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MUTAGENESIS VIA EXPOSURE TO PHYSICAL AND CHEMICAL MUTAGENS IN MICROTUBERS OF GLORY LILY

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Abstract: The mutagenic treatment of glory lily microtuber in VM_1 showed decrease in biometrical characters *viz.,* sprouting percentage, plant height, stem girth, tuber length, tuber girth and tuber weight with the increase in dose of mutagens. In VM_2 generation, the shift in mean values for plant height, number of leaves per plant and stem girth observed was in the positive direction with few exception. The treatment DES at 1.00 per cent recorded the maximum value for tuber length. All the mutagenic treatments except DES and EMS at 1.00 per cent significantly reduced the girth of the tuber (3.68 cm). The weight of the tuber was highest (8.85 g) in DES at 1.00 per cent.
Keywords: Glory lily, mutation, microtuber, mutagens, variation.

Introduction

Glory lily (*Gloriosa superba* L.), a glorious herbaceous climber with underground tuber belonging to Colchicaceae family.Tubers and seeds are an expensive export commodity. Colchicine & Gloriosine are two commonly used phytochemicals present in glory lily which is used for treating gout and rheumatism (Peranantham, *et al.,* 2014). *Gloriosa superba* is one of the endangered species among the medicinal plants (Patiland Gavale, 2016). In India, only Tamil Nadu has the largest area under glory lily cultivation (upto 6000 acres) spread over seven districts viz., Karur, Tirupur, Dindigul, Salem, Ariyalur, Perambalur and Nagapattinam and holds monopoly in production of glory lily seeds with an annual production of over 600 - 700 tonnes. The growing demand (600 million tonnes) for the seeds of Glory lily in the international market and the wider popularity it has gained among the Indian farmers necessitates attempts to induce new variability with high yield, high colchicine content, dwarf stature and leaf blight resistant of the plant as well (Ved, 2007). Glory lily cultivation has succeeded for nearly 25 years and approximately 15,000 ton of seeds has so far been produced and traded. Traditional or conventional breeding has not been attempted so far as there are only local ecotypes of *G. superba* under cultivation and genetic wealth is limited.

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The genetic variability of Glory lily is low owing to the continued vegetative propagation through tubers which has reduced the vigour, tolerance to biotic and abiotic stress, causing low yields (Rajadurai, 2001). Therefore, generation of variability through mutagenic treatments is of paramount importance for improvement of Glory lily.

Materials and methods

Sprouted microtubers (first generation tubers developed from seeds) of uniform size weighing 1-2 g were selected for mutagenic treatment.

Gamma radiation

The tubers were subjected to gamma radiation at varying levels (0.50, 1.00, 1.50 kR). Gamma irradiation was carried out at Gamma chamber - 900 installed at the Sugarcane Breeding Institute, Coimbatore. The irradiated tubers were planted immediately in the well prepared field. The untreated tubers were maintained as control.

Ethyl methyl sulfonate (EMS)

For experimental studies, Ethyl Methyl Sulfonate ($CH_3SO_2OC_2H_5$) was procured from M/s. Sigma Aldrich Company, U.S.A. The molecular weight and density of EMS was 24.60 and 1.17 g ml^{-1} respectively. EMS concentration of 1.00, 1.50 and 2.00 per cent were used in the present study.

Diethyl Sulphate (DES)

Diethyl Sulphate ($C_4H_{10}SO_4$) procured from Sisco Research Laboratory Pvt. Ltd., Mumbai was used as another mutagen. Molecular weight and density of DES was 154.19 and 1.33 g ml^{-1} respectively. Diethyl Sulphate concentration of 1.00, 1.25 and 1.50 per cent were used in the present study.

Sprouted tubers were dipped in above said concentration of chemical muagens for 12 hours at room temperature with intermittent shaking. Treated tubers were washed thoroughly and planted in the field.

Study of VM_1 generations

The sprouts produced from the planted tubers following mutagenic treatment was represented as first vegetative generation; which was designated as VM_1 generation plants. Observations on the variations in biometrical parameters namely number of days for sprouting of tubers, sprouting percentage, plant height, number of leaves per plant, stem girth, length, girth and weight of the tuber were recorded on 120 days after planting (DAP).

Study of VM_2 generation

Tubers collected from the VM_1 plants were planted in the well prepared bed made of Sand: Soil: FYM: Cocopeat (2:1:1:1) under the shade net, which was designated as VM_2 generation plants. Observations on the variation in biometrical parameters were recorded on 150 days after planting (DAP) as that of VM_1 generation.

Statistical analysis

The mean value of different quantitative characters in VM_1 generation were analysed statistically by adopting the standard procedures (Panse, and Sukhatme, 1984).

Results and discussion

Effect of mutagens on VM_1 generation in microtuber

The effect of mutagenic treatments on quantitatively inherited characters showed that the mutagens induced essentially random changes to the genotypes treated (Brock, 1972). At the treatment level, the mutagens exhibited an appreciable amount of variation in quantitatively inherited characters in addition to the visible mutations. The mutagens have been found to induce physiological disorders, chromosomal aberrations as well as point mutation in the biological material in M_1 generation (Gaul, 1977).

In the present study, it was observed in mutant progenies of microtubers, the sprouting percentage linearly reduced with the increase in concentration of gamma rays (Table. 1). The highest (93.33 per cent) sprouting percentage of microtuber was observed in control (MT_1), 0.50 kR gamma rays (MT_2) and 1.00 per cent DES (MT_8). The lowest sprouting percentage of tubers (53.33 per cent) was observed in 1.50 per cent DES (MT_{10}).

The decrease in sprouting percentage due to higher doses and an increase in sprouting percentage at lower doses caused by ionizing radiation have been observed in *G. superba* by Chandra and Tarar (1988) and Rajadurai (2001). The decrease in sprouting might be due to lethality caused in vegetative organs, physiological injuries and mutagenic reaction with the nucleic acids like DNA by alkylating their phosphate group. The hydrolytic products also affect the other cell constituents. In *G. superba,* reduction of sprouting percentage by higher doses of gamma rays, EMS and DES may 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. Mutagenic treatments revealed a gradual decreasing trend in germination from lower to higher doses (Sunil, *et al.,* 2011). At lower concentrations, the effect may be reversed. These

findings were made by Chandra and Tarar (1988) and Rajadurai (2001) in glory lily and Farooqi *et al*. (1990) in davana.

Earliness in sprouting was observed in the lower doses of gamma rays and EMS. The days taken for sprouting were earlier (15.69 days) in 1.00 per cent EMS whereas late emergence was observed in 1.50 per cent DES (23.20 days). The plant height after 120 days of sprouting showed a gradual reduction with an increase in the dose of mutagens. There was a significant difference among the mutagenic treatments on plant height. The mean plant height ranged from 5.70 cm to 10.96 cm in control (MT_1) and 1.00 per cent EMS (MT_5). The maximum value (192.28 per cent) has exhibited increase over control. Ionizing radiations as well as the effect of alkylating agents may cause destruction or damage to apical meristems or partial failure of the internodes to elongate so as to result in decreased number of proliferating cells (Patel and Shah, 1974). According to various reports, mutagenic agents reduce viability and sprouting speed (Krasaechai, (1992), Estrada-Basaldua *et al.* (2011) and Navabi, *et al*.(2016). Similar findings have been reported in other crops like ashwagandha (Rajamani, 1996) and Glory lily (Rajadurai, 2001).

Gamma rays at 1.50 kR recorded lower number of leaves (8.00), while at 0.50 kR gamma rays, higher number (10.28) was recorded. A gradual reduction of leaves per plant was observed in high to lower dose of gamma ray treatment, when compared with control. Similar findings were reported in aswagandha by Bharathi, *et al*., 2013. The reduction in leaves following mutagenic treatment was similar to the findings of Mensah and Eruotor (1993) in lima bean and Rajadurai (2001) in glory lily. Inhibition of vegetative growth may be due to radiation effect on the chromosomal material (Ehrenberg *et al*., 1969) genetic injury induced in dividing cells and deficiency of some physiological prerequisite to cell division (Stein and Sparrow, 1973).

A marginal reduction in the length and girth of tuber was observed with an increase in the concentration of mutagens. The length and girth of tuber was relatively higher (18.05 and 3.36 cm) in gamma ray treatment at 0.50 kR while the lowest length and girth (9.75 and 2.65 cm) was observed in 1.50 per cent DES. Higher doses of mutagens would have caused greater degree of inhibitory effect on tuber growth. In the cells of growing tuber, the mitotic aberrations caused would have resulted in the inhibitory effect on the tuber growth due to higher doses of mutagens as reported in crops like ashwagandha (Rajamani, 1996), glory lily (Rajadurai, 2001) and coleus (Velmurugan, 2007). Lower doses of DES (1.00 per cent) favored for an increase in weight of tuber, while in others, the weight of tuber was reduced.

The lowest weight of tuber was observed in the control. Thamburaj (1984) and Rajadurai (2001) obtained similar result in cassava and *G. superba* respectively.

Effect of mutagens on VM_2 generation in microtuber

Medium sized tubers produced by VM_1microtubers were carried forward to VM_2 generation. The sprouting percentage in the selected mutagenic dosages revealed that there was an exponential fall in sprouting and survival percentage in the VM_2 generation with increased dose of mutagens (Table.2). The highest (80.00 per cent) sprouting percentage of microtuber was observed in 1.00 per cent EMS (MT_5), which showed 109.09 per cent increase over control. In comparison to the VM_1 generation, decreased sprouting over their respective treatments was observed in VM_2 generation. This indicates the presence of ample mutated genes in VM_2 than in VM_1 generation. Conflicting views about the seed germinability in VM_2 generation of many crop plants have been reported. In *Gloriosa*, Rajadurai (2001) observed the reduction in germination percentage as the generation advanced, while Rajamani (1996) observed an increased seed germination percentage in M_2 generation in ashwagandha.

The reduction in sprouting of *G. superba* may be due to physiological imbalance and damages caused at molecular level, which results in chromosomal aberration causing cytological changes. The present findings are in line with the work of Chandhra, and Tarar (1988) and Rajadurai (2001) in glory lily.

The reduction of plant height in VM_2 was low at lower doses of mutagens. Among the mutagenic treatments, 1.00 per cent EMS (MT_5) produced tallest plant (49.00 cm), while the shortest plant (21.30 cm) was observed in control (MT_1). The estimates of variance ranged from 3.11 to 17.86 in 1.50 per cent DES (MT_{10}) and 1.00 per cent EMS (MT_5). The highest co-efficient (17.24 per cent) was registered in 1.00 kR gamma rays (MT_3) while the lowest (7.44 per cent) was noticed in 1.50 per cent DES (MT_{10}). Higher reduction in plant height may be due to the inhibitory effect of the mutagens which causes a breakage of apical dominance.

The inhibitory effect of mutagens on the length of seedling was evident from the decrease in length of root and shoot with increasing dose / concentration of gamma rays and EMS. Similar observations were made by several workers in sunflower (Jayakumar, and Selvaraj 2003). The stimulatory effect was observed in lower doses / concentrations of gamma rays and EMS on the length of root, shoot and seedling. The hypothetic origin of these stimulations by irradiation and EMS treatments was due to in cell division rates as well as an activation of growth hormone, e.g., auxin (Zaka, *et al*., 2004).

There was a significance in the mutagenic treatments for imparting changes in number of leaves per plant and stem girth. However unlike in the VM_1 generation, the number of leaves and stem girth showed a marginal increase in the lower doses of mutagens in VM_2 generation. Maximum number of leaves (36.50) was obtained in 0.50 kR gamma rays (MT_2), while minimum of 14.46 was observed in control (MT_1). The estimate of variance ranged from 1.98 (control) to 18.50 (1.50 per cent EMS). An increase in co-efficient was noticed among the treated population when compared to the untreated control. It was maximum (25.98 per cent) in 1.50 per cent DES (MT_{10}) and minimum (9.72 per cent) in the control (MT_1).

The mean stem girth ranged from 0.86 to 1.45 cm and all the treatment except 0.50 kR gamma rays (MT_2) shifted the mean values in positive direction. Among the treatments, the maximum variance (0.19) was observed in 1.00 kR gamma rays (MT_3). The co-efficient estimate revealed that 1.00 kR gamma rays (MT_3) recorded the maximum variance (41.77 per cent). The co-efficient of variation was minimum (15.05 per cent) in 2.00 per cent EMS (MT_7).

This may be due to stimulatory growth of apical shoots caused by the mutagens, which in turn would have promoted the number of leaves. Similar results of reduced plant height and number of leaves per plant were also reported in *Gloriosa* by Rajadurai (2001).

In the present study, the tuber length was inconsistent among the mutagenic treatments (Table.3). The tuber length ranged from 24.54 cm to 29.75 cm in 2.00 per cent EMS (MT_7) and 1.00 per cent DES (MT_8). The highest variance (14.59) and shift in variance (3.97) was observed in 1.50 per cent EMS (MT_6). Higher (14.62 per cent) and lower (5.10 per cent) estimates of co-efficient of variation were recorded in 1.50 per cent EMS (MT_6) and 1.00 per cent DES (MT_8) respectively.

All the mutagenic treatments except DES and EMS at 1.00 per cent significantly reduced the girth of the tuber (3.68 cm). The lowest girth (3.40 cm) was recorded in DES at 1.50 per cent. The co-efficient of variation showed consistent variation among the mutants and the maximum estimates (11.29 per cent) was exhibited in 1.00 per cent EMS (MT_5). The decrease in the girth of the tuber may be due to physiological imbalance caused at the cellular level as a result of irradiation. The tuber weight was highest in DES at 1.00 per cent (8.85 g) followed by the treatment gamma rays at 0.50 kR (8.12 g). Higher estimates of variance (39.50) were observed in 1.00 per cent EMS (MT_5). The variance was low (0.17) in 1.50 per cent DES (MT_{10}). Similarly, the greater co-efficient of variation (20.13 per cent) was

exhibited in 1.00 per cent EMS (MT_5) and a lesser value (1.54 per cent) was expressed in 1.50 per cent DES (MT_{10}).

Generally, weight of tuber increased gradually with increasing doses of gamma rays as reported by Ilyas and Naz (2014) in turmeric. In *G. superba*, the increase in length and weight of microtubers in DESat 1.00 per cent may be due to increase in the enzyme level which activates metabolism of cells responsible for translocation of source to sink. Rajadurai (2001) in *G. superba* observed similar results caused by EMS and DES.

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Table 1. Effect of physical and chemical mutagens on microtubers of glory lily in VM_1 generation

S. No	Treatments	Sprouting (%)	Days to sprouting	Plant height (cm)	Number of leaves/plant	Stem girth (cm)	Tuber length (cm)	Tuber girth (cm)	Tuber weight (g)
MT_1	Control	93.33	19.28	5.70	7.83	0.72	16.76	2.93	13.85
MT_2	0.50 kR γ rays	93.33	18.27	8.81	10.28	0.43	18.05	3.36	17.15
MT_3	1.00 kR γ rays	86.66	18.35	6.92	10.15	0.73	15.27	3.22	16.16
MT_4	1.50 kR γ rays	73.33	19.42	5.76	8.00	0.77	14.99	2.74	14.47
MT_5	1.00 % EMS	86.66	15.69	10.96	10.09	0.80	15.31	3.17	17.25
MT_6	1.50 % EMS	86.66	18.33	9.70	9.70	0.83	14.30	3.06	16.28
MT_7	2.00 % EMS	80.00	19.23	8.51	9.00	0.99	13.43	2.76	14.85
MT_8	1.00 % DES	93.33	21.76	9.72	9.76	0.73	16.30	3.18	16.75
MT_9	1.25 % DES	66.66	22.00	9.15	9.55	0.77	14.66	2.71	16.08
MT_{10}	1.50 % DES	53.33	23.20	8.00	9.55	0.77	9.75	2.65	15.20

Table.2 Effect of mutagens on plant characteristics in VM_2 generation of glory lily derived from microtuber

S. No	Treatments	Sprouting (%)	Per cent over control	Mean± SE	Variance	CV%	Mean± SE	Variance	CV%	Mean± SE	Variance	CV%
				Plant height			**Number of leaves per plant**			**stem girth (cm**		
MT_1	Control	73.33	100.00	21.30±1.08	12.88	16.85	14.46±0.36	1.98	9.72	0.95±0.05	0.03	20.61
MT_2	0.50 kR γ rays	66.66	90.90	30.91±1.25	11.03	10.74	36.50±1.37	15.14	10.66	0.86±0.12	0.04	24.01
MT_3	1.00 kR γ rays	60.00	81.82	23.32±1.03	16.18	17.24	24.33±0.70	7.52	11.27	1.04±0.12	0.19	41.77
MT_4	1.50 kR γ rays	53.33	72.72	21.76±1.94	11.32	15.45	21.00±2.00	8.00	13.46	1.15±0.14	0.13	32.28
MT_5	1.00 % EMS	80.00	109.09	49.00±1.49	17.86	8.62	18.00±1.34	12.66	19.77	1.45±0.08	0.07	19.04
MT_6	1.50 %	66.66	90.90	35.31±1.02	11.45	9.58	17.75±1.52	18.50	24.23	1.25±0.08	0.05	19.14

	EMS											
MT_7	2.00 % EMS	53.33	72.72	26.36±1.09	12.07	13.18	17.70±1.27	16.23	22.76	1.05±0.05	0.02	15.05
MT_8	1.00 % DES	66.66	90.90	37.15±1.21	11.90	9.28	26.54±1.06	12.47	13.30	1.14±0.06	0.03	16.98
MT_9	1.25 % DES	60.00	81.82	34.04±1.35	16.56	11.95	23.50±1.29	13.42	15.59	1.23±0.09	0.08	22.93
MT_{10}	1.50 % DES	53.33	72.72	23.70±0.45	3.11	7.44	15.90±1.24	17.09	25.98	0.95±0.11	0.10	34.22

Table.3 Effect of mutagens on tuber characteristics in VM_2 generation of glory lily derived from microtuber

S. No	Treatments	Mean± SE	Variance	CV%	Mean± SE	Variance	CV%	Mean± SE	Variance	CV%
		Tuber length			Tuber girth			Tuber weight		
MT_1	Control	26.76±0.94	10.62	12.17	3.68±0.11	0.17	11.27	23.85±0.38	1.73	5.52
MT_2	0.50 kR γ rays	28.05±0.95	3.61	6.77	3.66±0.10	0.14	10.22	31.97±2.02	16.39	12.66
MT_3	1.00 kR γ rays	26.70±1.32	12.36	13.16	3.62±0.17	0.12	9.91	31.44±1.26	11.20	10.64
MT_4	1.50 kR γ rays	25.49±1.01	13.28	14.29	3.45±0.11	0.09	8.81	27.65±1.08	14.24	13.64
MT_5	1.00 % EMS	26.31±0.94	8.96	11.37	3.68±0.13	0.17	11.29	31.21±1.89	39.50	20.13
MT_6	1.50 % EMS	26.12±1.15	14.59	14.62	3.65±0.08	0.06	6.85	28.36±1.15	12.03	12.22
MT_7	2.00 % EMS	24.54±0.91	7.59	11.22	3.57±0.09	0.10	8.85	27.28±0.90	8.13	10.45
MT_8	1.00 % DES	29.75±0.75	2.30	5.10	3.68±0.06	0.02	4.68	32.70±1.07	9.27	9.31
MT_9	1.25 % DES	27.55±1.05	8.85	10.79	3.58±0.05	0.02	4.57	31.08±1.62	21.07	14.76
MT_{10}	1.50 % DES	27.16±1.08	9.48	11.33	3.40±0.14	0.08	8.31	26.75±0.20	0.10	1.54