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RESEARCH ARTICLE

PHYSICAL AND CHEMICAL MUTAGENESIS OF VM1 GENERATION IN TUBERS OF GLORY LILY (*GLORIOSA SUPERBA*)

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ABSTRACT

Induced mutagenesis was conducted in *Gloriosa superba* by using both chemical and physical mutagens to study various morphological characters in six genotypes *viz*., Arupukotai, Chittor, Dharapuram, Mulanur, Nellore and Vedaranyam. The tubers were subjected with four doses of each chemical and physical mutagens were administered at various concentrations of 1, 1.5, 2, 2.5 % ethylmethane sulphonate (EMS) and 2.5, 3, 3.5, 4 gray Gamma rays respectively. Experiment was conducted at Variyankaval village, Udayarpalayam taluk of Ariyalur, district Tamil Nadu. Nine characters were recorded in the study among 48 mutants. Plant height was recorded highest in EMS 1% in Chittor (146.58) and 50% flowering was more in EMS 1% in Mulanur (114.64). EMS 1% in Mulanur produced more number of pods per plant (35.57) and seeds per pod (70.64). EMS 1% in Chittor recorded highest fresh seed yield (208.34) and dry seed yield (65.24). Length of tuber (21.64) and tuber weight (71.85) was high in EMS 1% in mulanur. Quantification of colchicine content in 48 mutants was done by High Performance Liquid Chromatography (HPLC) method. Colchicine content was high in Arupukotai (0.8567) followed by Chittor (0.6821) in EMS 1% and lowest at 4 gray in Vedaranyam (0.0501)

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INTRODUCTION

Glory lily *(Gloriosa superba),* which is originated from tropical Africa, is grown as one of the important medicinal crop in India and other Asian countries like Sri Lanka, Bangladesh, Myanmar and Malaysia. It is mainly cultivated for its seed and tubers which are used for pharmaceutical purpose. It is commonly known as Flame lily, Creeping lily, Gloriosa lily etc. Tubers and seeds are the expensive export commodity as it contains phytochemicals like Colchicine and Gloriosine which are used for treating gout and rheumatism (Peranantham *et al*., 2014). *Gloriosa superba* is one of the endangered species among the medicinal plants (Patil and Gavale, 2016). In India, Glory lily is majorly cultivated in tamil nadu in the districts of Dindigul, Salem, Karur, Tirupur, Ariyalur etc. and from North-West Himalayas to Assam regions. Demand for the cultivation of Glory lily is gaining importance among Indian farmers due to its demand in international market and medicinal importance in pharmaceutical use. Due to high medicinal importance, *Gloriosa superb* which is a vegetatively propagated crop is subjected to mutagenesis for better seedset, germination and colchicines content to exploit better mutant than conventional genotypes. The growing demand of about 600 million tonnes of seeds in the international market there has been wider popularity among the Indian farmers and therefore necessary attempts has to be made to induce new variability with high yield, high colchicine content, dwarf stature and leaf blight resistant of the plant as well (Ved, 2007).

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Habitat/ Ecology

In Australia, scattered naturalized populations exist in the understorey of coastal dry sclerophyll forest and sand dune vegetation throughout south-east Queensland and New South Wales. It is considered a rampant and dangerous invasive weed in Australia, dominating the coastal dunes at the expense of native species and leading to deaths of native animals and birds when ingested. In India, Gloriosa is distributed in the Western Ghats but the density is rapidly decreasing due to excessive uprooting by the Herbal Medicine producers.

Uses

Flame lily has a wide variety of uses, especially within traditional medicine as practised in tropical Africa and Asia (including Ayurvedic medicine in India). It contains the alkaloid colchicine, which has been used effectively to treat acute gout, intestinal worms, infertility, wounds and other skin problems. The roots and leaves used as an antidote for snake

bite, as a laxative, and to induce abortion. It has proven useful in the treatment of chronic ulcers, arthritis, cholera, colic, kidney problems and typhus. Colchicine, an alkaloid extracted from the tubers and seeds gives high price in the market and used in scientific research. Glory lily extract is useful against many skin diseases. It is used to rectify the many respiratory disorders. Colchicine is widely used as an experimental tool in the study of cell division, as it can inhibit mitosis and has been used in the treatment of cancer. The sap from the leaf tip is used for pimples and skin eruptions. Tribals of Patalkot apply the powder of rhizome with coconut oil in skin eruptions and related diseases. Gloriosa paste can be applied for curing inflammation like wound, lymphadenopathy, piles and skin related problem. It is also effective in poisoning. Their powder helps in easy digestion of food. It is also helpful in relieving from menstrual disturbance. It's also providing strength to the body. *Gloriosa superba* is widely cultivated as an ornamental for its stunning flowers.

MATERIALS AND METHODS

The tuber materials used in this investigation were collected from six different regions of Tamil nadu and Andhra Pradesh. List of genotypes is given below in Table 1.

Mutagenesis by physical irradiation (Gamma rays)

Induced mutation with Gamma irradiation was first discovered in organisms in 1930 (Miller, 1930). Subsequent studies demonstrated that gamma rays treatment was one of the most efficient mutagenesis in plants. The sprouted tubers were subjected to gamma radiation at Indira Gandhi Centre for Atomic Research Radiological Safety and Environmental Group, Kalpakkam**,** Chennai. The irradiated tubers were planted immediately in the well prepared field. The untreated tubers were maintained as control. The doses of Gamma rays are 2.5, 3, 3.5, and 4 gray.

Chemical mutagenesis by Ethyl methyl sulfonate (EMS)

EMS is one of the most frequently used chemical agents for creating mutations. This alkylating agent can efficiently induce chemical modification of nucleotides, which results in various point mutations, including nonsense and missence mutations. For experimental studies, Ethyl Methyl Sulfonate (CH3SO2 OC2H5) was procured from M/s. Sigma supplied by Lakshmi Scientific Company, Chidambaram. The molecular weight and density of EMS was 24.60 and 1.17 g ml-1 respectively. The doses of EMS are 1, 1.5, 2 and 2.5 percent

RESULTS AND DISCUSSION

Study of VM1 generation

The sprouts produced from the planted tubers following mutagenic treatment was represented as first vegetative generation, which was designated as VM1 generation plants. Sprouted tubers of uniform size weighing 50-60 gm were selected for mutation. The mutated tubers were sown in August- 2014 in Randomized Block Design with three replications for evaluation. Experimental plot area was tilled and furrows of 30 cm deep were made at a distance of 1.5m, 20 days before planting, Irrigation was done at weekly intervals along with recommended agronomic and plant protection practices. Observations were recorded from five randomly selected plants per replication for nine morphological characters viz., Plant height (cm), Number of days of 50% flowering, Number of pods per plant, Number of seeds per pod, Fresh seed yield per plant(g), Dry seed yield per plant (g), Length of tuber (cm), Weight of tuber (g), and Colchicine content. Statistical parameter like mean, standard error and critical difference for all the characters were calculated by standard methods of analysis. Variations among different mutants for vegetative, floral and yield characters were observed Table 2.

Table 1. Collection of tubers from different Agro climatic zones of Tamil Nadu and Andhra Pradesh

S.No.	Locations	Agro climatic zones	State
1.	Arupukotai local	Southern zone	Tamil Nadu
2.	Dharapuram local	Western zone	
3.	Mulanur local	Western zone	
4.	Vedaranyam local	Cauvery Delta zone	
5.	Chittor local	South zone	Andhra Pradesh
6.	Nellore local	South zone	

Quantification of Colchicine

a)Extraction Method

Five hundred milligrams of dried and powdered seed samples were macerated with 25 ml of methanol at room temperature for 24 hours and sonicated for 45 minutes in an ultrasonic bath. The extract was filtered and adjusted to a final volume of 25 ml with methanol. An aliquot of the extract was filtered through 0.22 µm filter before HPLC analysis.

b)Standards preparations

Pure colchicine from SIGMA, supplied by Lakshmi Scientific Company, Chidambaram was used as reference substance. Accurately weighed six different colchicine standards viz, 50, 100, 200, 400, 800 and 1600 µg/ml was run for getting retention time and peak area. Then, the tuber samples of different mutants were run in HPLC to quantify the amount of colchicine.

c) HPLC Analysis

Quantitative determination of colchicine was carried out by comparing the retention time of the sample with that of the standard. Shimadzu HPLC system equipped with a binary pump 1525 (Max.Pressure: 6000 psi.) and a porous silica with 5µm diameter C18 4.6 × 150 mm column was used for separation. The mobile phase consisted of Acetonitrile: 3% Acetic acid (60:40), at a flow rate of 1ml/min with an injection volume of 20 µl. The peaks eluted were detected at 245 nm and identified with authentic standards. Amount of colchicine present in dry weight of sample was calculated using the following formula, given by scottrpw, 1996 and expressed in per cent dry weight.

$$Cp(s) = \frac{Ap(s)}{Ap(st)} X\ Cp\ (st)$$

Cp (s) is the concentration of the solute in the mixture.
Ap (s) is the area of the peak for the sample in HPLC chromatogram.
Ap (st) is the area of the peak for the standard in HPLC chromatogram.
Cp (st) is the concentration of standard used for injecting in HPLC.

Table 2. Effect of mutagens on tuber characterstics in VM_1 genaration of glory lily

Treatments	Genotypes	Plant ht(cm)	50% flowering	No. of Pods per plant	No. of Seeds per pod	Fresh seed yield per plant(g)	Dry seed yield per plant (g)	Length of tuber (cm)	Weight of tuber (g)	Colchicine
	Arupukotai	140.21	108.35	27.35	63.49	167.16	54.35	17.89	67.59	0.8567
	Chittor	146.58	111.12	29.42	69.54	208.34	65.24	19.64	70.54	0.6821
	Dharapuram	115.52	97.51	18.42	39.55	127.54	36.58	14.34	54.98	0.4457
EMS 1	Mulanur	144.92	114.64	35.57	70.64	177.49	60.92	21.64	71.85	0.5462
	Nellore	124.01	98.30	21.68	52.52	131.54	38.15	17.86	66.10	0.4287
	Vedaranyam	112.26	99.71	19.53	44.52	78.59	22.54	14.52	55.12	0.2231
	Arupukotai	135.24	98.65	22.85	55.25	158.68	46.52	14.25	61.24	0.6521
	Chittor	141.56	102.62	24.35	61.48	199.26	57.59	16.12	65.41	0.4968
EMS 1.5	Dharapuram	112.32	94.71	16.57	34.58	124.53	34.07	13.72	52.61	0.3024
	Mulanur	139.65	105.12	30.54	62.87	167.97	52.80	18.57	67.84	0.4258
	Nellore	121.58	95.86	17.42	48.69	126.53	33.46	16.24	64.58	0.3124
	Vedaranyam	109.34	96.82	16.20	41.02	75.34	17.20	13.53	53.20	0.1160
	Arupukotai	129.42	95.27	16.35	47.56	151.52	40.67	11.98	57.24	0.5231
	Chittor	135.45	97.58	19.58	53.87	192.98	51.54	14.25	61.56	0.4102
EMS 2	Dharapuram	109.24	91.25	13.68	32.76	120.64	30.56	12.64	51.23	0.2458
	Mulanur	133.45	98.65	24.14	55.24	160.62	46.47	15.28	62.54	0.2689
	Nellore	118.54	94.17	15.28	45.65	124.27	32.24	14.57	61.24	0.2251
	Vedaranyam	106.35	95.36	13.52	37.58	71.27	16.33	12.01	51.24	0.0968
	Arupukotai	123.24	87.29	15.43	41.24	145.68	34.26	10.52	52.24	0.4572
	Chittor	129.35	89.35	18.24	46.51	186.61	45.63	12.32	57.24	0.3523
EMS 2.5	Dharapuram	107.25	83.76	11.10	26.35	114.28	24.86	11.10	46.81	0.1962
	Mulanur	130.34	91.86	21.86	48.32	154.22	40.34	13.27	58.12	0.2001
	Nellore	115.26	86.75	13.10	39.14	118.34	26.52	12.76	56.75	0.1862
	Vedaranyam	104.26	88.35	12.01	31.57	65.58	10.20	10.11	46.31	0.0520
	Arupukotai	133.42	96.65	20.85	53.25	156.68	44.52	13.25	59.24	0.7025
	Chittor	139.56	100.62	22.35	59.48	197.26	55.59	15.12	63.41	0.5320
GM 2.5	Dharapuram	113.23	95.51	16.42	37.55	125.54	34.58	13.34	52.98	0.3564
	Mulanur	137.65	103.12	28.54	60.87	165.97	50.80	17.57	65.84	0.4424
	Nellore	122.10	96.30	19.68	50.52	129.54	36.15	16.86	64.10	0.3620
	Vedaranyam	110.26	97.71	17.53	42.52	76.59	20.54	13.52	53.12	0.1368
	Arupukotai	129.68	94.86	17.17	48.82	148.24	40.24	12.57	58.24	0.6352
	Chittor	134.86	97.74	19.62	54.68	189.35	51.86	14.68	62.42	0.4168
GM 3	Dharapuram	111.60	91.57	13.10	32.82	118.63	30.91	12.21	51.20	0.3154
	Mulanur	135.74	98.63	25.43	55.69	157.86	46.15	16.24	63.89	0.3857
	Nellore	120.86	94.26	16.24	45.03	121.30	32.54	15.62	62.42	0.3016
	Vedaranyam	108.59	95.45	14.93	37.61	68.24	16.24	12.27	51.30	0.1157
	Arupukotai	125.38	91.20	15.24	42.20	137.23	36.16	11.53	56.24	0.5421
	Chittor	130.26	94.31	16.34	48.62	177.52	47.14	12.98	60.13	0.3698
GM 3.5	Dharapuram	107.42	87.23	11.10	26.41	107.63	26.10	11.40	49.21	0.2764
	Mulanur	131.15	95.63	22.23	49.72	146.60	42.52	15.17	61.52	0.3241
	Nellore	117.28	92.68	13.20	39.26	110.71	28.86	14.62	60.34	0.2601
	Vedaranyam	104.21	93.57	12.28	31.41	57.20	12.16	11.42	49.03	0.0987
	Arupukotai	119.00	87.35	13.42	37.23	125.18	31.20	10.10	53.21	0.4624
	Chittor	124.35	89.52	14.51	43.14	165.41	42.65	11.03	56.48	0.3062
GM 4	Dharapuram	101.57	81.90	9.12	21.42	95.34	21.85	10.40	45.01	0.2231
	Mulanur	125.37	90.10	18.92	44.12	134.38	37.75	14.15	57.15	0.2654
	Nellore	111.28	88.54	10.13	34.75	98.74	23.21	13.43	55.10	0.2103
	Vedaranyam	98.62	89.32	10.24	26.24	45.98	10.27	10.23	44.20	0.0501
	Arupukotai	137.40	103.84	24.70	58.73	162.56	50.42	16.64	64.50	0.7600
	Chittor	143.12	106.75	26.50	64.96	203.10	61.28	18.77	67.64	0.5780
Control	Dharapuram	116.67	101.80	20.11	41.54	131.44	40.73	16.26	59.82	0.3840
	Mulanur	141.62	109.86	32.95	65.65	171.40	56.91	20.72	69.10	0.4960
	Nellore	126.76	105.59	23.09	55.12	135.46	42.36	20.35	68.50	0.3751
	Vedaranyam	113.73	106.18	21.42	47.59	82.97	26.41	16.93	57.02	0.1440
	General mean	92.17	71.37	14.28	35.11	103.21	28.35	10.62	43.50	0.3108
	Cv	2.61	2.96	3.07	2.52	3.10	3.25	3.28	3.08	3.22
	Se	1.39	1.22	0.25	0.51	1.85	0.53	0.20	0.77	0.01
	Sed	1.97	1.72	0.36	0.72	2.61	0.75	0.28	1.09	0.01
	Cd (5%)	3.90	3.42	0.71	1.43	5.18	1.49	0.56	2.17	0.02
	Cd (1%)	5.16	4.52	0.94	1.90	6.85	1.98	0.75	2.87	0.02

Plant height

The present study revealed that the increase in concentrations of EMS and Gamma ray resulted in decrease in plant height, which is inversely proportional as observed in soyabean and sunflower (Mensah *et al.,* 2013). Comparing the effect of EMS and Gamma ray on plant height at various concentrations revealed that lower concentration of EMS at 1 percent exhibited positive effect over control recording the highest plant height (146.58 cms) in Chittor genotype. Plant height is decreasing with increase in the concentrations of EMS in 1.5, 2 and 2.5 percent concentrations. Lowest plant height was recorded in Vedaranyam genotype at 4 percent Gamma irradiation (98.62 cms). The genotypes subjected to Gamma rays recorded lesser plant height than control exhibiting negative mutagenic effect 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).

50 percent flowering

Flowering of mutants was reduced with increase in concentrations of EMS and Gamma ray. 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). With an exception, 50 percent flowering was higher than control in EMS 1 percent in Mulanur genotype (114.64), Chittor genotype (111.12) and Arupukotai genotype (108.35). It was observed that lower concentrations of mutagens exhibited positive mutagenic effect on all the genotypes.

Number of pods per plant

Number of pods per plant was higher in Mulanur genotype (35.57) followed by Chittor genotype (29.42) and least number of pods was produced in Dharapuram genotype (9.12) in Gamma 4 percent. 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).

Number of seeds per pod

Genotypes treated with lower concentrations of EMS at 1percent produced more seeds per pod over control in Mulanur genotype (70.64) followed by Chittor genotype (69.54). Positive shift in mean values due to the enhancing effect of EMS was also reported earlier by many research workers (Khan and Wani 2006, Siddiqui *et al*., 2009, Pavadai *et al*., 2010) in other crops like green gram, rapeseed, soybean and their findings confirmed the present observation. Mutation exhibited both advantageous and deleterious effect for mean number of seeds per capsule. Reduction in number of seeds per capsule was observed with increase in the dosage of mutagens. Lowest number of seeds per pod was observed in Vedaranyam genotype (26.24) followed by Dharapuram genotype (21.42).

Fresh seed yield

Fresh seed yield was higher in Chittor genotype (208.34) followed by Mulanur genotype (177.49) treated with EMS at 1 percent concentration. Mutants treated with lower concentrations of EMS produced higher fresh seed yield than mutants treated with Gamma rays (Begum and Dasgupta, 2015). Similar findings were also observed by (Begum and Dasgupta, 2010). Dosage increase of mutagens resulted in reduced fresh seed yield was observed in mutants treated with both EMS and gamma rays. Lowest fresh seed yield was observed in Vedaranyam genotype (45.98) treated with Gamma 4 percent concentration. Fresh seed yield was lower than control in mutants treated with Gamma rays.

Dry seed yield

Dry seed yield was recorded highest in Chittor genotype treated with EMS at 1 percent concentration (65.24) and the lowest was recorded in Vedaranyam genotype treated with 4 percent Gamma ray (10.27). This is due to the negative mutagenic effect of Gamma and EMS on the fresh seed yield and dry seed yield.

Length of tuber

Length of tuber was higher in Mulanur genotype (21.64 cms) followed by Chittor genotype (19.64 cms) exhibiting positive mutagenic increase over the control in EMS 1 percent and the lowest was recorded in Arupukotai (10.10) in Gamma at 4 percent. 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).

Weight of tuber

Increased weight of individual tuber subjected to EMS at1 percent concentration in 6 genotypes proved that EMS is more effective than Gamma ray. Weight of tuber was higher in Mulanur (71.85) and Chittor genotype (70.54) was on par exhibiting positive mutagenic increase over the control in EMS 1 percent and least tuber weight was recorded in Dharapuram genotype (45.01) followed by Vedaranyam (44.20) in 4 percent gamma ray. Thamburaj (1984) and Rajadurai (2001) obtained similar result in cassava and *Gloriosa superba* respectively.

Colchicine content of tubers

Quantity of Colchicine increase in tubers of 6 genotypes treated with EMS at 1 concentration proved that EMS is more effective than Gamma ray. Colchicine content was higher in Arupukotai (0.8567) followed by Chittor genotype (0.6821) exhibiting positive mutagenic increase over the control at 1 percent EMS concentration. Less colchicines content was recorded in Vedaranyam (0.0501) in Gamma ray at 4 percent. Due to the deleterious effect of higher concentrations lead to the reduced colchicines content.

Conclusion

Based on the experimental study in M1 generation of mutagenic tubers of *G. Superba* following conclusions were drawn. Positive mutagenic effect was observed in all the characters in EMS 1 percent over control for Chittor, Mulanur and Arupukotai varieties. Whereas negative mutagenic effect was observed in all characters of Nellore, Dharapuram and Vedaranyam over control except in colchicine content which

exhibited positive mutagenic effect over control. Comparing the results obtained in the six varieties treated with different concentrations of EMS and GY resulted in increase of negative mutagenic effect with increase of concentrations of EMS and GY. From the obtained results and performance of the varieties at different concentrations, Mulanur, Chittor and Arupukotai varieties were considered for further study.

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