum durability of first florets 11.40 days were recorded in T₁₂ (Soaked EMS @ 0.25%) for the years 2016-17 and followed by 10.60 days in T₁₃ (Soaked EMS @ 0.25%) for the years 2016-17 which were at par with each other. However, minimum durability of first florets 8.20 days were recorded in T₀ (Recommended dose of nutrients through chemical fertilizers) for the years 2016-17.

Maximum Vase life (days) 10.77 days were recorded in T₁₂ (Soaked EMS @ 0.25%) for the years 2016-17 and followed by 9.95 days in T₁₃ (Soaked EMS @ 0.25%) for the years 2016-17 which were at par with each other. However, minimum Vase life (days) 5.13 days were recorded in T₀ (Recommended dose of nutrients through chemical fertilizers) for the years 2016-17. The significantly maximum number of cormels per plant were recorded in 0.25 per cent SEMS T₁₂ (18.21) followed by T₁₃ and T₇ while it was minimum in control (12.55). The results are in line with the work of Roychowdhury and Tah (2011) [17]. The increase in production of corms and cormels could be due to activation of enzymes and hormones responsible for such growth.

Conclusion

Mutation is recognized as one of the most important technology for the development of new varieties through genetic manipulation. Mutation technique by using ionizing radiations and other mutagens have successfully produced a large number of new promising varieties in different ornamental plants. Mutation breeding has been successfully applied for varietal improvement of many crop species. About 70% of the world's mutant varieties have been induced through gamma-rays. After the observation of the study it was concluded that maximum plants height was observed in

control while minimum height observed in the treatment with Gamma Rays 12Kr while maximum number of leaves and number of shoot per corm were recorded in treatments with Gamma Rays 2Kr and SOAKED EMS @ 0.25% respectively.

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