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Research Note

Induced chlorophyll mutations in *Gloriosa superba*

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Abstract
The present experiment was carried out to study the spectrum of chlorophyll mutations, mutagenic effectiveness and efficiency in *Gloriosa superba* seeds using both chemical and physical mutagens. Self pollinated seeds of glory lily ecotype Gs-15 were treated with different doses of gamma rays *viz.,* 10, 20, 30 and 40 (Gy), EMS *viz.,* 10, 20, 30 and 40 mM and DES 15, 20, 25 and 30 mM. Both physical and chemical mutagens had significantly contributed to the production of the spectrum of chlorophyll mutants with higher mutagenic effectiveness and efficiency. The treatment of mutagens resulted in the production of four types of chlorophyll mutants *viz.,* albina, xantha, chlorina and viridis. The mutagenic effectiveness and efficiency were calculated based on biological damage as well as chlorophyll mutation frequency on M_1 plants. The results indicated that the mutagenic efficiency was the highest, at lower and intermediate concentrations of mutagens. The frequency of xantha (8.36) mutants were the highest followed by viridis (7.01) and other types were observed in all the treatments. Among different treatments, gamma rays were effective than the other two chemical mutagens in inducing different chlorophyll mutations. A comparatively lesser number of albina (2.13) mutants were observed with respect to all the mutagens studied. The higher frequency of mutations was observed in gamma irradiated seedlings (28.28) as compared to EMS (17.57) and DES (11.96). Likewise, the mutagenic effectiveness was at an increased score (81.10%) with 10 Gy gamma irradiation treatment. With regard to the EMS, based on lethality, increased mutagenic efficiency (27.37%) was observed at 20 mM concentration. Conversely, lower doses of physical mutagen (gamma radiation) induced a higher mutagenic rate, while EMS registered a lower score of mutations.

Key words: Glory lily, seeds, EMS, DES, gamma rays, chlorophyll mutants, mutagenic effectiveness and efficiency

Gloriosa superba L. (Colchicaceae) also known as Malabar glory lily is a perennial tuberous climbing herb, extensively scattered in the tropical and sub-tropical parts of India, including the foothills of the Himalayas. In India, it is widely distributed and is the state flower of Tamil Nadu. It is an important medicinal plant, which contains two major alkaloids, colchicine and colchicoside used to treat cancer related diseases, arthritis and gout. As a result of incessant over-exploitation of tubers from the wild, the species of *Gloriosa superba* is on the verge of extinction and included as RET species [Badola, 2002]. Tamil Nadu has the largest area under glory lily cultivation (up to 6000 acres) spread over seven districts *viz*., Karur, Tirupur, Dindigul, Salem, Ariyalur, Perambalur and Nagapattinam and holds monopoly in the production of glory lily seeds with an annual production of over 600 -700 tonnes in an area of about 6000 acres (Chitra and Rajamani, 2010). Though clonally propagated, Gloriosa is a highly cross-pollinated species and only limited attempts were made to induce variability towards a specific trait improvement. Mutation breeding is envisaged as complementary to conventional breeding due to the existence of polyploidy, crossing barriers, poor seed set and germination. Remarkable achievements made during recent years in this field have highlighted the utility and usefulness of mutation breeding in crop improvement.

The selection and acceptability of any mutagen for usage in a breeding programme relies both on their efficiency and effectiveness. The frequency of mutations induced by

a unit mutagen is referred to as mutagenic effectiveness, while the relative percentage of mutations causing detrimental changes like lethality, injury, sterility, mitotic and meiotic chromosomal aberrations etc., are known as mutagenic efficiency (Konzak *et al*., 1965). Whenever a plant is treated with either chemical or physical mutagens or their combinations, both synergistic as well as antagonistic effects are reported.

It is quite obvious that for the conduct of any mutation experiment, both chemical and physical mutagens are being engaged on a larger scale. The rate of mutation and the mutation frequency greatly decide upon the success of the mutation breeding programme. Several contributing factors *viz*., nature of mutagen used, transfer of linear energy, the dosage of mutagen, type of plant tissue etc. are responsible for the induction of spectrum of mutants. The mutagenic effectiveness and efficiency are not inter-related in most of the mutation experiments. Basically, increased efficiency of a particular mutagen represents comparatively less biological damage (seedling injury, ovule sterility, pollen sterility, etc.,) which are dependent on the mutant used (Shah *et al.,* 2008). Therefore, the choice of effective and efficient mutagen is very much needed to recover the high frequency of the desirable mutations in a mutation programme. Hence, the present study was taken up to understand the induction of the spectrum of chlorophyll mutants through the usage of chemical and physical mutagens.

The experiment was conducted in the Department of Medicinal and Aromatic Crops, TNAU, Coimbatore during 2017-18. Selfed seeds of genotype Gs-15 were subjected to mutagenic treatments with both physical and chemical mutagens. Graded seeds (100 seeds/treatment) was immersed in one litre of boiled water (heated upto100^{o}C and removed from heat source) and left to be retained in the same water overnight and shade dried were used for the experiment to permeate the hard seed coat for easy absorption of the mutagens. The different doses of physical and chemical mutagens were fixed based on the preliminary trials conducted. The seeds were subjected to gamma irradiation at varying levels *viz*., 10, 20, 30 and 40 Gy at the Gamma chamber available at Sugarcane Breeding Institute, Coimbatore. Concurrently, a fresh solution of Ethyl Methane Sulphonate (EMS) and Diethyl Sulphate (DES) was prepared using phosphate buffer at pH 7.0 in different concentrations *viz*., EMS (10, 20, 30 and 40 mM) and DES(15,20,25 and 30 mM), respectively. The seeds were treated for 1 hr at room temperature, followed by decanting of the mutagens. Based on the germination percentage of the seeds, the LD50 dose for gamma irradiation, EMS and DES were 20.96 kR, 1.91% and 1.13% respectively. The treated seeds were immediately sown in raised nursery beds (sterilized coco peat and sand 1:1 ratio) under shade net (50 per cent) on rows at a distance of 10 cm and 5 cm within the row.

The progenies of M_1 generation was screened for induction of chlorophyll mutants (Gustafsson (1940) and Blixt (1964). The different types of chlorophyll mutants are classified as given below (**Fig. 1**).

Albina : Includes mutants lacking chlorophyll or possessing relatively lesser amounts of the pigment.

Xantha : It constitutes light yellow leaves caused due to consists of pale yellow coloured leaves due to interruption of chlorophyll expression.

Viridis : Characterized by pale green leaves in nursery stage, that consequently changes to regular green colour over a period of time and becomes viable.

Chlorina : These mutants were yellow in colour.

The following are the formulae used for the calculation of mutagenic effectiveness, mutagenic efficiency and mutation rate suggested by Konzak *et al*. (1965)

$$\text{Mutagenic effectiveness} = \frac{\text{Mutagenic frequency (M)}}{\text{Dose (Gy) / Concentration of the mutagen (c) x time (t)}}$$

Mutagenic efficiency = M x 100/L; M x 100/I; M x 100/S

Where,
M - Chlorophyll mutation frequency
Gy - Dose of gamma radiation
c - Concentration of the chemical mutagen (mM)
t - Duration of treatment with chemical mutagen (hrs)
L - Percentage of lethality
I - Percentage of injury
S - Percentage of sterility

$$\text{Mutation rate} = \frac{\text{Total of efficiency or effectiveness of particular mutagen}}{\text{Number of treatments of a particular mutagen}}$$

The data of all characters recorded in M_1 generation of glory lily seedlings were analyzed using Statistical Analysis System software (SASs) V. 9.1.

The effect of chemical and physical mutagens on induction of chlorophyll mutations indicated that the survival percentage of seedlings of glory lily decreased with the increase in the dosages of both physical and chemical mutagens (**Table 1**). All mutagenic treatments showed the inhibitory effect on seed germination percentage. The highest rate of survival was observed in the control (65.25 %) while the lowest (28.75%) was documented in the seeds treated with 40 Gy gamma irradiation. With regard to chemical mutagens, survival percentage ranged from 35.12 to 42.82 per cent (EMS) and 37.55 to 45.38 per cent (DES). The reduction in plant survival is attributed to altered enzyme activities, cytogenetic

Fig. 1. Chlorophyll mutants isolated in M_1 generation in *Gloriosa superba* L.

damage and physiological disturbances caused by the mutagen (Shinde, 2013). Similar findings of reduced survival percentage due to mutagens were reported in fenugreek (Hanafy *et al.,* 2018) and Jasmine (Ghosh *et al*., 2019).

The complex process of photosynthesis is regulated by varied biochemical reactions catalyzed by several enzymes. The reduction in photosynthetic rate is mainly attributed to the occurrence of mutation in the genes that encode the synthesis of enzymes that are responsible for the biosynthesis of pigments or accelerate the metabolic pathways leading to their production. The resultant progenies are entitled chlorophyll-deficient mutants (Johnson, 2016). The chlorophyll mutants were classified with reference to the pattern of colour of whole plant leaves. The spectrum and frequency of chlorophyll mutants produced in M_1 generation of gloriosa are presented in **Table 2**. The dose or concentration of mutagen greatly influences the chlorophyll mutation frequency. The results indicate that the relative percentage of chlorophyll mutants were the highest for Xantha (8.36) followed by viridis (7.01). A comparatively lesser number of albina (2.13) mutants were observed with respect to all the mutagens studied. Gamma ray irradiated seedlings exhibited increased mutagenic frequency (28.28) as compared to EMS (17.57) and DES (11.96). The maximum frequency of chlorophyll mutation was observed in Gamma rays (20Gy) (9.09) followed by EMS (2%) (7.32), Gamma rays (10Gy) (6.76) and Gamma rays (40Gy) (6.67).

Table 1. Survival percentage in M_1 generation of mutated *Gloriosa superba* seedling progenies

Treatment	Number of seeds sown	Germination percentage	Survival %	% over control	% reduction over control
EMS (10mM)	100	54.50	42.82	65.62	34.38
EMS (20mM)	100	50.42	40.48	62.04	37.96
EMS (30mM)	100	45.22	37.48	57.44	42.56
EMS (40mM)	100	43.50	35.12	53.82	46.18
Control	100	73.00	66.50		
DES (15mM)	100	55.00	45.38	69.55	30.45
DES (20mM)	100	53.24	43.3	66.36	33.64
DES (25mM)	100	48.75	39.12	59.95	40.05
DES (30mM)	100	46.35	37.55	57.55	42.45
Control	100	73.45	67.82		
Gamma (10Gy)	100	46.12	38.14	58.45	41.55
Gamma (20Gy)	100	42.75	35.47	54.36	45.64
Gamma (30Gy)	100	40.10	32.25	49.43	50.57
Gamma (40Gy)	100	35.50	28.75	44.06	55.94
Control	100	72.52	65.25	100	-

Table 2. Frequency and spectrum of chlorophyll mutants in the M_1 generation of *Gloriosa superba*

Treatment	Number of plants observed	Spectrum of chlorophyll mutants				Total number of chlorophyll mutants	Relative percentage of chlorophyll mutants				Total mutagenic frequency (%)
		Albina	Xantha	Chlorina	Viridis		Albina	Xantha	Chlorina	Viridis	
EMS (1%)	87	1	1	1		3	1.15	1.15	1.15	0.00	3.45
EMS (2%)	82	2	1	2	1	6	2.44	1.22	2.44	1.22	7.32
EMS (3%)	75	1	1	1	1	4	1.33	1.33	1.33	1.33	5.33
EMS (4%)	68	1				1	1.47	0.00	0.00	0.00	1.47
Total	312	5	3	4	2	14	1.60	3.70	4.92	2.55	17.57
DES (1.5%)	92	1	1	1		3	1.09	1.09	1.09	0.00	3.26
DES (2%)	85	1	1	1	1	4	1.18	1.18	1.18	1.18	4.71
DES (2.5%)	78			1	1	2	0.00	0.00	1.28	1.28	2.56
DES (3%)	70				1	1	0.00	0.00	0.00	1.43	1.43
Total	325	2	2	3		10	0.57	2.26	3.55	3.89	11.96
Gamma (10Gy)	74	1	2	1	1	5	1.35	2.70	1.35	1.35	6.76
Gamma (20Gy)	66	2	1	2	1	6	3.03	1.52	3.03	1.52	9.09
Gamma (30Gy)	52	1	1		1	3	1.92	1.92	0.00	1.92	5.77
Gamma (40Gy)	45	1	1		1	3	2.22	2.22	0.00	2.22	6.67
Total	237	5	5	3	4	17	2.13	8.36	4.38	7.01	28.28

Table 3. Mutagenic effectiveness and efficiency based on chlorophyll mutations in the M_1 generation of *Gloriosa superba*

Treatments	% Survival reduction (Lethality)	% Height reduction (Injury)	Mutagenic frequency (M)	Mutagenic effectiveness (M × 100/ Gy (or) C × T)	Mutagenic efficiency M × 100/L	Mutation rate in terms of effectiveness
EMS (10mM)	22.50	10.83	3.45	34.50	15.33	27.20
EMS (20mM)	26.73	16.56	7.32	36.60	27.37	
EMS (30mM)	32.16	22.29	5.33	26.67	16.58	
EMS (40mM)	36.43	24.20	4.41	11.03	12.10	
DES (15mM)	17.86	7.64	3.26	21.73	18.25	15.07
DES (20mM)	21.63	12.10	4.71	23.55	21.76	
DES (25mM)	29.19	20.38	2.56	10.24	8.78	
DES (30mM)	32.04	21.66	1.43	4.77	4.46	
Gamma ray(10Gy)	30.97	39.49	8.11	81.10	26.18	40.61
Gamma ray(20Gy)	35.80	43.95	9.09	45.45	25.39	
Gamma ray(30Gy)	41.63	52.23	5.77	19.23	13.86	
Gamma ray(40Gy)	47.96	60.51	6.67	16.68	13.90	
Control	-	-	-	-	-	-

The occurrence of mutations in the genes responsible for the intermingling of enzymes involved in the biosynthesis of photosynthetic pigment is attributed for the prevalence of chlorophyll mutations (Emerson,1929). It is rational to understand that, chlorophyll mutations are used as the index to assess the sensitivity as well as the genetic effects of the plants exposed to different types of physical and chemical mutagens. Generally, chlorophyll deficit mutants experience a dearth of well-defined grana structure of the chloroplasts (Benedict and Ketring, 1972). Research suggests that the differential response of embryonic cells results in the development of chimeras leading to stimulation of varied responses in the mutated plants as a whole. Whenever a plant tissue is exposed to a mutagen, specifically gamma rays, the survival rate of the plant is questioned mainly due to the inactivation of certain genes encoding the synthesis of auxins causing cell division, resulting in poor establishment and ultimate survival (Wain and Wightman, 1956; Mahure *et al.,* 2010) or chromosomal aberrations caused due to lethal effect of gamma rays (Banerji and Datta, 1990).

The mutagenic effectiveness was found to be higher (81.10%) at 10 Gy gamma ray irradiation followed by 20 Gy (45.45%). EMS with a concentration of 20 mM recorded 36.60 per cent of mutagenic effectiveness, followed by 10 mM (34.50%) (**Table 3**). Higher mutagenic efficiency was noted in EMS (20 mM) based on lethality (27.37%) closely followed by Gamma ray (10 Gy) (26.18%). Among the three mutagens, a higher mutation rate (40.61) was recorded by gamma rays as compared to EMS (27.20) and DES (15.07). The representation in **Fig. 2** specifies that mutagenic efficiency and effectiveness was the highest at reduced dosages of mutagens.

The frequency of gene mutation is represented as mutagenic effectiveness and the relative quantity of mutation with reference to the undesirable changes such as injury, sterility and lethality is known as mutagenic efficiency. To attain precise accuracy, the mutagen used should supersede the effects of chromosomal alterations and other undesirable effects. Therefore the effectiveness of a mutagen involves the frequency of mutation and dosage of mutagen. In the present investigation, higher mutagenic effectiveness observed in a lower concentration of gamma rays is in corroboration with the findings of Padmadevi (2009) in chrysanthemum, Ghosh *et al.* (2019) in Jasmine and Naveena *et al.* (2020) in hibiscus. Certain physical properties such as solubility, toxicity and reactivity of chemical mutagens curtail their efficiency (Spencer-Lopes *et al.,* 2018).

The rate of mutation is a representation of the average amount of heritable changes induced by a particular mutagen. Generally, the efficiency of a mutagen is

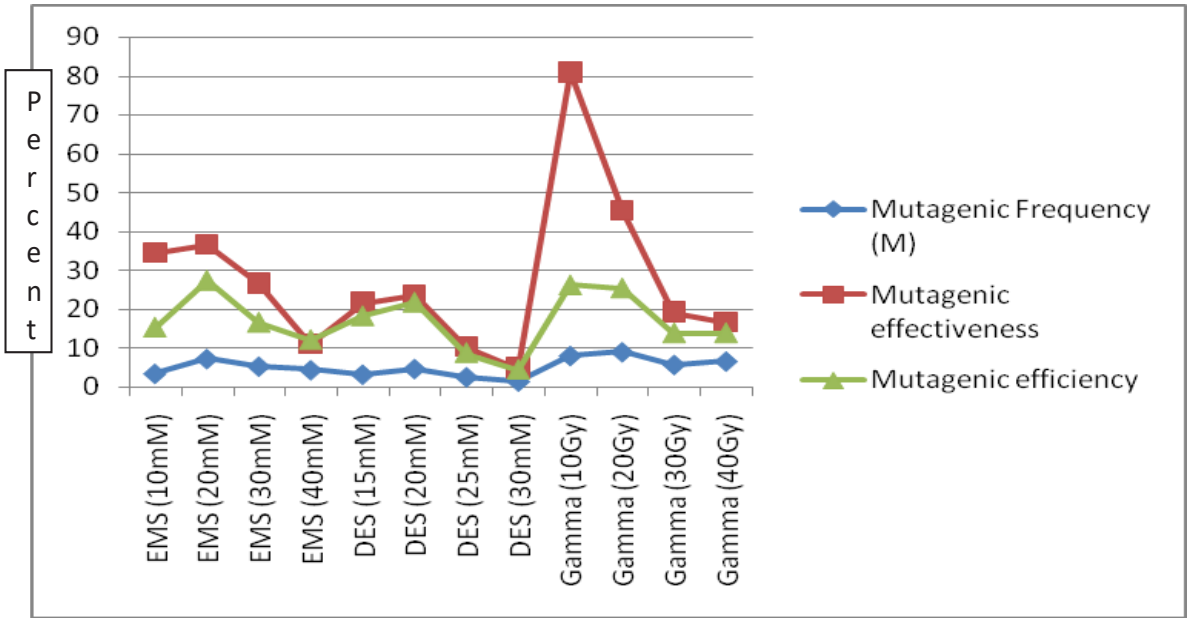

Fig. 2. EMS, DES and gamma radiation induced mutation frequency, effectiveness and efficiency in M_1 generation of *Gloriosa superba*

measured by the desirable changes that occur during the course of mutation. The highest mutagenic rate in terms of effectiveness was induced by lower doses of gamma radiation whereas, in the case of EMS, it is comparatively low (**Table 3**). Generally, the mutagen that gives a higher mutation rate also induces a high degree of lethality, sterility and other undesirable effects (Blixt *et al.,* 1964).

Thus from the current investigation, it is concluded that both physical and chemical mutagens were efficient in the production of chlorophyll mutants by treatment of seeds of *Gloriosa superba*. The mutagenic effectiveness was found to be higher (81.10%) at 10 Gy gamma ray irradiation followed by 20 Gy (45.45%). Likewise, higher mutagenic efficiency was noted in EMS (20 mM) based on lethality (27.37%). For the formulation of a successful breeding programme, induction of increased mutation rate along with least undesirable changes is more important. Moreover, the chlorophyll mutants are mostly developed as chimeric tissue, it is further suggested to perform research trials for handling the chimera formation and to advance the desirable mutants to future generations in order to bring out solid mutants for further improvement of glory lily.

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