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MUTATIONAL CHANGES IN *DELPHINIUM MALABARICUM* (HUTH.) MUNZ.: A POTENTIAL ORNAMENTAL PLANT

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ABSTRACT

Mutation breeding is an established method used for crop improvement and has played a major role in the development of many new flower color/shape mutant cultivars in ornamentals. The present study is aimed at inducing mutations in *Delphinium malabaricum* using chemical mutagens ethyl methane sulfonate (EMS), sodium azide (SA) and physical mutagen (gamma rays). It was observed that *D. malabaricum* manifested specific reactions to the treatments with EMS, SA and gamma rays. Identification and selection of mutations were carried out in the second generation (M_2). A variety of chlorophyll deficient mutants and high percentage of the flower color and morphological mutants were recorded. The maximum frequency of chlorophyll and flower color and morphological mutations were recorded in EMS treated plants when compared to the other two mutagens. The frequency values for the individual mutant types were varied and randomly distributed at different mutagenic treatments. The highest percentage of color mutants arose after treatments with 0.25% of EMS and the lowest at 20 kR of gamma rays. The mutants were quite distinct, as compared to the control and often had more attractive ornamental features compared to the starting material. The major commercial benefit of the application of this technology has so far been obtaining of novel flower mutants that can be used as an initial material for further breeding of new cultivars.

Key words: *Delphinium malabaricum,* mutation, EMS, SA, gamma rays, mutation frequency

INTRODUCTION

The genus *Delphinium* is one of the most important genera of the family Ranunculaceae, which represents a group of beautiful annuals, rarely biennial and perennial plants, commonly called Larkspurs. Among *Delphinium* blossoms, in shades of white, pink, scarlet, blue and purple, the blue flowering cultivars are the most common. The diversity of colors and shapes of their flowers bestows on this genus a very interesting ornamental potential. The genus has about 370 species (Blanché 1991), which grow wild on grazing meadows and stony slopes in the Himalayan region, with some species adapted to subtropical and others to subtemperate and temperate climatic conditions (Chowdhery & Wadhwa 1984; Polunin & Stainton 1984). In India, the genus *Delphinium* L. is represented by about 24 species (Rau 1993) mainly confined to the Himalayan regions excluding *Delphinium malabaricum,* which is the only species of the genus restricted to Northern Western Ghats of peninsular India. This plant has great ornamental value because of its attractive flowers of violet blue color. Novelty and uniqueness are two of the highly cherished objectives in ornamental plant improvement, and mutagenesis is an extremely useful technique for creating new genetic variability and for augmenting the existing one. Ornamental plants are ideal for the application of mutation techniques because economically important traits (e.g. flower characteristics or growth habit) are easily monitored after a mutagenic treatment.

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This method successfully produced quite a large variety of different plants having great demand, not only for their aesthetic appeal, but also for their economic value. Genetic variability has been induced through mutagenesis in several ornamental plants, but the information available on *D. malabaricum* is meager (Kolar et al. 2011), so induction of mutations was undertaken for the genetic improvement of this plant. In the present study, an attempt has also been made to understand the response of *D. malabaricum* to different physical and chemical mutagens, with a view to determining the type and dose of mutagen inducing maximum mutations.

MATERIALS AND METHODS

Induction of mutation

The seeds of *D. malabaricum* were collected in the locality Ajinkyatara at Satara district of Maharashtra, India. Dry and healthy seeds were used for inducing mutation by chemical mutagens – ethyl methane sulfonate (EMS), sodium azide (SA) and physical mutagen gamma rays. A total of 300 seeds were used for each concentration/dose of the treatment and control. Prior to the treatment the seeds were presoaked for 12 hours in distilled water and blotted dry. Non-treated seeds were used as control.

Mutagenic treatment

EMS and SA treatment. After presoaking, seeds were treated with freshly prepared aqueous solution of EMS and SA at concentrations: 0.01, 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30% for six hours at room temperature (23 ± 2 °C) with intermittent shaking. Immediately after the treatment, the seeds were washed thoroughly with distilled water for 30 minutes to leach out residual chemicals.

Gamma ray treatment. Seeds were irradiated from a ^{60}CO source at Bhabha Atomic Research Centre (BARC), Mumbai, with doses of 5, 10, 15, 20, and 25 kR at a dose rate of 28 Gy per minute.

Raising of mutant generation

After mutagenic treatment, seeds were sown in the experimental plots within the Botanical Garden, Department of Botany, Shivaji University, Kolhapur (MS) India. First generation (M_1) plants were grown to develop seeds of M_2 generation. M_2 populations were raised from a composite sample made by bulking 30 seeds from each M_1 plant of a treatment. The M_2 population was evaluated in two replications, each consisting of 150 seeds, with a total of 300 seeds in each treatment as well as in control. The M_2 plants were carefully screened for various mutations.

Selection of mutants

The treated and control M_2 plants were screened for chlorophyll mutations as well as for flower color and morphological variation. The chlorophyll mutations were classified according to Gustafson (1940) and Blixt (1961). Variations concerning the flower color were evaluated with the help of flower color shading chart of the Royal Horticultural Society, London, Flower Council of Holland, Leiden and the morphological variations were particularized by visual observations. The frequency and spectrum of mutations were calculated per 100 M_2 plants.

RESULTS AND DISCUSSION

Frequency and spectrum of chlorophyll mutants

Chlorophyll development is controlled by several genes located on different chromosomes as adjacent to centromere and proximal segments (Swaminathan 1964; Goud 1967) and also by non-chromosomal DNA (Levine 1972; Wildman 1973; Bonnett et al. 1993; Tambe et al. 2010). Different chlorophyll deficient mutants were observed in M_1 populations (Kolar et al. 2011) and in the M_2 segregating populations. They belonged to albino green, xantha, aurea, chlorina, viridis, yellow viridis, tigrina, striata, maculata and variegated types (Table 1, Fig. 1). The most frequent were viridis (24.2%), striata (8.12%), and variegated (6.63%) followed by yellow viridis (5.7%) and chlorine (4.08%). The reason for the appearance of a greater number of viridis may be attributed to the involvement of polygenes in the chlorophyll formation (Gaul 1964). Albina green type mutants were observed only after EMS treatment but their frequency was very low, while xantha type was observed only after 5 kR (0.45%) and 10 kR (0.48%) gamma ray treatment. Among the three mutagens used, EMS induced the highest frequency of chlorophyll mutations, followed by SA and gamma rays. In the M2 population derived from seeds treated with EMS the highest percentage of chlorophyll mutation was 7.8% (at 0.25%) and in SA 5.6% (at 0.10%). The highest chlorophyll mutation frequency recorded after

gamma ray treatments was 3.7% (at 15 kR). In the Shah et al. (2006) report, the overall frequencies and spectrum of four types of induced chlorophyll mutants in chickpea were viridis (9.0%), followed by xantha (8.6%), chlorina (5.8%), albina (0.5%), and others (0.26%). The frequency of total chlorophyll mutants varied for EMS, SA and gamma ray treatments from 1.2 to 7.8%, with the highest for EMS (0.25%).

The relative efficiency of mutagens in inducing chlorophyll mutations depends upon their specific action on DNA. EMS treatment causes gaps or minor deficiencies through depurination, incorporating mistakes at replication and repair process of DNA. The high incidence of chlorophyll mutations through EMS treatment may be due to specificity to affect certain regions of the chromosomes. High frequency and a wider spectrum of chlorophyll mutants in chemical mutagen EMS have been reported by Bhattacharya (2003), Sharma and Sharma (1984), Marki and Bianu (1970), Kawai and Sato (1969) in carnation, lentil, flax and rice respectively. The spectrum and frequency of chlorophyll mutations are assessed in M_2 population easily and is being used as the primary index of effectiveness of mutagens and mutability of the genotypes towards the mutagens which in turn would be useful to generate the wide array of desirable mutations in the treated population (Nilan & Vig 1976; Gottschalk & Wolff 1983; Hansel 1968).

Mutations of color and morphology of flowers

For the isolation of induced mutants, use of M_2 plants is more effective, because about 99% of all mutants concern recessive characters, therefore, the mutated genes are not discernible in M_1 plants. The physical and chemical mutagens succeeded in inducing numerous flower colors and their morphological deviations in *D. malabaricum* (Fig. 2 & 3) in wide-range frequencies (Tables 2 & 3). Flower color mutants varied from blue to pale pink and white with several intermediate colorations, whereas the control possessed blue color flowers. They were classified as dark blue, purple, sky blue, sky blue with pink shade and pale pink. The induced changes in color and forms were observed on the flower sepals and petals. Besides clear colors, differently colored spots, streaks, and sectors were observed. Clear color changes were more stable than some spots and streaks on the sepals, that is, they persisted in the M_3 generation. These stable changes may be regarded as mutations of the flower color qualitative structural genes; the unstable changes that disappeared in the next generations as changes in quantitative or regulatory genes or by transposable elements both were activated under the influence of environmental factors (Jaenisch & Bird 2003; Boyko et al. 2007). The percentage of flower color mutation in different mutagenic treatments ranges from 10.9 to 21.5% progeny of seeds treated by EMS, 7.8 to 14.0% by SA and 6.5 to 13.6% by gamma rays. The highest percentage of flower color mutations (21.6%) was observed in the population derived from seeds treated with 0.25% EMS (Table 2), similarly to that within chlorophyll mutations. Change in flower color has been reported many times, for example, by Datta and Gupta (1984) in rose, Raghava et al. (1988) in gladiolus, Kapoor et al. (2014) in marigold and by El-Mokadem et al. (2014) in *Browallia speciosa*. The flower color is controlled by many genetic factors, including structural genes, transcriptional factors and genes governing the whole metabolic chains or by epidermal structure, which affect the final color, and is a consequence of different events (Davies & Schwinn 1997; Mol et al. 1998). As a multigene trait of both quantitative and qualitative character, flower color of the same species can be a highly variable trait resulting from activation or suppression of various genes of a baseline genotype.

Morphological analysis of mutants revealed different variations in the flower and plant morphology relative to the control (Fig. 3). These variations included cup shape (Fig. 3 b, c), rounded sepals (Fig. 3 d), tapered sepals (Fig. 3 e, f), lobed sepals (Fig. 3 g, h), increased number of sepals (Fig. 3 i, j, k), presence of double spur (Fig. 3 l), long pedicellate flowers (Fig. 3 n), compact arrangement of flowers on an inflorescence (Fig. 3 p), and so on. The variation in flower morphological attributes observed in the progeny of seeds treated by EMS, ranged from 4.3 to 9.8%, from 3.7 to 7.8% in SA and from 4.29 to 7.0% in gamma ray progeny. The highest percentage of flower morphological mutations were observed in the progeny of seeds treated with 0.25% EMS (9.80%) and the lowest in 5 kR gamma ray treatment (Table 3).

Fig. 1. Chlorophyll mutants in M_2 generation of *Delphinium malabaricum* (Huth) Munz. a – Control, b – Albina green, c – Aurea, d – Chlorina, e – Viridis, f – Yellow viridis, g – Tigrina, h – Striata, i – Maculata, j – Variegated

Fig. 2. Flower color mutants selected in M_2 generation of *D. malabaricum*. Control – a – blue flowers; Mutants – b-t: b – dark blue, c, d – light violet, e – fresh sky blue, f, g – sky blue with pink tinge, h, i – light blue with purple shade, j – blue with white tip, k, l – dark purple, m, n – light purple, o – blue with white petals, p, q, r – blue with purple shades, s – pale pink, t – white

Fig. 3. Flower morphological mutants selected in M_2 generation of *D. malabaricum*. Control – a – blue flowers; Mutants – b-p: b, c – cup shaped, d – flowers with round sepals, e, f – flowers with tapered sepals, g, h – flowers with lobed sepals, i, j, k – flowers with double number of petals, l – double spurred flower, m – control flower with short pedicel, n – mutant flower with long pedicel, o – control inflorescence showing loose arrangement of flowers, p – mutant inflorescence showing compact arrangement of flowers.

Table 1. Frequency and spectrum of chlorophyll mutants in M_2 generation of *Delphinium malabaricum*

Treatment	Concentration or dose of mutagen	Relative frequency(%) of chlorophyll spectrum per 100 M_2 plants											Total frequency (%)
		Albina	Albina green	Xantha	Aurea	Chlorina	Viridis	Yellowviridis	Tigrina	Striata	Maculata	Variegated	
Control		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
EMS	0.01%	0.00	0.00	0.00	0.00	0.00	0.41	0.00	0.00	0.82	0.00	0.00	1.23
	0.05%	0.00	0.00	0.00	0.43	0.43	0.87	0.87	0.00	0.00	0.00	0.43	3.07
	0.10%	0.00	1.38	0.00	0.00	0.00	0.00	0.46	0.42	0.00	0.00	0.00	2.27
	0.15%	0.00	0.46	0.00	0.00	0.00	0.93	1.40	0.00	0.93	0.00	0.00	3.75
	0.20%	0.00	0.00	0.00	0.00	0.00	0.52	0.52	0.00	0.52	0.00	0.00	1.56
	0.25%	0.00	1.66	0.00	0.55	0.11	2.22	0.00	0.00	1.11	0.00	1.11	7.77
	0.30%	0.00	0.00	0.00	0.62	0.62	1.88	0.62	0.00	0.00	0.00	0.00	3.77
SA	0.01%	0.00	0.00	0.00	0.00	0.00	4.22	0.00	0.00	0.00	0.00	0.00	1.40
	0.05%	0.00	0.00	0.00	0.00	0.00	2.53	0.00	0.00	0.00	0.00	3.79	3.79
	0.10%	0.00	0.00	0.00	0.00	1.38	0.00	0.00	0.00	2.77	1.38	0.00	5.55
	0.15%	0.00	0.00	0.00	0.00	0.00	1.49	0.00	0.00	1.49	0.00	0.00	2.98
	0.20%	0.00	0.00	0.00	0.00	0.00	1.56	0.00	0.00	0.00	0.00	0.00	1.56
	0.25%	0.00	0.00	0.00	0.00	0.00	3.33	0.00	0.00	0.00	0.00	0.00	3.33
	0.30%	0.00	0.00	0.00	0.00	0.00	1.69	0.00	0.00	0.00	0.00	0.00	1.69
Gamma Rays	5KR	0.00	0.00	0.45	0.00	0.45	0.90	0.00	0.00	0.00	0.00	0.45	2.25
	10KR	0.00	0.00	0.48	0.00	0.48	1.93	0.00	0.00	0.48	0.00	0.00	3.38
	15KR	0.00	0.00	0.00	0.00	0.61	1.23	1.85	0.00	0.00	0.00	0.00	3.70
	20KR	0.00	0.00	0.00	0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.85	1.28

Table 2. Frequency and spectrum of flower color mutations in M_2 generation of *Delphinium malabaricum*

Mutagen	Concentration or dose of mutagen	Flower color mutations (%)												
		Dark blue	Light violet	Fresh sky blue	Sky blue with pink tinge	Light blue with purple shades	Blue with White tip	Dark purple	Light purple	Blue with white petals	Blue with purple shades	Pale pink	White	Total
RHS color code		105A	85B	107B	89D	83C	99B	77A	85B	100A	82A	75D	155A	
Control (Blue – 95B)		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
EMS	0.01%	0.00	0.00	0.00	4.23	0.00	0.00	2.82	1.41	0.00	2.82	0.00	0.00	11.2
	0.05%	1.52	0.00	0.00	3.03	0.00	0.00	1.52	0.00	0.00	6.06	1.52	0.00	13.6
	0.10%	0.00	0.00	0.00	1.59	0.00	3.17	6.35	0.00	0.00	0.00	0.00	0.00	11.1
	0.15%	0.00	1.82	0.00	0.00	0.00	0.00	1.82	3.64	0.00	3.64	0.00	0.00	10.9
	0.20%	0.00	0.00	0.00	0.00	0.00	0.00	3.51	3.51	0.00	7.02	0.00	0.00	14.0
	0.25%	0.00	3.92	3.92	1.96	0.00	1.96	1.96	1.96	0.00	5.88	0.00	0.00	21.5
	0.30%	0.00	2.13	0.00	0.00	0.00	2.13	0.00	2.13	0.00	6.38	0.00	0.00	12.7
SA	0.01%	3.03	0.00	0.00	0.00	0.00	0.00	1.52	1.52	0.00	3.03	0.00	1.52	10.6
	0.05%	0.00	0.00	0.00	3.23	0.00	0.00	1.61	3.23	0.00	4.84	0.00	0.00	12.9
	0.10%	0.00	0.00	0.00	6.25	0.00	0.00	0.00	1.56	0.00	6.25	0.00	0.00	14.0
	0.15%	0.00	0.00	0.00	3.17	1.59	0.00	3.17	0.00	0.00	3.17	0.00	0.00	11.1
	0.20%	1.79	0.00	0.00	0.00	0.00	0.00	1.79	1.79	0.00	3.57	0.00	0.00	8.93
	0.25%	0.00	0.00	0.00	5.56	0.00	0.00	0.00	0.00	0.00	3.70	0.00	0.00	9.26
	0.30%	0.00	0.00	0.00	3.92	0.00	0.00	1.96	0.00	0.00	1.96	0.00	0.00	7.84
Gamma Rays	5KR	0.00	0.00	0.00	2.86	0.00	0.00	1.43	1.43	0.00	1.43	0.00	0.00	7.14
	10KR	3.03	1.52	0.00	0.00	0.00	0.00	0.00	3.03	1.52	4.55	0.00	0.00	13.6
	15KR	0.00	3.51	0.00	1.75	0.00	0.00	0.00	1.75	0.00	5.26	0.00	0.00	12.2
	20KR	4.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.17	0.00	0.00	6.52

Table 3. Frequency and relative percentage of flower morphological mutations in M_2 generation of *Delphinium malabaricum*

Mutagen	Concentration or dose of mutagen	Flower morphological mutations (%)								
		Morphological attributes								
		Cup shaped	Flowers with round sepals	Flowers with tapered sepals	Double spurred	Double petals	Compact arrangement	Long pedicellate flower	Lobed sepals	Total
Control		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
EMS	0.01%	0.00	2.82	0.00	0.00	0.00	2.82	0.00	0.00	5.64
	0.05%	1.52	3.03	0.00	0.00	0.00	0.00	0.00	0.00	4.55
	0.10%	1.59	1.59	1.59	0.00	1.59	0.00	0.00	0.00	6.35
	0.15%	1.82	3.64	0.00	0.00	0.00	0.00	0.00	0.00	5.45
	0.20%	0.00	0.00	5.26	1.75	0.00	0.00	0.00	0.00	7.02
	0.25%	0.00	5.88	3.92	0.00	0.00	0.00	0.00	0.00	9.80
	0.30%	0.00	2.13	2.13	0.00	0.00	0.00	0.00	0.00	4.26
SA	0.01%	0.00	3.03	0.00	0.00	0.00	0.00	1.52	0.00	4.55
	0.05%	1.61	1.61	1.61	0.00	0.00	1.61	0.00	0.00	6.45
	0.10%	1.56	3.13	0.00	0.00	0.00	3.13	0.00	0.00	7.82
	0.15%	0.00	1.59	1.59	0.00	1.59	1.59	0.00	0.00	6.35
	0.20%	0.00	1.79	0.00	0.00	0.00	3.57	0.00	0.00	5.36
	0.25%	0.00	0.00	0.00	0.00	1.85	1.85	0.00	0.00	3.70
	0.30%	0.00	0.00	0.00	0.00	0.00	1.96	1.96	0.00	3.92
Gamma rays	5KR	0.00	1.43	0.00	0.00	0.00	2.86	0.00	0.00	4.29
	10KR	1.52	1.52	0.00	0.00	0.00	0.00	0.00	3.03	6.06
	15KR	0.00	1.75	1.75	0.00	1.75	1.75	0.00	0.00	7.00
	20KR	2.17	2.17	0.00	0.00	0.00	0.00	0.00	0.00	4.35

Among the mutagens, EMS proved to be also most effective in inducing changes on morphological attributes followed by SA and gamma rays. The mutants were morphologically quite distinct, as compared to the control and some have more attractive ornamental features.

There are numerous reports on alteration of flower color of ornamental plants arising as a result of mutagenic treatment. Schum and Preil (1998) reported that 55% of the records on induced mutation in ornamental plants concerned changes in flower color and 15% in flower morphology. Laneri et al. (1990) found six different flower color mutants and 13 types of variations affecting flower morphology following irradiation of in vitro shoots of a pink cultivar of gerbera. For many species, mutation induction leads to changes of an ornamental value including an increase or decrease in petal number, for example, in rose (Walther & Sauer 1986).

CONCLUSION

Mutation breeding appears to have a specific advantage for *D. malabaricum*. The new flower colors combined with novelty in flower morphology, compactness, and leaf patterns obtained in the present study are desirable for current horticulture and may be utilized in a future breeding program.

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