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INDUCTION OF GENETIC VARIABILITY AND ISOLATION OF MUTANTS IN DOUBLE TYPE TUBEROSE (*Polianthes tuberosa* L.) VAR. SUVASINI

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ABSTRACT
The investigation was carried out to study the effect of various mutagenic treatments on vegetative and floral characters on different cultivars of tuberose and isolation of promising mutants. Four varieties *viz*., Kalyani Single, Kalyani Double, Suvasini and Prajwal were treated with 2 doses each of gamma rays *viz*., (0.5 Kr, 1.5 Kr), X-rays (0.6 Kr, 1.2 Kr) and Ethyl Methyl Sulphonate (0.1 per cent, 0.2 per cent). The plant height ranged from 18.64 cm to 29.52 cm, spike length ranged from 54.38 cm to 89.21 cm and total florets per spike ranged from 13.50 to 42.57 among different treatment combinations. Six mutants were obtained exhibiting dwarfness in plant height (18.64 cm) [cv. Prajwal treated with EMS (0.2%)], increase in number of petals per floret (8) (cv. Prajwal treated with 1.2 Kr X-rays), fusion of two floret into one [cv. Suvasini and cv. Prajwal treated with EMS (0.2%)], decrease in number of whorl per floret (2) [cv. Suvasini with 1.2 Kr gamma rays treatment] and presence of stamen in double type cultivar (cv. Suvasini with plant treated with 1.2 Kr X-rays).

INTRODUCTION

Floriculture is a dynamic industry and demands for novelty in existing crops and products. Development of new cultivars through conventional or modern techniques has been a prime objective in commercial floriculture. New colour, earliness, stem length, number of flowers, plant architecture, resistance to abiotic and biotic stresses, productivity and keeping quality are the main attributes required in new cultivars. These new cultivars in existing crops could be produced by the introduction, hybridization and through molecular techniques such as genetic engineering through the alternation of characteristics such as flower colour and plant form. Over the past 50 years, the use of induced mutations (through irradiation and chemical agents) has also played a major role in the development of superior crop varieties (Datta, 1997). Mutation is a method by which novelty can be created in already well established cultivar. There is no visual difference between artificially produced or induced mutants and spontaneous mutants found in nature (Broertjes, 1968).

Ornamental plants appear to be ideal system for application of mutation induction technique as many characters of economic importance i.e. flowering traits or growth habit are easily monitored after mutagenic treatment. Any change in the dominant genes is easily expressed in the first generation and thus the selection of mutant of directly perceptible characters like flower colour, shape, size *etc*., is generally very easy and can directly be put to

commercial use. Furthermore, many ornamental species are heterozygous and are often propagated vegetatively thus allowing the detection, selection and conservation of mutants within M1 generation (Van Hartan, 2002). In ornamentals, the first artificially induced commercial mutant cv. Faraday, a flower colour mutant in tulip, was released in 1949 in The Netherlands by W. E. de Mol from X-ray irradiated bulbs of cv. Fantasy, following irradiation in 1936. A second flower colour mutant cultivar in tulip cv. Estella Rijnveld, was released by the same researcher in 1954 (Van Hartan, 2002).

Tuberose (*Polianthes tuberosa* Linn.) is a popular fragrant cut flower of Tropical and Subtropical regions of India. There are very few cultivars of tuberose in production worldwide. In all the existing varieties, flower colour is limited to white only, although some varieties show pinkish tinge at bud stage. To develop more variation in biotic and abiotic traits such as disease resistance, flower shape, vase life etc., in tuberose there is an urgent need of well planned breeding programme using conventional and nonconventional breeding techniques. The present study was carried out to assess the vegetative and floral characteristics of potential varieties of tuberose as influenced by different mutagens and doses used and to screen mutants of existing cultivars through mutation induced variation for desirable traits.

MATERIALS AND METHODS

The tuberose cultivars Kalyani Single (V1), Kalyani Double (V2), Suvasini (V3) and Prajwal (V4)[Plate-1] which have been found promising were selected for the present investigation.

Healthy and uniform bulbs of appropriate size (1.5-2.0 cm in diameter) were used for mutagenic treatments and subsequently planting out. The bulbs of selected cultivars were obtained from the germplasm being maintained at the Model Floriculture Centre of the University. The bulbs were exposed to Gamma rays [0.5 Kr] (T1), Gamma rays [1.5 Kr] (T2), X-rays [0.6 Kr] (T3), X-rays [1.2 Kr] (T4), EMS [0.1%] (T5), EMS [0.2%] (T6) and control (T7). The Gamma irradiation facility of National Botanical Research Institute, Lucknow having Gamma chamber-900 with source of 60Co, X-ray machine of Department of Entomology (Narang) of the University were availed for treating the bulbs with physical mutagens. The bulbs of selected varieties were dipped and subjected to continuous shaking in freshly prepared solution of 0.1 and 0.2 per cent of EMS for 12 hours and then were dried under shade before planting in the field.

The experimental area was laid out in Randomized Block Design. Eighty four plots of 1x1 m2 were laid out to accommodate all the twenty eight treatments replicated three times. The bulbs were planted at a spacing of 30 x 30 cm at a depth of 5-7 cm in month of April. The plants were maintained under uniform cultural conditions throughout the period of investigation.

RESULTS AND DISCUSSION

Vegetative characters

Data presented in Table 1 on plant height revealed that there was a significant effect of cultivars, mutagenic treatments and their interactions on plant height. Treatment 0.2 per cent EMS (T6) treated plants gave maximum (26.13 cm) mean plant height which was significantly higher than rest of the treatments while minimum (22.34 cm) mean plant height was found in plants treated with 1.5 Kr gamma-rays (T2). Among interactions, maximum (29.52 cm) plant height was found with 0.2 per cent EMS in cv. Prajwal (T6V4) which was statistically at par with T2V3 (29.32 cm), T5V2 (28.82 cm), T4V2 (27.58 cm), T1V4 (27.04 cm) and while minimum plant height (18.64 cm) in cv. Suvasini with 0.5 Kr gamma rays treatment (T1V3).

There was differential response of mutagenic treatment on plant height which was highly influenced by cultivar. With respect to the control, there was a slight increase in plant height in most of the cultivars after mutagenic treatment. Increase in plant height was slightly more at higher doses (1.5 Kr gamma rays, 1.2 X-ray and 0.2 per cent EMS) compared to lower doses of mutagenic treatment except X-rays treatment. Usharani and Ananda Kumar (2015) reported identification of dwarf types in urdbean among 40kR, 60kR suited for lodging resistant. Fowler and Mac Queen (1972) hypothesized that most of the reported stimulatory effects of low doses of radiation was due to early modifications in axillary bud development and changes in initial rate of floral differentiation.

A perusal of data for number of leaves reveals that among treatments, 0.5 Kr gamma rays (T1) gave maximum (47.25) number of leaves which was significantly higher than rest of the treatments while minimum (38.25) number of leaves was found in 1.5 gamma rays (T2) treatment. Among interactions, maximum (59.73) number of leaves per plant was found with untreated (control) plants of cv. Suvasini (T7V3) which was significantly higher than rest of the treatment combinations

Table 1: Effect of different mutagenic treatments on vegetative characters

Treatment	Plant Height (cm)					Number of leaves per plant				
	V_1	V_2	V_3	V_4	Mean	V_1	V_2	V_3	V_4	Mean
T_1	20.92	24.73	18.64	27.04	22.83	44	39	54	52	47.25
T_2	19.28	21.7	29.32	19.05	22.34	37	39	34	43	38.25
T_3	23.83	25.49	19.85	20.43	22.4	36	44	42	42	41.98
T_4	24.3	27.58	22.22	23.36	24.36	37	49.87	45	42	43.47
T_5	23.58	28.82	21.68	24.53	24.65	51	47	44	42	46
T_6	22	26.51	26.49	29.52	26.13	54.59	35	32	39	40.15
T_7	22.08	26.41	26.41	24.66	24.89	43	39	59.73	42	43.95
Mean	22.28	25.89	23.52	24.08	23.94	43.23	41.84	44.39	43.14	43.15
		CD (5%)	SeM±				CD (5%)	SeM±		
Varieties		1.46	0.52				0.93	0.33		
Treatments		1.11	0.39				1.23	0.43		
Interaction		2.93	1.03				2.45	0.87		

Table 2 : Effect of different mutagenic treatments on vegetative characters

Treatment	Chlorophyll content index (μg/cm2)					Leaf length (cm)				
	V_1	V_2	V_3	V_4	Mean	V_1	V_2	V_3	V_4	Mean
T_1	46.94	44.95	46.04	43.37	45.32	16.1	11.33	31.79	27.52	21.69
T_2	45.54	45.13	44.83	42.33	44.46	21.63	22.17	30.96	8	20.69
T_3	43.09	46.26	49.43	49.98	47.19	7.94	19.27	13	14	13.55
T_4	49.72	48.2	49.54	47	48.62	11.35	20	20.86	14.13	16.58
T_5	44.45	48.01	47.46	44.64	46.14	22	21.19	28	21.46	23.16
T_6	45.3	45.82	47.08	46.55	46.19	26.58	22.15	17.19	22.5	22.1
T_7	46.39	49.13	48.54	49.86	48.48	13.54	25.4	22.21	30.54	22.92
Mean	45.92	46.79	47.56	46.25	46.63	17.02	20.22	23.43	19.74	20.1
			CD (5%)	SeM±			CD (5%)	SeM±		
Varieties			1.19	0.42			1.43	0.50		
Treatments			1.57	0.55			1.89	0.67		
Interaction			3.15	1.11			3.78	1.33		


Kalyani Single


Suvasini


Prajwal


Kalyani double

Plate 1: Tuberose varieties


Mutant 1 (with 1.5 gamma rays treatment in cv.Suvasini)


Mutant 2 (with 0.2 percent EMS treatment in cv.Suvasini)

Plate 2: Mutants of Tuberose cv Suvasini

Table 3: Effect of different mutagenic treatments on floral characters

	Spike length (cm)					Rachis length (cm)				
Treatment	V_1	V_2	V_3	V_4	Mean	V_1	V_2	V_3	V_4	Mean
T_1	56.63	54.38	81.92	66.79	64.92	27.08	26.71	28.29	31.63	28.43
T_2	72.08	73.83	88.08	64.13	74.53	23.38	25.58	30.21	23.75	25.73
T_3	71.41	73.5	80	79.33	76.06	27.29	31.96	32.21	26.38	29.46
T_4	64.58	87.83	67.25	71.75	72.85	26.45	26.25	22.08	30.67	26.45
T_5	75.75	74.04	77	76.13	75.73	26.25	27.62	26.13	28.46	27.11
T_6	89.21	63.33	58.83	73.8	70.94	25.92	23.56	24.96	23.92	24.59
T_7	78	66.04	74.42	82.92	75.34	25.33	25.33	37.13	24.04	27.96
Mean	72.91	70.42	75.35	73.35	72.92	26	26.72	28.72	26.98	27.1
			CD (5%)	SeM±			CD (5%)	SeM±		
Varieties			3.12	1.1			1.08	0.38		
Treatments			4.13	1.46			0.82	0.29		
Interaction			8.26	2.91			2.16	0.76		

while minimum (32) number of leaves was observed in cv. Suvasini with 0.2 per cent EMS (T6V3) treatment. Number of leaves per plant decreased in all cultivars with increased dose of mutagen irrespective of treatments when compared to control. Decrease in number of leaves per plant was lesser in lower dose as compared to higher dose of mutagenic treatment. Abraham and Desai (1976) in tuberose, Sobhana and Rajeevan (2003) in Dendrobium also reported decrease in number of leaves per plant with increase in dose of mutagen. Ahirwar *et al*. (2014) reported highly significant differences for all the morphological and yield traits in lentil. Gordon and Weber (1950) who reported that a decrease in auxin level in leaves of Zea mays after 25 or 100 rads of X-rays and concluded that destruction of enzyme system or inhibition of auxin synthesis due to irradiation could result in decrease in vegetative growth. Inhibition of mitotic activities and chromosome damage associated with secondary physiological damage could also be the cause for reduction in vegetative growth as reported by Sparrow (1961) who studied cytological effects of ionisation of different plants.

It is apparent from data presented in Table 2 that there was significant effect of cultivars, mutagenic treatments and their interactions on chlorophyll content of leaves. Among treatments, 1.2 Kr Xrays (T4) resulted in maximum (48.62 μg

Mutant 3 (with 1.2 Kr X-ray treatment in cv.Prjwal)

Mutant 4 (with 0.2 percent EMS treatment in cv.Prjwal)

Plate 3: Mutants of Tuberose cv Prajwal

Table 4: Effect of different mutagenic treatments on floral characters (contd..)

Treat-ment	Opened florets					Unopened florets					Total florets				
	V_1	V_2	V_3	V_4	Mean	V_1	V_2	V_3	V_4	Mean	V_1	V_2	V_3	V_4	Mean
T_1	11.13	6.04	25.45	30.37	18.25	10.21	7.46	12.13	12.2	10.5	21.34	13.5	37.58	42.57	28.75
T_2	7.18	12.48	11.95	18.01	12.4	9.48	5.2	10.46	8.97	8.53	16.66	17.68	22.41	26.98	20.93
T_3	28.27	9.13	30.93	16.67	21.25	13.3	17.79	9.14	11.47	12.92	41.57	26.92	40.07	28.14	34.17
T_4	19.3	12.37	20.51	12.8	16.24	15.17	6.17	12.9	8.92	10.79	34.47	18.54	33.41	21.72	27.03
T_5	11.37	19.33	26.53	18.61	18.96	15.16	8.77	11.1	12.13	11.79	26.53	28.1	37.63	30.74	30.75
T_6	18.39	15.28	21.14	21.23	19.01	8.89	6.75	11.33	8.85	8.95	27.28	22.03	32.47	30.08	27.96
T_7	25.55	14.16	29.72	25.42	23.71	12.73	10.88	8.33	10.32	10.56	38.28	25.04	38.05	35.74	34.27
Mean	17.31	12.68	23.75	20.44	18.55	12.13	9	10.77	10.41	10.58	38.28	21.68	34.52	30.85	29.13
		CD (5%)	SeM±			CD (5%)	SeM±					CD (5%)	SeM±		
Varieties		1.10	0.39			0.53	0.19					1.16	0.41		
Treatments		1.46	0.52			0.70	0.25					1.54	0.54		
Interaction		2.92	1.03			1.41	0.50					3.08	1.08		

Table 5: Effect of mutagenic treatments on floral characters

Treatment	Duration of flowering (days)					Vase life (days)				
	V_1	V_2	V_3	V_4	Mean	V_1	V_2	V_3	V_4	Mean
T_1	12.69	28.69	19.69	15.69	19.19	7.24	5.24	10.24	10.04	8.19
T_2	11.69	23.69	15.69	9.69	15.19	8.24	6.44	11.24	8.25	8.55
T_3	14.69	21.69	22.69	16.69	18.94	6.24	9.25	8.45	9.25	8.29
T_4	10.69	18.69	17.69	11.69	14.69	8.25	10.24	11.24	8.24	9.49
T_5	12.69	18.69	15.69	13.69	15.19	12.24	11.25	7.45	9.15	9.52
T_6	9.69	22.69	12.69	9.69	12.69	13.25	8.25	7.45	9.15	7.45
T_7	15.69	21.69	21.69	15.69	18.94	7.45	8.25	7.25	9.92	8.21
Mean	12.54	17.97	17.97	13.26	16.4	8.98	8.41	9.41	9.04	8.97
		CD (5%)	SeM±			CD (5%)	SeM±			
Varieties		0.53	0.19			0.66	0.23			
Treatments		0.70	0.25			0.87	0.308			
Interaction		0.14	0.49			0.17	0.62			

cm-2) chlorophyll content of leaf which was statistically at par with T7 (48.48 μg cm-2) and T3 (47.19 μg cm-2) while minimum (44.46 μg cm-2) chlorophyll content of leaf was found in 1.2 Kr gamma rays (T2). Among interactions, maximum (49.98 μg cm-2) chlorophyll content of leaf was found in cv. Prajwal with 0.6 Kr X-rays treated plants (T3V4) which was statistically at par with T7V4 (49.86 μg cm-2), T4V1 (49.72 μg cm-2), T4V3 (49.54 μg cm-2),T3V3 (49.43 μg cm-2), T7V2 (49.13 μg cm-2), T5V2 (48.01 μg cm-2),T4V2 (48.2 μg cm-2), T7V3 (48.54 μg cm-2), T5V3 (47.46 μg cm-2),T6V3 (47.08 μg cm-2), T4V4 (47 μg cm-2) and T1V1 (46.94 μg cm-2) while minimum (42.33μg cm-2) chlorophyll content of leaf was observed in cv. Prajwal with 1.5 Kr gamma-rays (T2V4 μg cm-2) treated plants. Significant variation in the chlorophyll content due to mutagenic treatment was also reported by Swaminathan (1964) in wheat while comparing mutation induction in diploids and polyploids and Kolar *et al*. (2011) in Delphinium malabaricum (Huth) Munz while studying gamma ray induced chlorophyll mutations. Variation in chlorophyll development seems to be controlled by many genes located on several chromosomes which could be adjacent to centromere and proximal segment of chromosome

Mutant 5 (with 1.2 Kr X-ray treatment in cv.Suvasini)

Mutant 5 mutant (with 0.2 percent EMS treatment in cv Prajwal)

Plate 4: Mutants of tuberose cvs Suvasini and Prajwal

(Swaminathan, 1964).

It is evident from the data presented in Table 2 that 0.1 per cent EMS (T5) gave maximum (23.16 cm) leaf length per plant which was statistically at par with T1 (21.69), T6 (22.10) and T7 (22.92) while minimum (13.55 cm) leaf length was found in 0.6 Kr X-rays (T3) treated plants. Among interactions, maximum (31.79 cm) leaf length was found in cv. Suvasini with 0.5 Kr gamma rays (T1V3) treated plants which was statistically at par with T2V3 (30.96 cm) while minimum (7.94 cm) leaf length was observed in the Kalyani Single with 0.6 Kr X-rays (T3V1) treated plants.

There was a differential response of cultivars for mutagenic treatments resulting in non linear decrease in leaf length in most of the cultivars when compared to control while EMS and gamma rays treatment resulted in increase in leaf length in cv. Kalyani Double (V1) and cv. Suvasini (V3). Banerji and Datta (2001) while analysing gamma rays-induced mutation in 'Lalima' chrysanthemum (Chrysanthemum morifolium), Dwivedi and Banerji (2008) in gamma induced mutant 'Pinki' of dahlia, and Dilta *et al*. (2003) in gamma rays induced mutation in chrysanthemum also reported decrease in leaf length with increase in dose of mutagen.

Reduction in leaf length are associated with abnormalities which are resulted due to disturbances by phytochromes was reported by Moh (1962) while studying biological effects of X-rays irradiation in Coffee. Sparrow (1961) while working on cytological effect of radiation concluded that decrease in vegetative growth was as a result of radiation induced cytological changes such as chromosomal damages, inhibited mitotic division, degeneration of nuclei, cell enlargement etc.

Floral characters: It is evident from the data pertaining to spike length presented in Table 3 that within treatments, maximum spike length (76.06 cm) was observed in 0.6 Kr X-rays (T3) while minimum (64.92 cm) spike length was found in 0.5 Kr gamma rays (T1). Among interactions, maximum (89.21 cm) spike length was found in cv. Kalyani Single with 0.2 per cent EMS (T6V1) while minimum (54.38 cm) plant spread was observed in cv. Kalyani Double with 0.5 Kr gamma rays (T1V2). Karki and Srivastava (2010) who studied the effect of gamma irradiation on various growth and flowering attributes on 20 varieties of gladiolus also concluded that lower doses *i.e*. 0.5 and 1.5 Kr was effective in improving some important vegetative and floral parameters. In case of rachis length, the 0.6 Kr X-rays rays (T3) gave maximum (29.46 cm) rachis length per plant while the minimum (24.59 cm) rachis length was found in 0.2 per cent EMS (T6). Among interactions, the maximum (37.13 cm) rachis length was found in cv. Suvasini with control (T7V3) while minimum (22.08 cm) rachis length was observed in cv. Suvasini with 1.2 Kr X-rays (T4V3) [Table 3].

The total number of florets was recorded maximum (34.27) in untreated plants (T7) while minimum (20.93) number of total floret per spike was found in 1.5 Kr gamma rays (T2) [Table 4]. Maximum (12.92) number of unopened florets per spike was in 0.6 Kr X-rays treatment (T3) while minimum (8.53) number of unopened florets per spike was found in 1.5 Kr gamma rays treatment (T2). The number of opened florets per spike were maximum (23.71) in untreated plants (T7) while minimum (12.40) number of opened florets per spike was found in 1.5 Kr gamma rays (T2). Among interactions, number of total florets per spike was maximum (42.57) in cv. Prajwal with 0.5 Kr gamma rays (T1V4) treatment while minimum (13.50) number of total florets per spike was found in cv. Kalyani Double with 0.5 Kr gamma rays (T1V2). Maximum (17.79) number of unopened florets per spike was found in cv. Kalyani Double with 0.6 Kr X-rays (T3V2) while minimum (5.20) number of unopened florets per spike was found in cv. Kalyani Double with 1.5 Kr gamma rays treatment (T2V2). Whereas, number of open florets per spike were maximum (30.93) in cv. Suvasini with 1.2 Kr X-rays treatment (T3V3) while minimum (7.18) number of opened florets per spike was found in cv. Kalyani Single with 1.5 Kr gamma rays (T2V1) [Table 4].

The flowering duration was significantly affected by mutagen doses and their interaction with variety (Table 5). The maximum flowering duration (19.19) was observed with 0.5 Kr gamma rays (T1), while minimum flowering duration (12.69 days) was found in 0.2 per cent EMS(T6). Among interactions, maximum flowering duration (28.69 days) was found in cv. Kalyani Double treated with 0.5 Kr gamma rays (T1V2) while minimum (9.69 days) flowering duration was observed in cv. Kalyani Single treated with 0.2 per cent EMS (T6V1), in cv. Prajwal treated with 1.5 Kr gamma rays (T2V4) and 0.2 per cent EMS (T6V4).

A perusal of data for vase life presented in Table 5 envisage that 0.1 per cent EMS (T5) gave maximum (9.52 days) vase life while minimum (7.45 days) vase life was found in 0.2 per cent EMS (T6). Among interactions, maximum (13.25 days) vase life was found in cv. Kalyani Single treated with 0.2 per cent EMS (T6V1) while minimum (5.24 days) vase life was observed in cv. Kalyani Double treated with 0.5 Kr gamma rays (T1V2). Banerji and Datta (2001) also observed decrease in number of flowers per plants while working with chrysanthemum cultivar 'Surekha'. The decrease in flower head production with higher doses is mainly due to decrease in plant growth as reported by Dwivedi and Banerji (2008) in dahlia cv. 'Pinki'. Stimulative effect of EMS could be due to its effectiveness to induce a high rate of mutations in both micro and higher organisms. Karki and Srivastava (20) studied the effect of gamma rays on different varieties of gladiolus and reported that

maximum vase life was observed in 1.5 Kr gamma rays treatment and there was decrease in vase life at higher doses *viz*., 2.5 and 3.5 Kr gamma rays.

Isolation of mutants: Six mutants having desirable variation were found. The desired mutants were:

Mutant 1: A stunted plant with only two whorls of petals was observed in cv. Suvasini in 1.5 Kr gamma-ray treatments (T2V3) (Plate 2).

Mutant 2: A plant having a spike in which lower two florets were fused to form one larger floret was observed in cv. Suvasini treated with 0.2 per cent EMS (T6V3) (Plate 2).

Mutant 3: A plant of cv. Prajwal treated with 1.2 Kr X-rays (T4V4) was having spike with more than six petal in few florets (Plate 3).

Mutant 4: A plant having a spike in which lower two florets were fused to form one larger floret was observed in cv. Prajwal treated with 0.2 per cent EMS (T6V4) (Plate 3).

Mutant 5: The flower of cv. Suvasini treated with 1.2 Kr X-ray (T4V3) had distinctly visible stamens (Plate 4).

Mutant 6: A plant of cv. Prajwal treated with 0.2 per cent EMS (T6V4) had extreme reduction in plant height (18.64 cm) (Plate 4).

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