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Induction of mutation in *Jasminum grandiflorum* with gamma rays and EMS and identification of novel mutants using molecular markers and SEM imaging

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ABSTRACT

Induced mutation is an exceptionally novel method of creating genetic variability and fast method of developing new cultivars especially in ornamental crops. The present experiment was conducted during 2016-2018 to assess the effect of the mutagens *viz*., gamma irradiation and EMS on *Jasminum grandiflorum* cv. White Pitchi for identification of novel mutants using molecular markers. Terminal cuttings were treated with 10, 15, 20 and 25 Gy of gamma rays and 25, 30, 35 and 40 mM of EMS. Morphological and flowering parameters namely, plant height, number of primary branches, leaf area, flower yield and flower bud length were reduced with increase in dosage of the mutagens. Lower doses of gamma rays were associated with earliness in flowering compared to untreated control. Comparative biochemical analysis, scanning electron microscope (SEM) imaging and molecular analysis were carried out to understand the nature of mutation. Molecular analysis based on ISSR data revealed that Jaccard's similarity index ranged from 0.91 to 1.00, indicating that there was not much significant variation at genetic level between the parent and the putative mutants. SEM analysis of one of the putative mutants revealed that the epidermal cells of exposed leaves appeared relatively deformed with sparse trichomes compared to unirradiated leaves.

Key words: *Jasminum* sp., mutagens, genetic variability, molecular analysis, ISSR.

INTRODUCTION

Jasmine (*Jasminum* sp.) belonging to family *Oleaceae* is one of the most important fragrant traditional flowers used by man since time immemorial. The name jasmine is of Arabic origin and is believed to have been derived from *Yasmin* or *yasmyn*. The genus *Jasminum* is reported to comprise of around 200-300 species out of which about 40 species are reported to occur in India (Misra *et al*., 14). The species *J. grandiflorum*, *J. sambac*, *J. auriculatum* and *J. multiflorum* are commercially cultivated in Tamil Nadu, Karnataka, Andhra Pradesh, Uttar Pradesh and some parts of Bihar and West Bengal. Flowers and buds of jasmine are used for making garlands, bouquets, *veni* and for religious offerings in the country. Besides India, it is grown commercially for usage as fresh flowers in Thailand, China, Sri Lanka and Philippines. Apart from domestic consumption, export of traditional flowers, particularly jasmine has increased over the past two decades along with export of other major cut flowers from the country. Demand of jasmine flowers from the non-resident Indians (NRIs) settled in Middle East countries and the United States of America has led to increased export demand for flower strings of *J. sambac*. Moreover, India is the largest exporter of jasmine oil in the world accounting for over 40 per cent of total world export. In Europe and Mediterranean countries, jasmine flowers are used in large scale for extraction of essential oils. Since these species are propagated only by asexual means, limited variability exists in them. Mutation breeding has been used as a successful tool to generate genetic variation and breeding new varieties in many crop plants during the past decades. Generally, both physical and chemical mutagens are employed in mutation experiments. Two major factors *viz*., the rate of mutation and the mutation efficiency influence the success of mutation breeding. Upgrading the well-adapted plant varieties by improving a few desirable traits is the principal strategy in mutation breeding (Ahloowalia *et al*., 1). Mutation and molecular breeding methods offer sound and attractive choice for plant breeders to raise desired traits in plants. Since the demand for jasmine flowers is growing day by day owing to its wide range of uses, there arises an urgent need for improving its production and productivity and also to develop newer types with novel traits. Keeping in view the importance of new cultivars with novelty, the present investigation was carried out to evolve some promising mutants through mutation breeding in jasmine.

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MATERIALS AND METHODS

The experiment was carried out at Department of Floriculture and Landscape Architecture, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu during 2016 to 2018. White Pitchi, an ecotype of *J. grandiflorum* which is a region-specific genotype popularly cultivated in the southern districts of Tamil Nadu was used in this study. Terminal cuttings (13-15 cm long with three pairs of nodes) were irradiated with 10, 15, 20 and 25 Gy of gamma rays at the dose rate of 5000 rad per minute in Gamma chamber - 1200 available at Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. Immediately after irradiation, the treated cuttings were planted in polybags filled up to 3/4th of the height with rooting medium consisting of red soil + farmyard manure + sand (1:1:1ratio) and the top 1/4th with sand. Untreated cuttings (control) were also planted for comparison. Another set of cuttings was also treated with EMS (25, 30, 35 and 40 mM). Initially, cuttings were soaked in water for 1 hour to improve the uptake of the chemical mutagen. After shade drying, the cuttings were incubated in respective concentrations of EMS solutions at room temperature (1 h) and then rinsed with running tap water for 1 hour to wash out the chemical residues. The cuttings were then planted in polybags filled with the rooting medium. The mutagenized M_1V_1 population was screened for deviation in the various traits in comparison with the control plants, with respect to vegetative growth and flowering parameters. Selected putative mutants were subjected to analysis of physiological parameters namely, total soluble sugars content (Hedge and Hofreiter, 10), IAA oxidase activity (Parthasarathy *et al*., 16) and soluble protein (Bradford, 3) to understand the physiological basis of the mutation expressed. Analysis was carried out to find out mean, range, standard error, coefficient of variation using MS Excel, 2010.

SEM imaging of leaves and stems of unirradiated control plants and the irradiated putative mutant (WP-DM-7) was carried out to detect cellular differences, since gamma irradiation is known to cause structural changes at cellular level. Stem samples (1 to 2 cm long) were treated with 2% glutaraldehyde solution for 4 h and then they were treated with 30, 50, 70 and 100% acetone serially at 10 min. interval for each treatment followed by rinsing the samples serially with 30, 50, 70 and 100% ethanol at 10 min. intervals. The samples were then dried using a vacuum desiccator and were subjected to gold sputtering using Quarum- Q15ORS for 30 seconds to improve their conductive nature. Finally, the samples were placed over an aluminium stub through a double sided adhesive carbon tape and viewed with a field emission scanning electron microscope at 50 µM with accelerating voltage of 7kV and the images were photographed with an image capturing system.

The M_1V_1 generation plants of *J. grandiflorum* cv. White Pitchi were further raised for the next vegetative generation namely, M_1V_2 through vegetative propagation using terminal stem cuttings and placed under poly-tunnels with average temperature of 28-30°C and relative humidity 80% to induce rooting. After root formation, the M_1V_2 putative mutants were transferred to grow-bags filled with potting mixture and evaluated for various growth parameters. In M_1V_2 generation, seven putative mutants which expressed distinct mutations in the plant growth, flower yield and disease resistance to Alternaria leaf blight were subjected to confirmation using molecular markers (Table 3). For extraction of DNA, a modified DNA extraction method was adopted (Doyle, 5). Seven RAPD and ISSR markers each were used for amplification (Tables 1 and 2). Only the clear and unambiguous bands were scored. Markers were scored for the presence and absence of the

Table 1. List of ISSR markers used for assessing mutant diversity.

S. No.	Marker	Marker sequence 5' to 3' end	Annealing temperature (ºC)
1.	UBC 817	CACACACACACACACAA	50
2.	UBC842	GAGAGAGAGAGAGAGACG	50
3.	UBC 880	GGAGAGGAGAGGAGA	50
4.	GA(9A)	GAGAGAGAGAGAGAGAGAA	50
5.	GT(8CC)	GTGTGTGTGTGTGTGTCC	52
6.	AG(8CT)	AGAGAGAGAGAGAGAGCT	50
7.	GA(8T)	GAGAGAGAGAGAGAGAT	50

Table 2. List of RAPD markers used for assessing mutant diversity.

S. No.	Marker	Marker sequence 5' to 3' end	Annealing temperature (ºC)
1.	OPG-09	CTGACGTCAC	36
2.	OPE-10	AGGGCCGTCT	
3.	OPE-01	CCCAAGGTCC	
4.	OPE-02	GGTGCGGGAA	
5.	OPE-04	GTGACATGCC	
6.	OPX-02	TTCCGCCACC	
7.	OPK-07	AGCGAGCAAG	

Table 3. List of putative mutants of White Pitchi (*J. grandiflorum*) subjected to molecular analysis.

Putative mutant (s)	Mutation observed
1. WP-DM-4	Dwarf mutant
2. WP-EFM-1	Early flowering mutant
3. WP-APM-2	Altered phyllotaxy mutant
4. WP-LCTM-6	Long corolla tube mutant
5. WP-HYM-4	High yielding mutant
6. WP-LBR-2	Leaf blight resistant mutant
7. WP-PBM-4	Profuse branching mutant

corresponding bands among the different genotypes. The scores '1' and '0' were given for the presence and absence of bands, respectively. The data were used to generate genetic similarity coefficient matrices on the basis of Jaccard's coefficient. The dendrogram (cluster diagram) was generated by unweighted pair group method with arithmetic average (UPGMA) algorithm using Sequential Agglomerative Hierarchial and Nested (SAHN) technique. The clustering result was used to construct a dendrogram following TREE module (Ghosh *et al*., 7).

RESULTS AND DISCUSSION

The observations recorded on the plants clearly revealed that the mean plant height ranged from 47.77 cm (25 Gy) to 51.99 cm (10 Gy) for the gamma irradiation treatments and from 51.24 cm (40 mM) to 71.82 cm (25 mM) for the EMS treatments, with the control recording the maximum plant height (78.75 cm). Among all the treatments, maximum co-efficient of variation (8.23%) was observed in 15 Gy. The mean number of primary branches ranged from 2.78 (25 Gy) to 3.98 (15 Gy) for the gamma irradiation treatments and from 2.86 (40 mM) to 4.38 (30 mM) for the EMS treatments. The mean internodal length ranged from 2.92 cm (25 Gy) to 5.26 cm (10 Gy) for the gamma irradiation treatments and from 6.03 cm (40 mM) to 6.98 cm (25 mM) for the EMS treatments, with the maximum value in control (7.10 cm). Maximum co-efficient of variation (9.54%) was observed in 25 Gy. The mean number of leaves ranged from 69.42 (25 Gy) to 75.11 (10 Gy) for the gamma irradiation treatments and from 65.51 (40 mM) to 71.60 (25 mM) for the EMS treatments. The maximum number of leaves (71.69) were observed in control. The highest variability (11.61 %) was observed in 15 Gy. Mean leaf area ranged from 9.39 cm^2 (25 Gy) to 11.70 cm^2 (10 Gy) for the gamma irradiation treatments and from 10.03 cm^2 (40 mM) to 13.70 cm^2 (25 mM) for the EMS treatments. Control plants exhibited maximum leaf area of 14.20 and 14.58 cm^2 for gamma rays and EMS treatments, respectively. The maximum leaf area (14.58 cm^2) was observed in control. Maximum co-efficient of variation (23.30 %) was observed in 10 Gy (Table 4). Plant height showed a progressive reduction with increase in dosage of mutagens which may be due to the fact that inactivation of auxin and decrease in auxin content with increase in radiation doses was responsible for reduction in plant height (Tewari *et al*., 18). In case of primary branches, the lower dosages of both gamma rays and EMS recorded higher values over control. This may be due to hermetic effects of low dose ionizing radiation on plants that accelerated cell proliferation, stimulated growth and increased yield. Increase in vegetative characters with the increase in dose of mutagen to a certain optimum level was reported in tuberose (Kainthura and Srivastava, 11).

Irradiation of plants with mutagens at varying doses had pronounced effects on flowering attributes. The mean number of days to flowering ranged from 107.19 days (15 Gy) to 115.53 days (25 Gy) for the gamma irradiation treatments and 114.21 days (40 mM) to 124.34 days (35 mM) for of EMS treatments. In case of total flower bud length, the mean values ranged from 3.28 cm (25 Gy) to 3.34 cm (15 Gy) for gamma radiation treatments and from 3.30 cm (40 mM) to 3.46 cm (25 mM) for EMS treatments. Among the treated population, maximum co-efficient of variation (5.51%) was observed in 20 Gy. Corolla tube length recorded had mean values ranging from 1.53 cm (25 Gy) to 1.57 cm (15 Gy) for gamma radiation treatments and from 1.52 cm (40 mM) to 1.60 cm (25mM) for EMS treatments. The highest variability (3.24%) was observed in 20 Gy (Table 4). Yield of flowers did not follow any particular trend where the highest monthly yield (126.26 g $plant^{-1}$) was recorded in Aug. 2017 with 15 Gy, followed by 10 Gy treated plants (125.54 g $plant^{-1}$). Untreated control plants recorded yield of 123.61 g $plant^{-1}$ during the same period. In case of EMS treatments, highest monthly yield (129.53 g $plant^{-1}$) was recorded with 25 mM and lowest in control (125.97 g $plant^{-1}$) in Aug. 2017. The variability as measured in terms of co-efficient of variation was observed to be maximum (5.69%) in 15 Gy (Table 5). In the present study, flowering parameters showed positive response at lower dosages of gamma rays and EMS, whereas very high dosages were found to execute negative influence on the parameters. Lower dosages of mutagen treatments advanced flowering as well as gave higher flower yield as compared to control. It is known that low and intermediate concentrations

Table 4. Vegetative and flowering characters of mutagen treated plants of *J. grandiflorum* genotype White Pitchi in M_1V_2 generation.

Treatment	Vegetative characters										Flowering characters					
	Plant height (cm)		No. of primary branches		Internodal length (cm)		Number of leaves		Leaf area		Days to flowering		Flower bud length (cm)		Corolla tube length (cm)	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
							Gamma rays (Gy)									
Control	74.40 ±0.89 (9.65)*	64.25-90.56	3.71 ±0.13 (23.04)	2-5	6.99 ±0.10 (12.16)	5.54-9.12	70.34 ±0.79 (8.79)*	58-82	14.20 ±0.58 (10.08)	12.23-16.21	116.75 ±1.25 (3.02)*	113-124	3.25 ±0.06 (7.72)	2.59-3.40	1.52 ±0.003 (1.09)	1.51-1.57
10 Gy	51.99 ±0.14 (2.17)	50.14-54.58	3.67 ±0.11 (21.79)	2-6	5.26 ±0.04 (6.08)	4.49-6.11	75.11 ±0.49 (6.96)	62-84	11.70 ±0.96 (23.30)	9.12-17.56	110.83 ±1.85 (4.12)	102-113	3.32 ±0.004 (0.41)	3.35-3.39	1.55 ±0.008 (1.84)	1.54-1.62
15 Gy	50.81 ±0.42 (8.23)	43.25-50.87	3.98 ±0.13 (25.10)	3-7	4.51 ±0.03 (5.49)	4.11-5.13	68.15 ±0.28 (4.67)	54-72	9.98 ±0.45 (12.88)	8.23-12.03	107.19 ±5.36 (12.04)	98-123	3.34 ±0.005 (0.55)	3.32-3.37	1.57 ±0.003 (1.83)	1.52-1.65
20 Gy	49.18 ±0.17 (2.74)	42.23-49.59	3.16 ±0.08 (21.24)	2-5	3.66 ±0.02 (5.73)	3.35-4.15	72.23 ±0.41 (7.61)	56-74	9.74 ±0.52 (14.26)	8.25-12.31	111.32 ±1.09 (2.65)	107-111	3.33 ±0.05 (5.51)	2.96-3.38	1.56 ±0.006 (3.24)	1.48-1.63
25 Gy	47.77 ±0.13 (2.18)	41.58-47.58	2.78 ±0.11 (28.41)	2-5	2.92 ±0.03 (9.54)	2.53-3.64	69.42 ±0.86 (18.96)	48-72	9.39 ±0.12 (13.96)	8.14-10.86	115.53 ±5.31 (11.88)	110-124	3.28 ±0.03 (4.17)	2.94-3.30	1.53 ±0.005 (2.53)	1.48-1.67
							EMS (mM)									
Control	78.75 ±2.16 (12.00)	64.25-96.37	3.94 ±0.16 (17.86)	3-5	7.10 ±0.25 (14.55)	5.54-9.12	71.69 ±0.44 (2.17)	72-76	14.58 ±0.34 (10.11)	12.23-16.86	118.75 ±1.25 (3.02)	114-126	3.28 ±0.03 (9.55)	2.56-3.39	1.51 ±0.03 (9.55)	1.49-1.58
25 mM	71.82 ±1.04 (5.26)	67.39-80.12	3.43 ±0.12 (14.90)	3-4	6.98 ±0.01 (0.81)	6.94-7.11	71.60 ±0.83 (3.67)	70-78	13.70 ±0.96 (13.20)	9.12-17.11	117.34 ±3.63 (9.25)	97-125	3.46 ±0.006 (1.39)	3.34-3.45	1.60 ±0.006 (1.39)	1.52-1.67
30 mM	64.37 ±0.67 (4.18)	61.53-70.55	4.38 ±0.18 (14.83)	4-6	6.64 ±0.08 (4.93)	5.89-6.87	70.35 ±1.62 (9.49)	58-80	12.98 ±0.45 (10.19)	8.23-13.03	118.66 ±4.48 (7.18)	102-131	3.44 ±0.006 (1.45)	3.36-3.49	1.55 ±0.006 (1.45)	1.47-1.65
35 mM	55.94 ±0.78 (5.79)	52.36-61.23	3.82 ±0.17 (19.02)	2-5	6.31 ±0.03 (1.88)	6.15-6.53	68.52 ±1.28 (7.69)	58-70	10.74 ±0.52 (8.22)	8.25-12.78	124.34 ±3.63 (12.37)	111-135	3.40 ±0.004 (0.93)	3.35-3.47	1.53 ±0.004 (0.93)	1.50-1.64
40 mM	51.24 ±0.05 (2.68)	48.78-23.24	2.86 ±0.21 (29.08)	2-5	6.03 ±0.08 (5.21)	5.55-6.43	65.51 ±0.70 (3.71)	60-72	10.03 ±0.12 (9.87)	8.14-11.86	114.21 ±5.79 (5.67)	100-136	3.30 ±0.007 (1.61)	3.22-3.41	1.52 ±0.007 (1.61)	1.47-1.62

*Figures in parenthesis indicate CV (%)

Table 5. Peak season flower yield (g/plant) of mutagen treated plants of *J. grandiflorum* genotype White Pitchi in M_1V_1 generation.

	June 2017	July 2017	Aug 2017	Sept 2017	Oct 2017	May 2018
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
			Gamma rays (Gy)			
Control	74.64 ±2.38 (7.82)*	106.69 ±2.51 (5.76)	123.61 ±1.33 (2.65)	105.83 ±1.47 (9.33)	89.03 ±1.47 (4.04)	70.06 ±2.15 (7.85)
10 Gy	75.06 ±2.25 (9.12)	101.01 ±5.99 (15.69)	125.54 ±2.29 (4.69)	104.02 ±3.32 (5.83)	87.84 ±1.93 (4.67)	72.70 ±2.32 (8.46)
15 Gy	76.82 ±2.36 (7.92)	108.34 ±3.63 (9.03)	126.26 ±2.71 (5.69)	107.81 ±2.84 (7.10)	90.22 ±1.91 (5.61)	73.97 ±1.58 (5.74)
20 Gy	74.20 ±2.40 (9.51)	100.87 ±5.93 (15.57)	124.89 ±2.38 (5.02)	100.03 ±1.89 (9.40)	87.70 ±1.89 (5.72)	72.31 ±2.35 (8.60)
25 Gy	73.88 ±4.75 (17.25)	109.01 ±4.36 (10.59)	121.86 ±1.77 (3.86)	105.35 ±3.57 (8.98)	87.53 ±2.08 (6.31)	73.32 ±1.36 (4.76)
			EMS (mM)			
Control	75.44 ±2.21 (7.18)	104.67 ±2.96 (6.94)	125.97 ±1.00 (1.95)	106.61±3.79 (8.72)	89.76 ±1.74 (4.75)	69.91 ±1.95 (7.04)
25 mM	84.03 ±2.32 (7.67)	103.12 ±4.62 (11.85)	129.53 ±2.00 (4.19)	112.03 ±1.15 (2.73)	93.82 ±1.37 (3.97)	76.49 ±1.68 (6.05)
30 mM	76.88 ±2.55 (8.56)	106.60 ±3.35 (9.31)	128.10 ±1.37 (4.95)	108.32 ±2.96 (7.37)	90.57 ±1.69 (5.01)	74.74 ±2.01 (7.23)
35 mM	89.82 ±4.75 (17.25)	115.12 ±4.62 (11.80)	131.76 ±1.88 (4.12)	117.22 ±1.90 (8.87)	96.27 ±1.90 (5.71)	77.52 ±2.01 (7.44)
40 Mm	80.03 ±2.32 (7.67)	110.10 ±4.03 (9.76)	127.61 ±2.42 (5.10)	111.76 ±1.25 (2.97)	92.93 ±1.38 (3.94)	75.32 ±1.36 (4.67)

*Figures in parenthesis indicate CV (%)

of mutagens generally stimulate cell growth rate and produce earlier flowering in specific cases (Funk *et al*., 6). On the other hand, elevated concentrations seemed to inhibit the cell growth, decrease growth rate and delay the flowering date (Kapoor *et al*., 12). The stimulating effect of lower doses of gamma rays and EMS may also be due to the stimulation of cell division and elongation, or the alteration of metabolic processes that affect the synthesis of phytohormones or nucleic acids (Chandrashekar, 4).

SEM imaging indicated that the leaves of control plants exhibited many unicellular unbranched trichomes on the adaxial surface (upper) epidermis, while those of the putative mutant exhibited no trichomes (Fig. 1). Further, the epidermal cells of the putative mutant appeared relatively deformed compared to control leaves. The stem of control plants showed normal arrangement of vascular channels and cell layers whereas the putative mutants, lacked a proper arrangement for these traits. The first layer of epidermis comprises of epidermal structures including trichomes and stomata. Any change as a result of mutations are preserved and passed down the cell lineage that forms the outer skin of the plant. Therefore, study of distribution of trichomes could be a suitable indicator of the magnitude of mutation as a result of exposure to physical mutagens like gamma rays (Nazki *et al*., 15). Scanning electron micrographs taken in the present study revealed no major deformations on the surface of leaves but only a drastic reduction in the number of unicellular unbranched trichomes in the putative mutant and this is probably due to the stress condition imposed by gamma rays.

Early flowering and high yielding putative mutants recorded higher soluble protein content (16.24 mg/g, 14.18 mg/g) as compared to control (13.28 mg/g). Dwarf mutants recorded lower IAA oxidase content (11.71 µg unoxidised auxin g^{-1} h^{-1}) as compared to untreated control (17.18 unoxidised auxin $g^{-1}h^{-1}$). Total soluble sugar content was found to be higher in profused branching (69.20 mg/g) and higher yielding (78.76 mg/g) putative mutants compared to control (53.18 mg/g) (Table 6). Selected promising

Table 6. Physiological parameters of isolated putative mutants in M_1V_1 generation of *J. grandiflorum* genotype White Pitchi.

Characters	Soluble proteins(mg/ g)			IAA oxidase activity (µg of unoxidised auxin g^{-1} h^{-1})			Total soluble sugar (mg/g)		
	Mean ± SE	Range	CV (%)	Mean ± SE	Range	CV (%)	Mean ± SE	Range	CV (%)
Control (Untreated)	13.28 ±0.48	12.96-13.30	0.59	17.18 ±0.16	16.43-17.56	2.24	53.18 ±1.46	43.56-58.93	7.53
Dwarf stature (12 Nos.)	13.94 ±0.26	13.85-14.24	4.04	11.71 ±0.35	11.48-12.86	1.14	58.95 ±2.61	48.45-60.79	7.21
Early flowering (17 Nos.)	16.24 ±0.21	15.63-16.87	2.95	16.12 ±0.18	12.85-16.56	3.22	62.65 ±3.10	52.59-67.12	8.14
Profuse branching (17 Nos.)	13.49 ±0.48	12.75-13.54	8.01	14.22 ±0.34	12.45-16.47	5.38	69.20 ±2.94	54.92-75.33	7.83
High Yielding (18 Nos.)	14.18 ±0.50	12.97-15.34	8.18	15.94 ±0.88	12.43-17.22	14.11	78.76 ±1.29	63.23-82.25	3.26

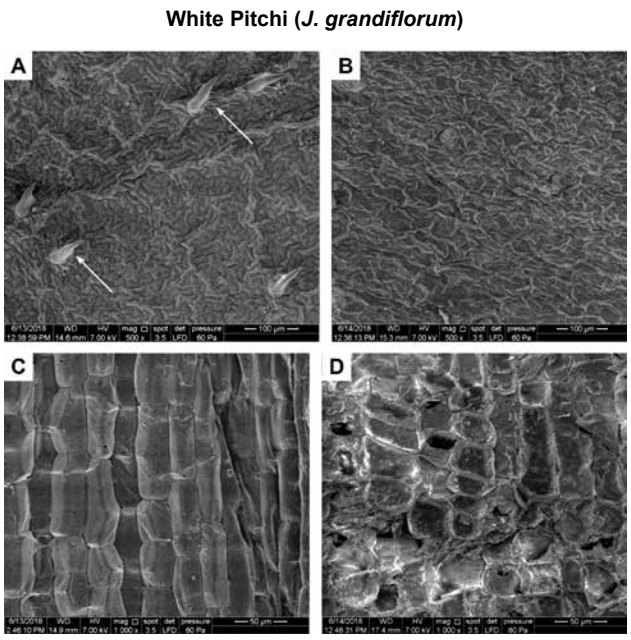

Fig. 1. Scanning electron micrographs of leaves (Top) and stem (Bottom).

A : Unicellular unbranched trichomes from the upper epidermis
B : Absence of trichomes on the exposed leaves
C : Regular arrangement of cells
D : Disrupted cell arrangement with irregular cell boundaries

mutants were analyzed for physiological parameters to find out the basis of positive shift compared to the untreated control plants. In case of dwarf mutants, IAA oxidase activity was found to be lower compared to their respective untreated parents. This is normally expected, since higher the IAA oxidase activity, lesser is the production of auxins in the growing point, leading to suppressed growth. On the other hand, higher soluble proteins and total soluble sugar contents were observed in some of the putative mutants, indicating improved potentials for photosynthetic efficiency and flower production. Increase in protein may be due to the repression of gene expression as suggested in *Amaranthus* species (Gorinstein *et al*., 8). The observation implicates that the higher levels of soluble sugar content induced by gamma rays and EMS act as protective agents against protein oxidation. These results are in conformity with earlier findings in jasmine (Saranraj and Kannan, 17) and fenugreek plants (Hanafy and Akladious, 9).

The UPGMA dendrogram programme which was constructed with coefficient similarity matrix based on RAPD data classified the samples into two main clusters, Cluster A and Cluster B (Fig. 2). Cluster A was again divided into two sub-clusters. Sub-cluster I comprising of the putative mutant WP-LCTM-6 alone, whereas Sub-cluster II consisted of the mutants WP-DM-4, WP-EFM-1, WP-LCTM-6, WP-HYM-4 and WP-PBM-4. Cluster B consisted of the parent (White Pitchi) and the putative mutant WP-APM-2. Lowest similarity (79%) was found between WP-EFM-1 and parent and also among WP-LCTM-6 and WP-DM-4. Dendrogram based on ISSR data revealed that there were two main clusters, where cluster A was divided into two sub-clusters (Fig. 3). Sub-cluster I consisted of WP-EFM-1 and WP-LCTM-6. Sub-cluster II included the mutants WP-DM-4, WP-PBM-4 and WP-HYM-4. Main cluster B was divided into parent, WP-APM-2 and WP-LBR-2. Based on ISSR data, Jaccard's similarity index ranged from 0.91 to 1.00 revealing that there was

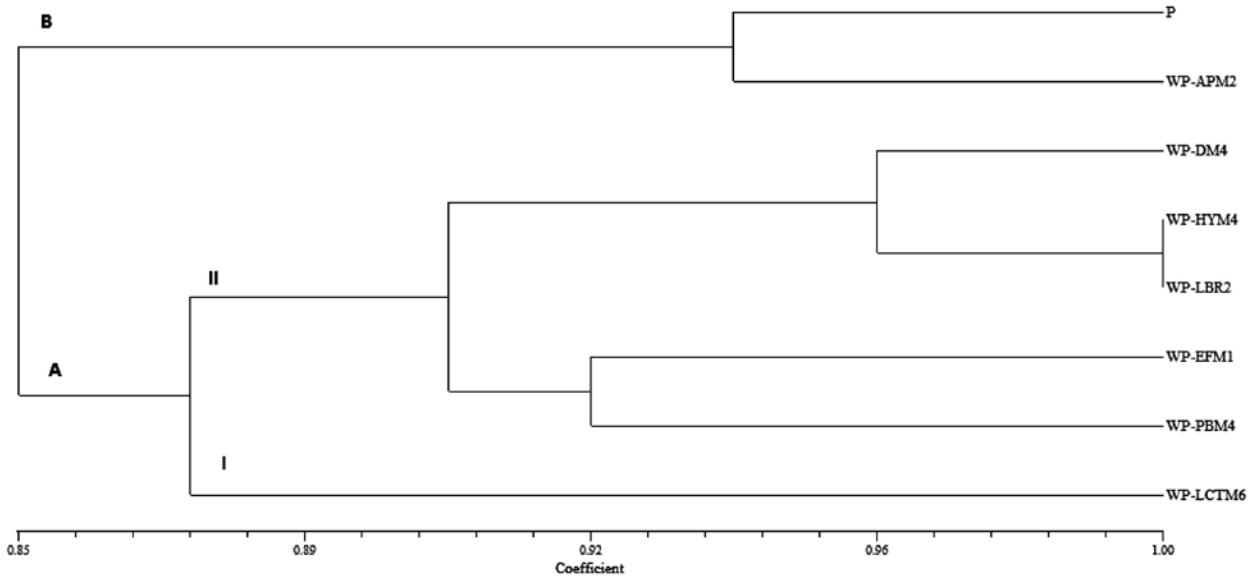

Fig. 2. Dendrogram based on UPGMA of Jaccard's similarity matrix representing the genetic relatedness among mutants of *J. grandiflorum* genotype White Pitchi using RAPD primers.

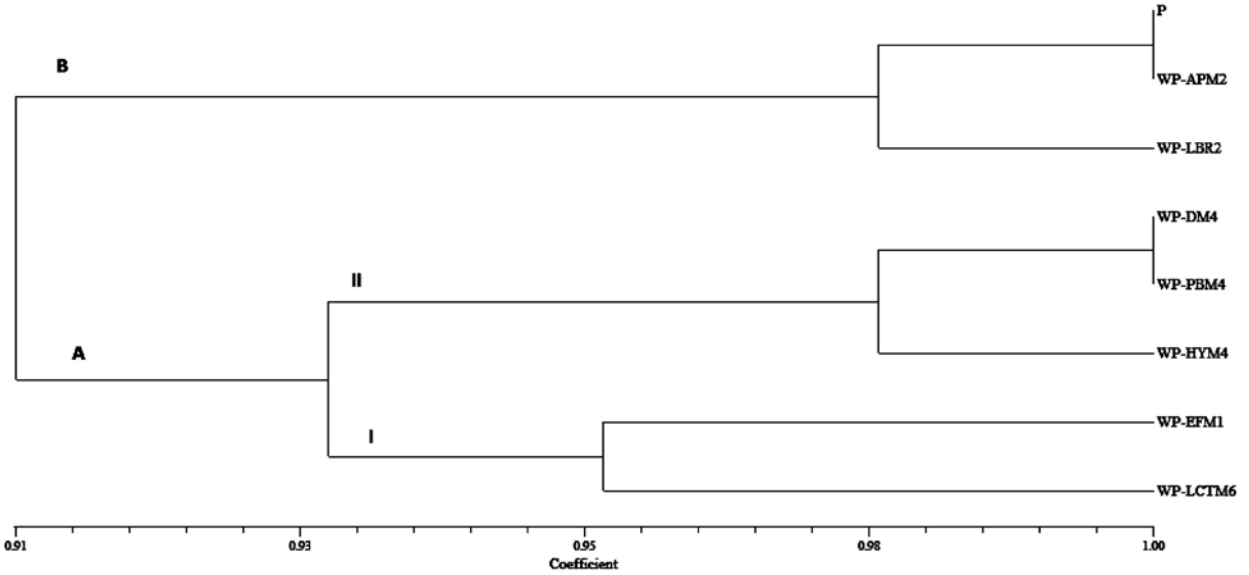

Fig. 3. Dendrogram based on UPGMA of Jaccard's similarity matrix representing the genetic relatedness among mutants of *J. grandiflorum* genotype White Pitchi using ISSR primers.

no significant variation among parent and putative mutants. The main changes observed between the RAPD and ISSR profiles were the presence and absence of different bands as well as variation in their intensities according to different genotypes (Figs. 4 and 5). RAPD and ISSR methods were also used to study genetic variability among mutants in different crops like chrysanthemum (Minano *et al*., 13) and rose (Bala and Singh, 2). Although the mutants varied in their morphology, bands specific for the respective traits could not be distinguished.

DECLARATION

The authors declare no conflict of interest.

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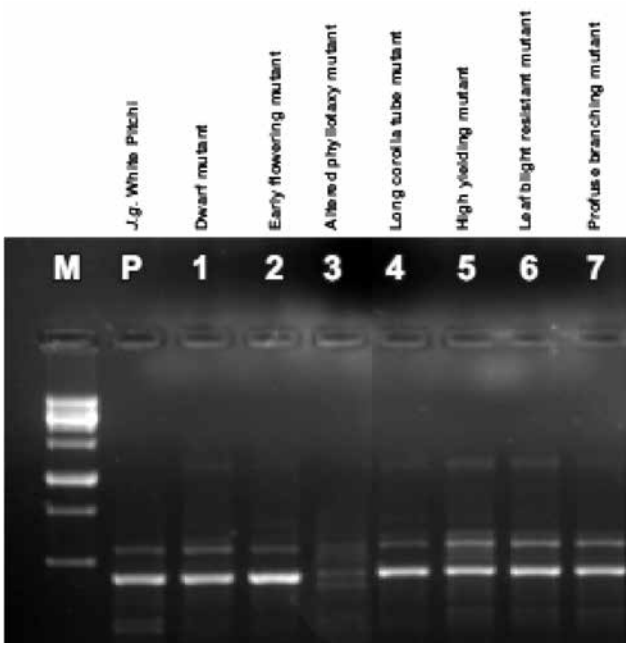

Fig. 4. RAPD profile of putative mutants of *J. grandifllrum* genotype White Pitchi using primer OPX-02.

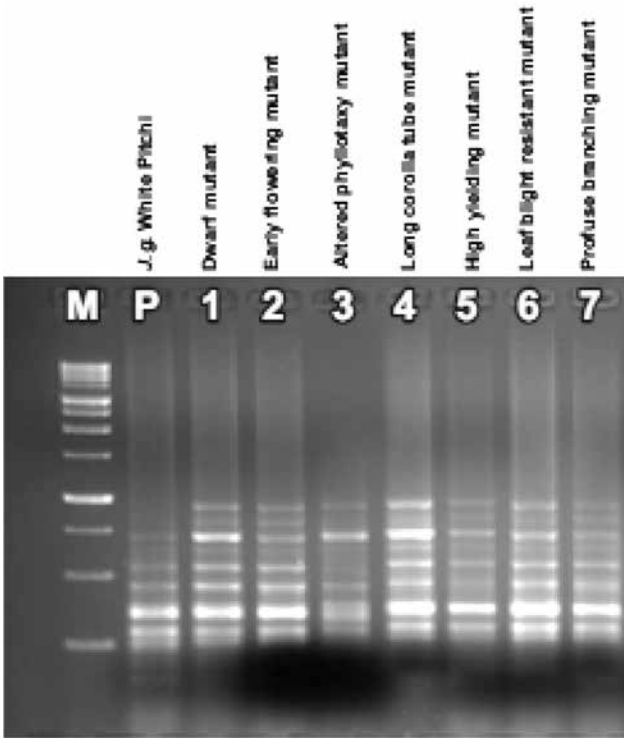

Fig. 5. ISSR profile of putative mutants of *J. grandifllrum* genotype White Pitchi using primer UBC 880.

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