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Mutation studies in chrysanthemum cultivar Poornima white

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Abstract
Rooted cuttings of chrysanthemum cultivar Poornima White were subjected to the different doses of both physical (gamma rays) and chemical mutagens (EMS) with the objective of determining the optimum dose for inducing the variation in both vegetative and flower characteristics. The experiment was planned out in poly house with eleven treatments and three replications. Effect of mutagen was significantly varied with dose of mutagen and type of mutagen, survival percentage of mutated plants was decreased with increasing dose of mutagen. Minimum survival percentage of plants was observed in 15 Gy gamma irradiation treatment and there was no survivability of plants in 20 Gy gamma irradiation treatment. Changes in morphological and flower characteristics were observed in mutagen treated plants. Reduction in flower size and increase in flower yield in one plant which was treated with 5 Gy gamma irradiation, dwarf plants were observed in 10 Gy gamma irradiated plants and chlorophyll variegated plants were observed at 0.1% EMS treatment. Molecular analysis was done by using five SRAP primers to detect the genetic polymorphism between parents and mutants and highest similarity of ninety per cent was observed between PWM2 and PWM1.

Keywords: Mutation studies, chrysanthemum cultivar, Poornima white

Introduction
Chrysanthemum (*Dendranthema grandiflora* Tzvelve.) is one of the most important commercial floricultural (cut and loose flower) crops in the world. A part from the commercial value, chrysanthemum is also having high ornamental (pot and garden flower) values. Wide variation can seen in chrysanthemum with respect of growth pattern, flower size, colour and shape of bloom because of it's diversity (Barbosa, 2003) [1]. These characteristics make the chrysanthemum suitable for every purpose as a flower crop. In India, it is commercially cultivated in Karnataka, Maharashtra, Tamil Nadu, Andhra Pradesh, and West Bengal. Mutagenesis allows the possibilities for creation of genetic variability with high ornamental value (Cantor *et al.*, 2002) [2]. Mutation breeding is one of the established breeding methods by which one can induce genetic variability in vegetatively propagated crops and it also offers advantages over conventional breeding methods for the improvement of one or more traits within a short span of time. Mutation derived varieties have had a significant impact on the array and choiced of genetic resources available in modern agriculture (Ahloowalia *et al.*, 2004). The major objective of any mutation breeding programme is to obtain better and noble genotype through the creation of genetic change in the existing cultivars or varieties. The advantage of mutagenesis in chrysanthemum is mainly the ability to change one or a few characters of an excellent cultivar without changing rest of the genotype. The induced mutagenesis in plants has been used to create genetic variations for large number of traits by both physical and chemical mutagens when seeds and vegetative planting materials are used (Gustafsson, 1960). Mutation breeding through mutagenesis for development of novel cultivars in Chrysanthemum is best way, due to it's self-incompatibility nature (Bajpay and Dwivedhi, 2019). In this experiment we successfully tried induced mutagenesis in chrysanthemum cultivar Poornima White by using both physical and chemical muatgens and detected the genetic polymorphism between chrysanthemum variety Poornima White and it's mutants by using SRAP primers.

Materials and methods
From the mother plants in polyhouse in Department of Floriculture and Landscape Architecture, 10 cm terminal cuttings were taken and basal leaves were removed and slant cut was given just below the nodal portion. Cut portion of the cuttings were dipped in Keradix rooting powder to stimulate rapid and prolific rooting of cuttings.

Soon after the treatment, cuttings were placed in protrays which were filled with cocopeat for rooting, further these cuttings were used for mutagenic treatments.

Mutagenic treatment

Rooted cuttings were irradiated with four doses of gamma rays (5, 10, 15 and 20Gy) in Gamma Cell-200 (Cobalt-60 source emitting 3600 rads per minutes) at Bhabha Atomic Research Centre (BARC), Trombay, Mumbai. EMS was used as chemical mutagen, rooted cuttings were treated with different concentration of EMS and planted in polyhouse for evaluation based on morphological characters to know the optimum dose/ concentration of mutagen with the aim of inducing mutations for developing novel mutants. After mutation, cutting were planted immediately along with control (without mutation) cuttings in polyhouse, The experiment was conducted in poly house with 11 treatments replicated thrice at a spacing of 30x30 cm. Observations were recorded for vegetative and flower characteristics, at field of Department of Floriculture and Landscape Architecture, Kittur Rani Channamma College of Horticulture, University of Horticultural Sciences, Bagalkot, Karnataka. Cultural practices such as watering, nutrition and control of disease and pests were practiced according to the standard procedure for chrysanthemum culture of University of Horticultural Sciences, Bagalkot. The crop was kept weed free by manual weeding as necessary. Morphological and flower characters were recorded at matured stage to know the effect of mutagen. Plant height (cm), Number of primary branches, Stem girth (mm), Plant spread E-W (cm), Plant spread N-S (cm), Number of leaves, Leaf area (mm^2), Days to flower bud initiation, Days taken for flowering, Duration of flowering (Days), Flower diameter (cm), Flower stalk length (cm), Individual flower weight (g), Number of flowers per plant, Flower yield per plant (g), Vase life (days), Total chlorophyll content ($nmol/cm^2$) were measured to know the effect of mutagen and variegation in leaves were observed and matched with Royal Horticulture Society color chart. After identification of mutants, DNA extraction (Mishra *et al.*, 2011) [11] was done by using (2%) CTAB method, using the leaf samples collected from the parents and the mutants and PCR analysis was carried out by using the genomic DNA from the parent and its mutants which were obtained through induced mutagenesis. Five SRAP primer combination was used for molecular characterization of parent and mutants. After PCR, 100 bp ladder marker was used for estimation of band sizes. Fragments amplified by the primer used and molecular weights in base pairs (bp) were scored for their presence or absence (Echt *et al.,* 1992) [4]. The scored band data was subjected to statistical analysis using the computer programme NTSYS-PC Ver. 2.2 software (Rohlf, 2000) [15]. UPGMA (Unweighted Pair Group Method with Arithmetical averages) was used to generate the resultant similarity matrix tree. Five SRAP primers used for molecular characterization of mutants are presented below.

Five SRAP primers used for molecular characterization of mutants are presented below

Forward primer		Reverse primer		Primer combinations	
Name	**Sequence**	**Name**	**Sequence**	**Forward**	**Reverse**
Me_1	TGAGTCCAAACCGGATA	Em_2	GACTGCGTACGAATTTGC	Me_1	Em_{10}
Me_2	TGAGTCCAAACCGGAGC	Em_3	GACTGCGTACGAATTGAC	Me_2	Em_3
Me_3	TGAGTCCAAACCGGAAT	Em_6	GACTGCGTACGAATTACA	Me_3	Em_7
		Em_7	GACTGCGTACGAATTCAA	Me_2	Em_2
		Em_{10}	GACTGCGTACGAATTCAT	Me_2	Em_6

Statistical analysis

The observed data were statistically analysed by control with other treatments in completely randomized design and the significance at 1% was observed.

Results and Discussion

The morphological and flower characters were studied after mutagenesis (both gamma rays and EMS) and revealed that the variegation in foliage and also change in plant stature and shape of flowers in plants treated with 5 Gy, 10 Gy and 0.1% EMS over control. Maximum plant height was found in control (56.90 cm) which was statistically on par with 0.05% EMS treatment (54.70 cm). The reduction in height at lower doses might be due to the biosynthesis of primary metabolites may have reduced by mutagenic treatment. Maximum stem girth (11.07 mm) was observed in 0.05% EMS treatment followed by 5 Gy gamma rays irradiation treatment (9.89 mm), there were no previous reports about stem girth in mutation breeding of chrysanthemum. Maximum plant spread in East-West (42.10 cm) was observed in 0.05% EMS treatment maximum plant spread in North-South (40.00 cm) was observed in 5 Gy gamma rays irradiation treatment followed by 0.05% EMS treatment (36.50 cm) Similar results were obtained by Singh and Bala (2015) [16] in chrysanthemum. Number of leaves were maximum (272.96) in 5 Gy gamma rays irradiation treatment followed by control (255.51) but on contrary, Singh and Bala (2015) [16] in chrysanthemum reported that the gamma irradiation significantly reduced the number of branches per plant compared to control. these results were quite similar to the results obtained by Patil *et al.,* 2017 [14] in chrysanthemum. There was no significant difference observed in leaf area among all treatments. number of leaves was more in lower dose of mutagenic treatment. Similar results were seen in tuberose by Kayalvizhi *et al.* (2017) [8]. Minimum number of days taken for flower bud initiation (96.10 days) was observed in 0.2% EMS treatment followed by control (96.40 days) Similar reports were found in previous studies conducted by Singh and Bala (2015) [16] in chrysanthemum. Control plants showed minimum number of days taken for flowering (112.60 days) which was statistically on par with 5 Gy gamma rays irradiation treatment (113.10 days). Similar experiments were done by Dobanda (2004) [3] and Patil (2014) [13] and they observed gamma ray lower doses induced earliness with respect to opening of first floret. Whereas, opening of first floret was delayed with increase in doses. Initiation of flowering may be affected as a result of mutagenic treatments because many biosynthetic pathway are believed to be altered, which are directly as well as indirectly associated with the flowering physiology (Mahure *et al*., 2010 and Ismael and Mohmoud 2015) [10, 5]. 0.05% EMS treatment showed maximum duration of flowering (53.33 days) in mutated population of Poornima White followed by control (51.00 days), these findings were quite similar to the results obtained by Patil *et al.,* 2015 in chrysanthemum. maximum flower diameter (5.36 cm) was observed in 0.05% EMS treatment followed by 0.3% EMS treatment (5.21 cm) These results were quite line with the results obtained by Kapadiya *et al.,* 2014 [6] in chrysanthemum, Patil *et al.,* 2017 [14] in chrysanthemum and Kayalvizhi *et al.,* 2017 [8] in tuberose. 10

Gy gamma rays irradiation treatment showed maximum flower stalk length (6.01 cm) was observed in followed by control (5.73 cm) Maximum individual flower weight (1.84 g) was observed in 0.05% EMS treatment which was statistically on par with control (1.72 g), In Poornima White, 0.05% EMS treated plants showed maximum number of flowers per plant (164.20) followed by control (156.00) Flower yield per plant (377.33 g) was maximum in 0.05% EMS treatment followed by control (272.40 g) These results were not similar to the previous observations in chrysanthemum. Varietal response about flower yield per plant to the mutagenic treatment varied, it might be due to the genetic make up of the variety. there was no significant difference observed for vase life among treatments. Molecular analysis of Poornima White and it's mutants by using five primer combinations of SRAP produced a total of 22 scorable bands which were used to estimate the genetic variation in control and it's variants. These bands were well cleared, readable and reproducible polymorphic bands. A total of 22 bands were observed, in which 13 bands were polymorphic, 4 were unique bands and 5 monomorphic bands. The number of bands produced from each primer varied from 3-6 with an average of 4.4 bands per primer (Table 3). Among five primers, Me1 + Em10 and Me2 + Em2 produced maximum number of polymorphic bands (4 bands each) followed by Me3 + Em7 (3 bands). While Me2 + Em3 and Me2 + Em6 produced one polymorphic bands (each). The dendrogram was established by UPGMA (Unweighted Pair Group Method with Arithmetical averages) method of clustering using Dice similarity co-efficient. Cluster analysis based on a total of 22 bands with 13 polymorphic bands using 5 SRAP primers disclosed that, the Poornima White and it's mutants clustered in the dendrogram (Fig. 1). Poornima White and it's mutants were grouped into two clusters. Cluster I constituted with Poornima White, cluster II contains three mutants. The cluster II in dendrogram was further divided into two sub clusters, sub cluster I had two variants PWM1 and PWM2 and sub cluter II had one variant PWM3. the major cluster I had Poornima White, major cluster II further divided into two sub clusters. Sub cluster I had two variants (PWM1 and PWM2) and similarity index between them was 0.90 and sub cluster II had one variant PWM3 with similarity index 0.57. Kaul *et al.* (2011) [7] used RAPD analysis to detect genetic polymorphism among the variants and the control, found that the genetic similarity among them ranged from 0.06 to 0.79 revealing high genetic diversity. Palai and Rout, 2011 [12] used ISSR primers to detect the new variety of chrysanthemum developed through spontaneous sporting and resulted that rate of polymorphism showed significant differences as compared to other existing varieties.

Table 1: Effect of mutagenic treatment (gamma rays and EMS) on morphological characters of chrysanthemum var. Poornima White

Treatments	Survivability (%)	Plant height (cm)	Stem girth (mm)	Plant spread in E-W (cm)	Plant spread in N-S (cm)	Number of Primary branches	Number of leaves	Leaf area (mm^2)	Days to flower bud initiation
Control	89.99 (71.72)	56.90	7.96	37.30	33.30	4.00	255.51	491.92	96.40
5 Gy	54.44 (47.54)	47.60	9.89	33.80	40.00	5.00	272.96	486.52	96.50
10 Gy	45.55 (42.44)	24.70	4.51	16.30	14.80	1.66	113.00	397.42	106.00
15 Gy	35.55 (36.58)	45.70	7.34	31.80	31.20	2.66	224.70	480.93	98.20
20 Gy	0.00 (0.28)	-	-	-	-	-	-	-	-
0.05% EMS	75.55 (60.53)	54.70	11.07	42.10	36.50	4.33	253.73	492.59	99.30
0.1% EMS	65.55 (54.15)	43.20	5.11	26.10	28.30	3.33	232.48	483.05	97.60
0.2% EMS	61.10 (51.44)	43.50	4.57	29.30	27.50	2.90	243.13	430.43	96.10
0.3% EMS	59.99 (50.77)	42.10	4.99	21.10	21.60	2.80	230.13	424.58	103.00
0.4% EMS	51.10 (45.63)	40.00	4.28	26.70	23.40	2.90	232.60	437.25	104.50
0.5% EMS	39.99 (39.19)	32.20	6.07	20.60	19.80	2.30	221.26	305.11	105.60
S. Em±	2.92	2.05	0.93	2.20	1.83	0.49	10.23	NS	1.45
C. D. at 1%	11.64	8.25	3.76	8.86	7.36	1.97	41.16	NS	5.83

Table 2: Effect of mutagenic treatment (gamma rays and EMS) on morphological characters of chrysanthemum var. Poornima White

Treatments	Days taken for flowering	Duration of flowering (Days)	Flower diameter (cm)	Flower stalk length (cm)	Individual flower weight (g)	Number of flowers per plant	Flower yield per plant (g)	Vase life (days)
Control	112.60	51.00	4.60	5.73	1.72	156.00	272.40	3.80
5 Gy	113.10	49.00	3.30	5.23	1.62	123.00	185.30	3.76
10 Gy	123.36	33.46	4.50	6.01	1.23	22.60	46.56	3.80
15 Gy	114.66	48.20	4.56	5.53	1.18	100.30	187.20	3.93
0.05% EMS	116.16	53.33	5.36	4.35	1.84	164.20	377.33	3.73
0.1% EMS	116.70	49.03	4.86	4.29	1.70	69.06	139.20	3.60
0.2% EMS	113.46	49.56	5.08	4.24	1.70	88.69	153.68	3.76
0.3% EMS	122.50	49.60	5.21	4.46	1.24	35.43	106.26	3.60
0.4% EMS	120.53	47.06	5.15	4.57	1.21	76.11	123.50	3.66
0.5% EMS	116.26	43.03	4.41	3.45	1.19	45.00	85.14	3.40
S. Em±	1.74	1.06	0.13	0.24	0.08	12.57	3.09	NS
C. D. at 1%	7.00	4.28	0.53	0.96	0.33	50.58	12.42	NS

Table 3: Polymorphism in chrysanthemum variety Poornima White and it's variants related to SRAP primers

S. No	Primer	Number of bands produced	Number of polymorphic bands	Unique bands	Number of monomorphic bands	Per cent of polymorphism (%)
1	Me1+Em10	5	4	1	0	80
2	Me2+Em3	4	1	3	0	25
3	Me3+Em7	3	3	0	0	100
4	Me2+Em2	4	4	0	0	100
5	Me2+Em6	6	1	0	5	16.66
		22	13	4	5	

Table 4: Unique bands produced by SRAP primer combinations in chrysanthemum variety Poornima White and it's variants

S. No	Primer	Number of unique bands Produced	Number of unique bands	Variant code
1	Me1+Em10	1	1	PWM3
2	Me2+Em3	3	3	PWW3

PWM: Poornima White Mutant

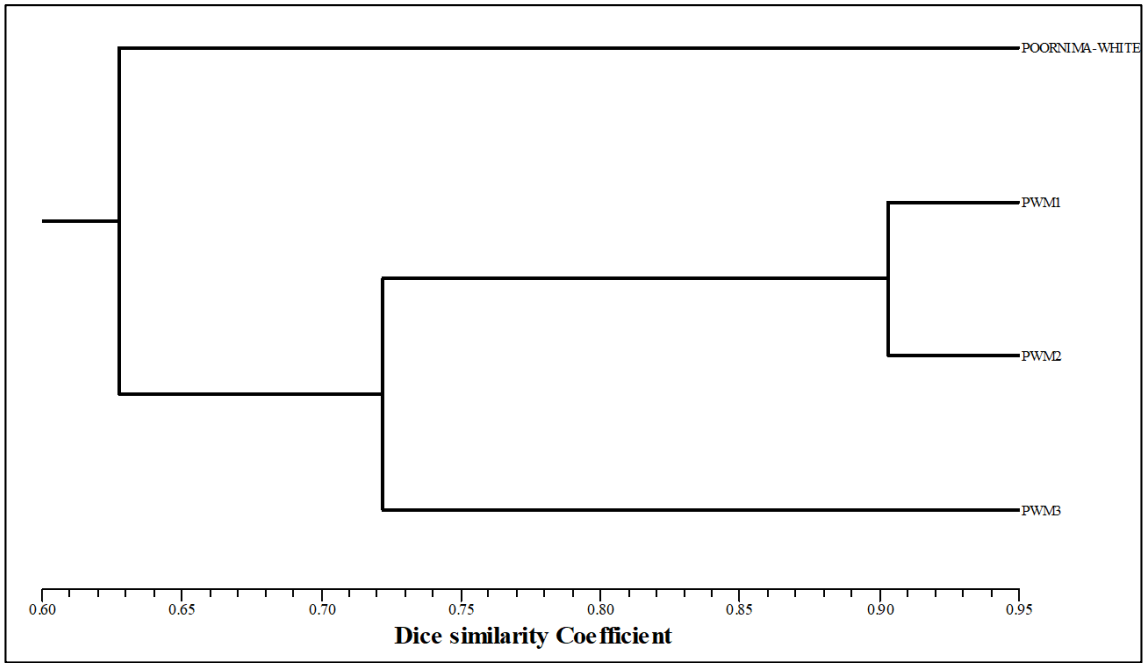
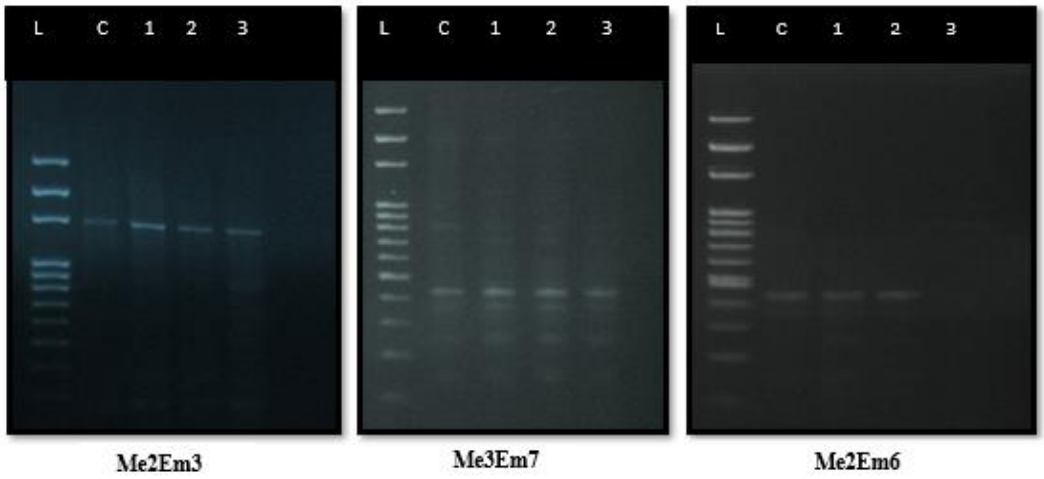


Fig 1: Dendrogram showing genetic relationship in chrysanthemum variety Poornima White (Parent) and it's mutants based on SRAP marker according to unweighed pair group method with arithmetic average (UPGMA) analysis

Fig 2: Mutants of *ex vitro* mutated population of chrysanthemum var. Poornima White



L: Ladder
C: (Control plant) Chrysanthemum variety Poornima White
1-3: Mutants of chrysanthemum variety Poornima White

Fig 3: SRAP profiles generated by different primers using chrysanthemum variety Poornima white and its mutants

Conclusion

The results revealed that induced mutagenesis on rooted cuttings of Chrysanthemum cv Poornima White treated with 10 Gy gamma irradiation doses induced dwarfness and also 5 Gy gamma irradiation indudces the higher flower yield.leaf variegation was observed in 0.1% EMS treatment over control. While, higher radiation doses of gamma rays increased aberrations in morphological and flower characters. It may conclude that lower dose of physical and chemical mutagenesis is useful for inducing changes in morphological and flower characteristics in chrysanthemum cv. Poornima White. Even though less number of primers were analyzed for molecular characterization of mutants, still it gave some information about polymorphism between parent and mutants. It proves SRAP primers will be useful for characterization of mutants at molecular level in chrysanthemum.

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