



Induction of novel variants in *Chrysanthemum morifolium* through electron beam radiation

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ABSTRACT

Un-rooted cuttings of chrysanthemum varieties Pusa Chitraksha, Pusa Anmol and Basanti were treated with Electron Beam (EB) radiation (20 Gy and 30 Gy) with an objective of inducing variability and evaluated at ICAR-DFR, Pune during 2020. A gradual and significant reduction in vital plant growth parameters was observed with increase in doses. Influence of EB radiation (at 30Gy) on plant height (-34.55%), plant spread (-28.60%), internode length (-39.04%), leaf length (-26.95%), leaf width (-29.28%), number of flowers per plant (-23.28%) and for flower diameter (-24.80%) in comparison to control was recorded. Delay in days to bud initiation and flowering at higher dose (30 Gy) were 13.47 days and 17.33 days, respectively over control. Among the varieties, Pusa Chitraksha was found superior for most of the vegetative growth and flowering parameters. However, it exhibited delay in bud initiation (95.80 days) and flowering (118.0 days). The trend is similar for other two varieties at higher dose. In the interaction effect (Variety x EB dose), plant height (17.00 cm) was very less in variety Basanti at 30 Gy dose. Flower diameter (3.26 cm) was reduced with increased EB dose (30 Gy) in Pusa Aditya compared to control (5.24 cm). Pusa Chitraksha exhibited variation in flower colour (Magenta to white/ cream colour) and Pusa Aditya produced red colour flower at 30 Gy. Based on the study, it can be inferred that Electron Beam at 20-30 Gy had potential to create variation in chrysanthemum.

Keywords: Chrysanthemum, Dose, Electron Beam, Irradiation, Mutants

Floriculture industry is always driven by novelty which includes variation in form, colour, shape, size, etc. Conventional breeding plays an important role in the development of new varieties. Of all the alternate breeding methods, induced mutation techniques are most suitable for chrysanthemum improvement. It is the only acceptable way of classically increasing variability in plant species that do not produce seeds (Ahloowalia and Maluszynski 2001), and to develop novel colours and variations in vegetatively propagated ornamental plants (Broertjes and Van Harten 1988, Datta and Teixeira Da Silva 2006 and Kondo *et al.* 2008).

The type of mutagen and the treatment method are important factors for obtaining success in mutation breeding. Besides X-rays and gamma rays, the electron beams are also capable of inducing variability in plants. The electron beam has directivity and high processing performance. The

rate of imparted energy (the ratio of energy given to the material per unit length) is extremely higher than that of other radiations. Moreover, the absorbed dose rate is very large because the electron beam has high directivity to the direction of acceleration (Kashiwagi and Hoshi 2012). High energy electron beams from the LINACS are useful in a variety of applications, besides development of new crop varieties through mutation breeding. Electron beam based radiation processing is an extremely safe alternative to radioisotope based facilities (like cobalt-60) as there is no radioactivity involved. However, electron beam have not been sufficiently characterized in terms of mutagen for mutation breeding until now. Very limited reports are available on the induction of mutagenesis using Electron Beams in chrysanthemum (Sun *et al.* 2007). The scope of conventional methods of breeding is limited under Pune conditions as many varieties failed to set seed and on the other hand chrysanthemum is highly amenable to mutation breeding. Hence, an attempt was made to explore the potential of electron beam (10 MeV) in inducing variability in chrysanthemum and to obtain novel mutants as there were no reports/studies till date in chrysanthemum involving use of electron beam radiation in India.

MATERIALS AND METHODS

The experiment was conducted at ICAR-Directorate

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of Floricultural Research, Pune using three varieties of chrysanthemum, viz. Pusa Chitraksha (PC), Pusa Aditya (PA) and Basanti during 2020. Un-rooted terminal cuttings (7-9 cm) were used as the experimental material. These cuttings were irradiated with electron beam (at 20 Gy and 30 Gy) at the Electron Accelerator based Radiation Processing Facility developed by Raja Ramanna Centre for Advanced Technology (RRCAT), Indore. This facility is based on the indigenously developed 10 MeV, 5 kW electron linear accelerators (LINACS). The irradiated cuttings were subjected to rooting in plug trays containing Cocopeat:Perlite: Vermiculite (4:2:1v/v) and kept in shadenet. After 21 days the rooted cuttings were transplanted into the pots (8") filled with media containing Red Soil: FYM (3:1 v/v). Standard cultural operations were followed throughout the period.

The mortality percent was calculated at an interval of 7 and 14 days. All the varieties were evaluated for vegetative growth, flowering and any other kind of variation due to EB radiation. Factorial CRD (completely randomized design) with two factors namely dose of electron beam radiation (20 Gy and 30 Gy) and varieties (Pusa Chitraksha, Pusa Aditya and Basanti) with 3 replications for each treatment were used for the experiment. The data were analyzed using SAS software for analysis of variance (ANOVA), after which means were compared using the least significant difference (LSD).

RESULTS AND DISCUSSION

Optimization of EB dose: Determination of optimum dose is crucial to achieve the objective of creating variability and thereby the novel mutants. The dose of any physical mutagen is directly proportional to the extent of variability and inversely proportional to the survival of the plants. The LD₅₀ dose varies with variety, season, type of plant materials, etc. The optimum dose was determined by calculating the survival percentage of rooted cuttings after 21 days. Maximum survival rate was recorded in control and minimum in 30 Gy. However, based on mortality rate and induction of variability 30 Gy was found to be optimum. The reduction in survival rate may be due to damage to the auxin metabolic path that hampered rooting. According to Datta (2009) the optimum dose of gamma ray irradiation for rooted cuttings was estimated to be in between 10-30 Gy. Kiong *et al.* (2008) also revealed that alteration in metabolic pathways of the cells caused due to chromosomal damage leads to reduction in survival. Increase in radiation dose causes chromosomal damage which may be responsible for reduction in survival percentage. Significant differences in respect of survival per cent were not observed among the three varieties studied but it was found that the survival decreased in all the varieties with increase in dosage (from 20 to 30 Gy).

Effect of electron beam radiation on morphological parameters: There was a significant difference in plant growth characteristics when the un-rooted cuttings of three varieties were exposed to different doses of Electron Beam

(EB) radiation. The variety, dose and their interaction effects were also highly significant.

Plant height: A perusal of data (Table 1) revealed that there were significant differences among the varieties (Factor A) with respect to plant height. Maximum height (41.98 cm) was observed in Pusa Chitraksha (V₁) followed by Pusa Aditya (V₂) (30.93 cm) and 23.92 cm in Basanti (V₃). Significant reduction in plant height was observed when un-rooted cuttings were exposed to EB radiation. Plant height was maximum (38.03 cm) in control and gradually reduced with the increase in dosages. Minimum height (24.89 cm) was observed in D₃ (30 Gy dose). There were significant differences among the interaction of electron beam dose and variety. Maximum plant height was recorded in the interaction between variety Pusa Chitraksha (V₁) and Control (D₁) (47.54 cm) which was significantly higher than the individual factors (Variety and Dose). Whereas, least interaction value (17.00 cm) was observed in variety Basanti (V₃) with 30 Gy (D₃). Interaction (dosage and variety) effects revealed that the plant height is inhibited with increase in EB dose in all the varieties. Similar type of results were also exhibited by Gamma Irradiation where it had an inhibitory effect on the plants and resulted in the production of free radicals which have deleterious effects on physiological, morphological and anatomical aspects according to the irradiation level. It was reported that the radiation possess powerful agents that can alter physiological and biochemical properties of plants depending on the absorbed doses (Ling *et al.* 2008). Plant growth inhibition at higher doses may be due to the damage to the meristematic region or inhibition of meristematic growth (Joshi-Saha *et al.* 2015 and Yadav 2016).

Plant spread: Pusa Chitraksha recorded maximum plant spread (31.28 cm), whereas minimum was in Basanti (19.27 cm). Among the dose factor, maximum plant spread was in control (28.98 cm) followed by 20Gy (D₂ -24.26 cm). The interaction among variety Pusa Chitraksha and control recorded maximum plant spread (35.72 cm) and least (16.88 cm) in Basanti at 30 Gy. Melki and Marouani (2010) reported that low doses of radiation promote plant development but inhibited with increasing doses.

Internode length: The Electron Beam had a pronounced effect on internode length and it decreased with the increase in doses. Among varieties, it was maximum in Pusa Chitraksha (1.98 cm) and minimum in Basanti (1.32 cm). With respect to EB doses, it was observed maximum in control (D₁) with 2.10 cm. Among the interaction effects the variety Basanti and Pusa Aditya recorded the maximum dwarfness (1.0 cm and 1.24 cm respectively), whereas maximum internode length was observed in Pusa Chitraksha in control (2.36 cm). The reduction in internodal length resulted in decrease in plant height (with increased dose of electron beam radiations). Similar inference was reported with the use of Gamma rays for induction of variability by Singh and Bala (2019) in chrysanthemum.

Leaf characters: EB radiation had a pronounced effect on leaf length and leaf width. Pusa Chitraksha recorded

Table 1 Effect of electron beam irradiation on plant growth and floral characters in chrysanthemum

Particular	Treatment	Plant height (cm)	Plant spread (cm)	Internode length (cm)	No. of primary Branch	No. of secondary branch	Leaf length (cm)	Leaf width (cm)	Days to initiation	Days to flowering	No of flowers per plant	Flower diameter (cm)
Factor A (Variety)	V1 (Pusa Chitraksha)	41.98	31.28	1.98	4.00	8.60	5.31	3.78	95.80	118.00	98.33	4.66
	V2 (Pusa Aditya)	30.93	23.37	1.78	4.73	13.00	4.49	2.76	78.86	100.20	76.73	4.34
	V3 (Basanti)	23.92	19.27	1.32	4.33	9.66	4.52	2.66	82.66	105.06	62.06	4.52
Factor B (Dose)	D1(Control)	38.03	28.98	2.10	3.00	7.93	5.49	3.62	78.66	98.60	89.33	5.16
	D2(20Gy)	33.92	24.26	1.70	4.40	10.53	4.82	3.02	86.53	108.73	79.26	4.47
	D3(30 Gy)	24.89	20.69	1.28	5.66	12.80	4.01	2.56	92.13	115.93	68.53	3.88
Interaction (A × B)	V1xD1	47.54	35.72	2.36	2.60	7.20	6.1	4.20	90.4	110.60	107.80	5.26
	V1xD2	43.84	31.62	1.98	3.80	8.00	5.38	3.84	96.8	119.00	98.20	4.54
	V1xD3	34.58	26.52	1.60	5.60	10.60	4.46	3.30	100.2	124.40	89.00	4.18
	V2xD1	36.56	27.96	2.26	3.60	9.20	5.16	3.42	71.00	92.20	89.40	5.24
	V2xD2	33.14	23.48	1.84	5.00	13.40	4.62	2.72	78.40	99.60	76.80	4.52
	V2xD3	23.10	18.68	1.24	5.60	16.40	3.70	2.16	87.20	108.80	64.00	3.26
	V3xD1	30.00	23.26	1.68	2.80	7.40	5.22	3.24	74.60	93.00	70.80	4.98
	V3xD2	24.78	17.68	1.30	4.40	10.20	4.46	2.52	84.40	107.60	62.80	4.36
	V3xD3	17.00	16.88	1.00	5.80	11.40	3.88	2.24	89.00	114.60	52.60	4.22
CV		4.30	5.13	9.32	19.92	17.43	5.58	7.52	1.94	2.88	4.28	4.92
Variety (CD 0.05)		1.03	0.94	0.11	0.64	1.35	0.19	0.17	1.24	2.31	2.52	0.16
Dose (CD 0.05)		1.03	0.94	0.11	0.64	1.35	0.19	0.17	1.24	2.31	2.52	0.16
V×D (CD 0.05)		1.79	1.62	0.20	1.11	2.34	0.34	0.29	2.15	4.01	4.36	0.28

maximum leaf length (5.31 cm) and leaf width (3.78 cm) followed by Basanti (4.52 cm and 2.66 cm) and Pusa Aditya (4.49 cm and 2.76 cm). Significant results were also found among the dosages of radiation. The plants in control recorded maximum leaf length (5.49 cm) and leaf width (3.62 cm). Leaf length and width was considerably reduced with the increase in radiation doses (from 20 to 30 Gy). Perusal of interaction effects revealed that the variety Pusa Chitraksha (V_1) with control (D_1) recorded the maximum leaf length (6.10 cm) and leaf width (4.20 cm). Whereas, least leaf length (3.70 cm) and width (2.16 cm) was in variety Pusa Aditya (V_2) with 30 Gy radiation (D_3). Joshi-Saha *et al.* (2015) also obtained similar trends in leaf characteristics with Electron Beam irradiation in chickpea. The reduction in leaf size of plants treated with higher doses of radiations may be due to inactive or decrease in auxin content or disturbances in auxin synthesis (Misra *et al.* 2009, Bala and Singh 2015).

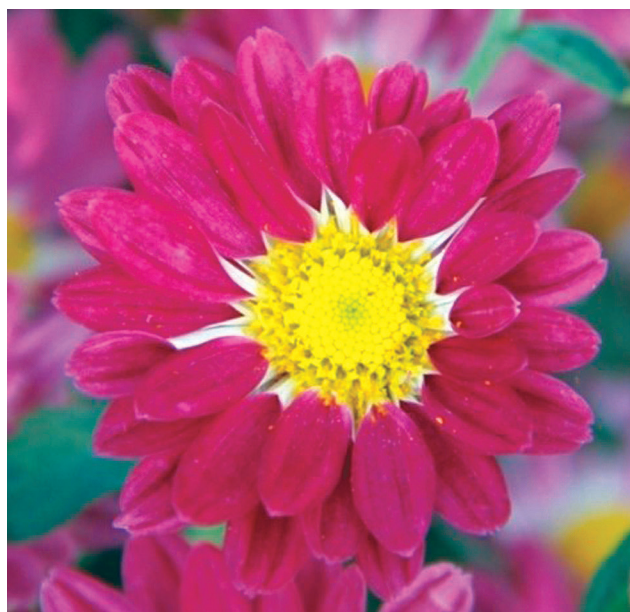
Branching habit: Significantly maximum number of primary and secondary branches was recorded in variety Pusa Aditya (4.73, 13.0) followed by Basanti (4.33, 9.66). The trend was just the reverse of other parameters as mentioned above. Here the numbers increased with the dosage of irradiation. Maximum number of branches were recorded in the plants where the cuttings were subjected to 30Gy EB (5.67 and 12.80) followed by 20 Gy (4.40 and 10.53). The increase was about 89% over the control for primary branches. Among the interaction effect, maximum number (5.8) of primary branches was observed in variety Basanti (V_3) with 30 Gy radiation (D_3). Whereas, Pusa Aditya (V_2) with 30 Gy dose (D_3) exhibited maximum number of secondary branches. In the present study the radiation resulted in more branching rather than apical growth. Similar results with higher irradiation dose were reported in fennel (Verma *et al.* 2017).

Effect of electron beam radiation on floral traits: Changes in flower shape, size and colour exhibit direct effect of radiation. In the present study, the flowering characters were also affected by the different doses of electron beam irradiation. Treatment with higher doses delayed most of the flowering characters like days to bud initiation, days to flowering and number of flowers per plant. High frequencies of changes in flower colour were observed in the present study (Fig 1). Such phenomenon might result from the appearance of LII tissue on the surface, *i.e.* cell layer rearrangement (Broertjes and Van Harten 1988).

Flower bud initiation and flowering: The flowering habits are the inherent characteristics of a variety. Variation, however, due to different factors like growing environment, season, cultural practices, etc is common among all the varieties. A perusal of data revealed that maximum number of days for bud initiation and flowering was recorded in Pusa Chitraksha (95.80 days and 118 days, respectively) (Table 1) while minimum in Basanti (82.66 days and 105.06 days).

With the increase in the dosage, there was delay in bud initiation and thereby flowering. Minimum days for bud initiation and flowering was observed in control (78.66 and 98.60, respectively), whereas maximum delay was recorded with 30Gy radiation (92.13 and 115.93 days, respectively). Irradiation with Gamma rays also revealed the same results as of EB as reported in chrysanthemum by Banerji (2002) and in marigold (Singh *et al.* 2009). Pusa Chitraksha (V_1) when irradiated with 30 Gy dose (D_3) of EB recorded the maximum number of days for flower bud initiation (100.2 days) and flowering (124.40 days). Whereas minimum days were recorded in variety Pusa Aditya (V_2) in control (D_1) for bud initiation (71.00) and flowering (92.20).

Number of flowers: Number of flowers per plant is crucial parameter as it determines the overall yield of the crop. Irradiation with electron beam resulted in suppressive



Pusa Chitraksha (Control)



Pusa Chitraksha at 30Gy

Fig 1 Variation in flower colour in variety Pusa Chitraksha at 30 Gy electron beam irradiation.

effect on number of flowers per plant. Pusa Chitraksha (98.33) recorded maximum number of flowers per plant, whereas Basanti recorded minimum (62.06). The plants that were not irradiated (control) exhibited maximum number of flowers (89.33) while minimum were observed in 30 Gy (68.53). Among the interaction effects the variety Basanti (V_3) with 30 Gy (D_3) recorded minimum number of flowers (52.60), whereas maximum was recorded in variety Pusa Chitraksha with control (107.80). This may be due to the deleterious effects of irradiation on plant growth hormones.

Flower diameter: Flower diameter was found to be maximum in Pusa Chitraksha (4.66 cm) which was at par with Basanti (4.52 cm) (Table 1). The EB radiation had inhibitory effect on flower diameter. Maximum diameter was recorded in Control (5.16 cm) which got reduced to 3.88 cm upon exposure to 30 Gy irradiation. Un-irradiated (control) plants of Pusa Chitraksha (5.26 cm) followed by Pusa Aditya (5.24 cm) recorded maximum flower size. Least flower diameter (3.26 cm) was recorded in Pusa Aditya with 30Gy irradiation. The probable cause of the reductions in flower diameter might be due to reduced petal size or abnormalities and variations in floral characters due to hindered development by irradiation. Similar results were reported by Bala and Singh (2015) in rose.

Flower colour: As mentioned in the foregoing paras, novelty is very crucial in floriculture world. Flower colour, form and off-season availability are few areas of novelty. It was found that EB radiation is a potential source to induce novelty in the form of changed flower colour. The flower colour of Pusa Chitraksha got changed from reddish purple to white/light cream (Fig 1). Change in flower colour due to EB radiation either in Pusa Chitraksha or Pusa Aditya may be harnessed for development of improved varieties with novel flower colours.

Chrysanthemum is highly amenable to mutation breeding and based on this fact physical mutagens (Gamma rays) were extensively used for creation of variability. In the similar pattern, the findings of the present investigation conclude that the radiation from Electron Beam could be a great source for creating variability as it resulted in more pronounced effect in inducing variability in chrysanthemum. EB dose of 30 Gy was found to be optimum for un-rooted cuttings of chrysanthemum to induce variability. Use of EB can be recommended as it is environmentally safe and less harmful to the research workers. Novel variations obtained would be evaluated further in multiple generations and desirable colour and form can be advanced further for commercial exploitation in the form a variety/selection.

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