Congenial in Vitro γ-Ray Induced Mutagenesis Underlying the Varied Array of Petal Colours in Chrysanthemum (Dendranthemum Grandiflorum Kitam). 'Candid'

Ambreena Din
SKUAST Kashmir: Sher-E-Kashmir University of Agricultural Sciences and Technology Kashmir
Zahoor Ahmed Qadri
SKUAST Kashmir: Sher-E-Kashmir University of Agricultural Sciences and Technology Kashmir
MUNEEB AHMAD WANI (wanimuneeb05@gmail.com)
Sher-E-Kashmir University of Agricultural Sciences and Technology Kashmir https://orcid.org/0000-0002-7161-0901
Z.A. Rather
SKUAST Kashmir: Sher-E-Kashmir University of Agricultural Sciences and Technology Kashmir
Shameen Iqbal
SKUAST Kashmir: Sher-E-Kashmir University of Agricultural Sciences and Technology Kashmir
Sajid Ali Malik
SKUAST Kashmir: Sher-E-Kashmir University of Agricultural Sciences and Technology Kashmir
Sadaf Rafiq
SKUAST Kashmir: Sher-E-Kashmir University of Agricultural Sciences and Technology Kashmir
I.T. Nazki
SKUAST Kashmir: Sher-E-Kashmir University of Agricultural Sciences and Technology Kashmir

Research Article

Keywords: Chrysanthemum, colour mutants, mutagenesis, gamma irradiation, mutants

Posted Date: June 22nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-617238/v1

Abstract

Chrysanthemum (*Dendranthemum grandiflorum kitam.*) is amongst the top ten cut flowers globally. The flower colour of ancestral species is restricted to white, yellow, and pink and is acquired from anthocyanins, carotenoids, and the dearth of both pigments, respectively. An extensive array of flower colours, like orange, dark red, purplish-red, and red, has been bred by enhancing the variety of pigments or the amalgamation of both pigments. In recent times, green-flowered cultivars having chlorophylls in their ray petals have been produced and have grown a reputation. Furthermore, violet /blue flowers have been bred via transgenic interventions. Flower colour is considered as critically acclaimed feature of any flower cultivar especially chrysanthemum. Creating newer chrysanthemum cultivars with novel features, for instance, new flower colours in a time and input optimised approach, is the eventual ambition for breeders. Exploring the molecular mechanisms that control flower pigmentation may present imperative suggestions for the rational manoeuvring of flower colour. To generate a diverse array of flower colour mutants in chrysanthemum cv. “Candid” through mutagenesis, *in vitro* grown micro shoots were exposed to 10, 20, 30, and 40 Gy gamma irradiation at 100 Gy per minute and were evaluated for different parameters. The rhizogenesis parameters declined with the increase in irradiation dose from 0 Gy to 40 Gy, while as, 10 Gy dose proved to record minimum decline in contrast to the control. Survival, leaf size, and the number of leaves plant^{-1} after the 8th -week interval also decreased with the increasing trend of gamma irradiation dose but recorded a minimum decline in plants raised from shoots irradiated with 10 Gy gamma irradiation dose with respect to the control. Apparently, the minimum delay in the number of days to floral bud appearance took under 10 Gy compared to control. The highest number of flower colour mutants were recorded under 10 Gy (light pink, orange-pink, white and yellow). Demountable mutation frequency based on flower colour was desirable in plants irradiated with the slightest dose of 10 Gy

Introduction

Chrysanthemum is a prevalent and essential cut flower crop grown worldwide in China, Japan, France, the USA, India, and the UK. It is a keyfloricultural crop, and ranks 2nd in terms of cut flowers following rose, amongst the floricultural crops traded in the international flower market (Kumar et al. 2006). The complex genetic hetrozygosity makes the cultivated chrysanthemum an unlimited source of new flower form and cultivars. The common garden chrysanthemum is hexaploid with 54 chromosomes (Wolff 1996). It is propagated vegetatively and has a robust self-incompatibility system (Richards 1986); therefore, new varieties are complicated to acquire by crossing. Usually, new varieties have been developed from spontaneous mutations in vegetative reproduction, sports, being some variations more stable than others (Miñano et al., 2009). In recent years induced mutations along with the somaclonal variations resulting from the tissue culture practices have been in work as an innovative source of variability (Schum 2003, Datta et al. 2005, Jain et al. 2006, Zalewskam and Miller 2007, Chatterjee et al. 2006, Barakat et al. 2010) Even though comprehensive efforts have been undertaken to expand uniqueness in chrysanthemum cultivars by induced mutations employing physical and chemical

mutagens (Broertjes and Van-Harten 1978), there is a constant need to investigate the chances of a new cultivars for horticulture trade. Mutation breeding by radiation has been extensively used to improve well-established plant cultivars and develop novel variants within superior consumer characteristics. While the majority of cultivated chrysanthemum varieties are polyploids with huge genetic heterogeneity, mutants with allied flower shape, floral size, and colour, are frequently recovered. Associated flower colours with chimeric tissue can be simply stimulated by radiation and isolated with *in vitro* apparatus (Kumar et al., 2006). Mutation procedures are used because chrysanthemum is a hexaploid plant and vegetative propagated, making it difficult to conduct the hybridization (Broertjes and Van-Harten 1978). Genetic variation is essential in any plant breeding programme for crop improvement. Mutation breeding is an efficient way to produce heritable change, particularly for the flower colour. Increasing demand for the new form of chrysanthemum leads to research for obtaining new varieties. Mutation breeding by radiation, a farming appliance of nuclear technology, and has been widely exploited to develop well-adapted plant varieties by one or a few important qualities (Kumar et al. 2006, Jain and Spencer (2006, Dwimahyani and Widiarsih 2010, Chatterjee et al. 2006) Commercially important traits in horticulture plants have been altered positively by the various physical mutagens. Gamma rays are widely used among the physical mutagens for inducing mutations in flowering plants due to their easy application and high efficiency. The physical irradiations have been used efficiently to induce mutation in chrysanthemum and the optimal dose range from 1.0 to 3.0 Krads correlated to the type of genotype (Dilta et al., 2003). While going for mutation breeding programmed various factors like choice of material, character to be improved, type of mutagens and its dose to be used, an experimental procedure to be chosen should be considered. Thus through mutation breeding, it is possible to induce a genetic variation for quantitative and qualitative characters that is heritable of sufficient magnitude and frequency of interest in the breeding programme. Accordingly, the genetic variability produced by mutation was studied to develop a novel cultivar in chrysanthemum having momentous customer fondness. Consequently, with deliberation to above facts, the current study “Congenial in vitro γ- ray induced Mutagenesis underlying the diverse array of petal colours in chrysanthemum (*Dendranthemum grandiflorum kitam*) cv. ‘Candid’ ” was undertaken with an objective to generate a diverse array of flower colour mutants through mutagenesis.

Materials And Methods

Tissue culture developed micro shootlets of *chrysanthemum* cv. ‘Candid’ (parent cultivar, Figure-1) were exposed to Cobalt^{60} gamma irradiation doses of 0, 10, 20, 30, and 40 Gy at 100 Gy per minute and were allowed to raise vegetatively mutated generations first and second at 5-week intervals. Finally, shoots obtained from vegetatively mutated generation 2 were allowed for rooting and consequent acclimatization. Rooted shoots were allowed to grow in pots in the field to obtain new enviable colour mutants, and rooting parameters were recorded in terms of percentage rooting and the number of roots per shoot. Survival (%), leaf area plant^{-1} (cm^2), number of leaves plant^{-1} were recorded at 4th and 8th weeks growth in the field. Days to flower bud appearance were recorded at the initiation of flower bud appearance. Plant height was noted at the end of full flower bloom. Flower colour was recorded in terms

of the difference between the parent flower and mutants obtained. The frequency of mutation was calculated based on flower colour, as the ratio between such desired or undesired colour mutant and whole plants irradiated with each gamma irradiation dose.

The investigation was carried out in completely randomised design with four replications and the data composed on various observations during the trail was preceded to analysis of variance (ANOVA). To assure model assumptions for ANOVA percent age data were subjected to square root transformation as recommended by Steel and Torrie (1980) and the average data was classified by Duncan's multiple range test (Gomez & Gomez, 1983).

Results And Discussion

The irradiation doses had an enervating effect on all the rooting parameters in comparison to control (Table 1). A significant decrease in mean rooting percent and the number of roots shoot^{-1} in all the irradiation treatments compared to control was observed. Among irradiation treatments, the minimum decline in rooting and number of roots were recorded under 10 Gy dose, followed by 20 and 30 Gy irradiation doses. Maximum decline in rooting and the number of roots were registered with 40 Gy dose (Fig. 2-a, and b, Fig. 1, 3a and 3b). Rhizogenesis is a procedure of dedifferentiation of definite pre-determined cells near the vascular bundles. Any harm to cell division capability will have an unconstructive outcome on the dedifferentiation of cells and consequent reorganization into root primordia. This could cause malfunction of rooting or deferred emergence of roots. Singh et al. 1999 also reported that increased doses of gamma irradiation (from 20 to 50 Gy) decreased the rooting percentage of carnation cv. 'Espana.' Radiation treatments also delayed root initiation significantly in comparison to control. Sooch *et al.*2000 observed delayed root initiation of carnation shoots of cv. 'Scania' under 1.00, 1.50, and 2.00 K-rads gamma irradiation doses. The deleterious effects of radiations also showed a significant decline in root number per shoot under 10 to 30 Gy treatments. El-sharnouby and El-Khateeb 2005 also reported that most of the gamma irradiation treatments (10, 20, 30, and 40 Gy) without or with NAA in the rooting medium decreased the number and the length of roots in the carnation cultivars "Medea," "Candela" and "Picaro." All the above-quoted studies seem closer to the findings recorded in the present study. Survival of rooted shoots at the end of 4 weeks was significantly minimum by the shoots treated with 40 Gy dose as against control, followed by 30 and 20 Gy dose (Table 2). Under a minimum dose of 10 Gy, there was a minimum decline in survival of shoots at the end of the 4 weeks over control. At the end of 8 weeks, shoots treated with 10 Gy dose recorded maximum survival, followed by 20 and 30 Gy dose (Fig. 2, 3c and 3d). While lowest survival per cent was recorded in 40 Gy irradiated shootlets corresponding to a sharp decline compared to control.

Table 1

Influence of ^{60}Co gamma irradiation on rhizogenesis in shoots of Chrysanthemum (*Dendranthemum morifolium* L.) cv. “Candid”

Dose	Rooting (%)	Root number shoot^{-1}
0 Gy	89.28a ± 2.38 (9.44) *	15.75a ± 0.50 -
10 Gy	85.71 b ± 0.00 (9.26) * (1.90) **	7.75 b ± 0.96 (50.79) **
20 Gy	79.76c ± 2.38 (8.93) (5.40)	5.50 c ± 0.58 (65.07)
30 Gy	74.99 d ± 2.38 (8.66) (8.26)	3.00d ± 0.00 (80.95)
40 Gy	73.80d ± 2.75 (8.59) (9.00)	2.75d ± 0.50 (82.53)
L.S.D $_{P\leq0.05}$	**0.18**	**0.90**

*Figures in the parenthesis are the square root transformed values of percentage data **Figures in the parenthesis are the per cent decrease in vegetative parameters over control

Table 2

Influence of ^{60}Co gamma irradiation on survival of rooted plantlets under polyhouse conditions in Chrysanthemum (*Dendranthemum morifolium* L.) cv. “Candid”

Dose	Survival (%)	
	Week 4th	Week 8th
0 Gy	95.23a ± 0.00 (9.76)*	95.23a ± 0.00 (9.76)*
10 Gy	85.71b ± 3.89 (9.25)* (5.22)**	82.14 b ± 2.38 (9.06)* (7.17)**
20 Gy	76.18c ±3.89 (8.72) (10.65)	67.85c ± 4.56 (8.23) (15.67)
30 Gy	61.90d ± 3.89 (7.86) (19.46)	53.56d ± 4.56 (7.31) (25.10)
40 Gy	53.56e ± 5.99 (7.31) (25.10)	40.47e ± 6.15 (6.34) (35.04)
LSD $_{(P\leq0.05)}$	**0.17**	**0.27**

*Figures in the parenthesis are the square root transformed values of percentage data **Figures in the parenthesis are the per cent decrease in vegetative parameters over control

Broertjes and Lock (1984) obtained 100% survival when chrysanthemum plantlets transferred to soil were irradiated with 2.5 or 5 kGy. The deleterious chimera load carried by the plants leads to mortality in post-irradiation proliferative generations. Another reason might be the formation of the low or reduced wax component on the post-irradiation plants. The wax module decides the pace of water loss through the cuticle and the vulnerability of tissue-cultured plants to desiccation accredited to a decline or lack of wax acting as an antitranspirant. The epicuticular wax is reduced or absent on the carnation leaves of *in vitro* cultured plants compare to glasshouse or field-grown plants (Sutter and Langhans1979). Still, during

acclimatization, the density of waxes boosts as the humidity recedes (Wardle et al. 1983). Since the irradiation impairs the plants' epidermal skin, low wax formation during the acclimatization process leads to mortality.

Influence of γ- rays on leaf area and number of leaves

Gamma irradiated treatments significantly recorded a decline in leaf number plant^{-1} and leaf size in both the intervals, i.e., 4 and 8 weeks, compared to control (Table 3). At the end of 4 weeks, significantly minimum leaf number plant^{-1} and size were registered under the highest dose of 40 Gy, followed by 30 and 20 Gy. The lowest gamma irradiation dose, 10 Gy, recorded a minimum decrease in leaf number and size compared to the control. At the end of 8 weeks, both leaf number as well as leaf size improved in all the gamma irradiation doses, including the control plants but recorded a similar trend of decline in both the parameters as in the 4-week interval with the successive gamma irradiation doses (Fig. 3e to 3h).

Table 3

Influence of ^{60}Co gamma irradiation on leaf number and leaf size in Chrysanthemum (*Dendranthemum morifolium* L.) cv. "Candid"

Dose	Leaf number plant^{-1}		Leaf size (length/width) (cm^2)	
	4th week	8th week	4th week	8th week
0 Gy	14.00a ± 0.82	15.75a ± 0.96	22.31a ± 3.30	28.52a ± 1.18
10 Gy	11.00b ± 0.82 (21.42)	13.00 b ± 0.82 (17.46)	20.42 a ± 1.45 (8.43)	27.80 a ± 0.89 (2.52)
20 Gy	9.25 c ± 0.50 (33.92)	11.75 bc ± 0.96 (25.39)	17.34 b ± 01.32 (22.24)	20.42 b ± 1.45 (28.40)
30 Gy	8.75 cd ± 0.50 (37.50)	10.75 cd ± 0.96 (31.74)	13.52 c ± 0.72 (39.37)	14.82 c ± 1.23 (48.03)
40 Gy	8.00 d ± 0.00 (42.85)	9.75 d ± 0.96 (38.09)	5.31 d ± 0.66 (76.18)	10.74 d ± 2.14 (62.34)
L.S.D $P \leq 0.05$	**0.92**	**1.41**	**2.69**	**2.37**

Figures in the parenthesis are the per cent decrease in vegetative parameters over control

Leaf area increment results from the growth of cells mainly controlled by growth regulators (auxins). Higher exposure to gamma irradiation agitate synthesis of auxins, hence leads to decreased leaf area. Simard *et al.*1992 and Cassels et al. 1993 recorded biological damage in carnation on increasing the dose of radiation. Misra and Bajpai, 1983a in gladiolus; Gupta et al., 1982 in costus; Gupta et al., 1974 in tuberose; Acharya and Tiwari 1996; Siranut et al., 2000 in chrysanthemum; Srivastava et al. 2007 in gladiolus; Misra et al. 2009 in chrysanthemum and Kahrizi et al. 2011 in rose also accounted the decrease in number of leaves with the raise in dosage of gamma irradiation whereas, Kumari et al. 2013 reported decline in leaf size in terms of length and width of plants treated with higher doses of gamma

rays in variety “Otome Pink” and found that petiole length was shorter with increasing dose of mutagenic agents. Mahure et al.2010 recorded minor dosages like 10, and 20 Gy enlarged leaf area, but 30 Gy dwindled leaf area over control. In yet another study by Dilta et al. 2006 a reduction in leaf number was reported in *Dendranthemum grandiflorum kitam* cv. “Gulmohar” under gamma irradiation dose range of 1.0–3.0 kR.

Influence of gamma irradiation on days to floral bud appearance and plant height (cm) at flowering

With the increment of each dose of irradiation (Table 4), there was a considerable delay in days to bud appearance contrast to control plants (23.50). Under 10, 20, and 30 Gy doses, days to bud appearance were recorded 27.25, 37.00, and 39.25, respectively. Whereas, days to bud appearance under the last dose of 40 Gy were recorded significantly highest 40.75, representing maximum delay compared to control (Fig. 3i). The results in the present study may be due to the disturbances in the biochemical pathway, which assists in the synthesis of flower-inducing substances and hence delay in flowering.

Table 4

Influence of ^{60}Co gamma irradiation on the number of days to floral bud appearance and plant height at flowering in Chrysanthemum (*Dendranthemum morifolium* L.) cv. “Candid”

Dose	Number of days to floral bud appearance	Plant height at flowering(cm)
0 Gy	23.50a ± 1.73	53.25a ± 2.50
10 Gy	27.25b ± 1.50 (15.95)	49.00b ± 0.82 (7.98)
20 Gy	37.00 c ± 0.82 (57.44)	36.50c ± 0.58 (31.45)
30 Gy	39.25cd ± 1.89 (67.02)	34.00d ± 20.82 (36.15)
40 Gy	40.75d ± 2.22 (73.40)	31.50e ± 1.00 (40.84)
L.S.D $_{P\leq0.05}$	**2.58**	**2.03**

Figures in the parenthesis represent per cent increase in case of days to floral bud appearance and per cent decrease in plant height at flowering over control

The present study results concur with the findings of Datta and Banerji 1993 who observed delayed flowering behaviour after irradiating rooted cutting of small decorative type chrysanthemum cv. “Kalyani Mauve.” In another study Dilta et al. 2006 also observed a significant delay in days to bud formation, buds showing colour, and days for full bloom in the treated plants, often chrysanthemum cultivar as compared to control. Similar were the results recorded by Misra et al. 2009 in chrysanthemum cultivar

“Pooja.” Plant height is the genetic characteristics of the plants, and it was expressed as per the individual potential of a variety. With the increment of each dose of irradiation (Table 4), there was a major decline in plant height compared to control plants (53.25 cm). Under 10, 20, and 30 Gy doses, plant height was recorded at 49.00, 36.50, and 34.00 cm, respectively. Whereas, plant height at the flowering time under the last dose of 40 Gy was recorded significantly lowest 31.50 cm, representing the highest decrease compared to control (Fig. 3j). Reduction in vegetative characters by gamma rays treated plants depends on the nature and degree of chromosomal injury or morphological, cytological and physiological, disturbance induced by irradiation and the decline of interior auxin manufacture, leading to plummeting growth of the plant (Banerji and Datta 2002). Also, due to the inactivation and decrease in auxin synthesis and nature and degree of chromosomal aberration (Singh et al. 2011). The results in the present study are in concurrence with the findings of Banerji and Datta 1993. They observed a decrease in plant height with an increased dose of gamma irradiation in the rooted cutting of chrysanthemum cv. “Jaya” and “Lalima.” Kole and Meher 2005 also reported decreasing effects in zinnia might be due to physiological damage caused by mutagen at higher doses.

Influence of mutation frequency and gamma irradiation on flower colour

With response to the colour of flowers after irradiation, desired colour mutants were selected only from the plants irradiated with 10 Gy dose, which evolved 60 per cent of pink, 15 per cent of orange-pink, 10 per cent white, 5 per cent light yellow (5%) and remaining 10 per cent were as identical as control, i.e., showing original red colour (Fig. 3-a, b, c, and d). Higher doses of 20, 30, or 40 Gy produced either distorted red buds or distorted red (Fig. 3-e and f). Colour mutants under 20, 30, and 40 Gy were undesirable. The results in the present study may be due to physiological transformations which happen in the plant; hence, deferred flowering occurs at elevated doses due to inhibitory result. This can be ascribed to the fact that no chimeric growth was developed in the shoot due to mutagenesis. Tissue or shoot without chimeric growth leads to non-formation, diverse colour variations in petals as testified in chrysanthemum by Longton 1980. This quoted observation is in close conformity to the present study. Data regarding the mutation frequency in chrysanthemum flowers based on flower colour showed a highly desired mutation frequency amounting to 90 per cent when the plants were irradiated with 10 Gy dose. Whereas, under 20, 30, and 40 Gy doses flower mutation frequency, although recorded cent per cent, produced undesirable mutants. The results recorded in the present study are in accordance with the finding of Siavash et al.2009 who advocated increased mutation frequency when plants were UV irradiated.

Conclusion

The investigation concludes that irradiation 40 Gy dose resulted in a significant decrease in per cent rooting, subsequent decline in field survival, days to floral bud appearance, plant height at flowering, and mutation frequency. The highest number of desired mutants concerning flower colour (Light pink, Orange-Pink, White, and yellow) and the highest mutation frequency were observed in shoots irradiated with 10 Gy. Hence, 10 Gy gamma irradiation treatment is congenial for mutagenesis in Chrysanthemum Cv. “Candid.”

Abbreviations

GA_3 : Gibberelic acid: MS : Murashige; Skoog's medium BAP: 6-Benzyl amino purine;; Gy : Grays; PANBIT: Panoramic Batch Irradiator

Declarations

Acknowledgements

We want to express our sincere gratitude to UGC, GoI for the MANF fellowship.

Conflict of interest

No conflict of interest.

Declarations

Not applicable

References

1. Acharya NN, Tiwari DS (1996) Effect of MMS and gamma rays on seed germination, survival and pollen fertility of *Hamatocactus setispinus* in M_1 generation. Mysore Journal of Agricultural Science 3(1):10–13
2. Banerji BK, Datta SK (1992) Gamma ray induced flower shape in chrysanthemum cv. 'Jaya'. Journal of Nuclear Agriculture Biology 21(2):73–79
3. Banerji BK, Datta SK (2002) Induction and analysis of gamma ray-induced flower head shape mutation in 'Lalima chrysanthemum (*Chrysanthemum morifolium*). Indian Journal of Agricultural Science 72:6–10
4. Barakat MN, Abdel FRS, Badr M, El-Torky MG (2010) *In-vitro* culture and plant regeneration derived from ray florets of *Chrysanthemum morifolium*. Afr J Biotech 9(8):1151–1158
5. Broertjes C, De-Jong J (1984) Radiation induced male sterility in daisy type of *Chrysanthemum morifolium* Ramat. *Euphytica.* 33,433 – 34
6. Broertjes C, Van-Harten AM (1978) Application of mutation breeding methods in the improvement of vegetatively propagated crops. An interpretive literature review. In: **In**: Developments in crop science (2). Elsevier Scientific Publishing Company, Amsterdam, p 316
7. Cassels AC, Walsh C, Periappuram C (1993) Diplontic selection as a positive factor in determining the fitness of mutants of *Dianthus* 'Mystere derived from X-irradiation of nodes in *in vitro* culture. Euphytica 70:167–174
8. Chatterjee J, Mandal AKA, Ranade SA, Teixeira da Silva JA (2006) Datta SK. Molecular systematics in *Chrysanthemum x grandiflorum* (Ramat.) Kitamura. Sci Hortic 110:373–378

9. Chatterjee J, Mandal AKA, Ranade SA, Teixeira-da-Silva JA, Datta SK (2006) Molecular systematics in *Chrysanthemum x grandiflorum* (Ramat) Kitamura. Sci Hortic 110:373–378
10. Datta SK, Banerji BK (1993) Gamma ray induced somatic mutation in chrysanthemum cv. 'Kalyani Mauve'. Journal of Nuclear Agricultural Biology 22:58–61
11. Datta SK, Misra P, Mandal AKA (2005) *In-vitro* mutagenesis a quick method for establishment of solid mutant in chrysanthemum. Current Science 88(1):155–158
12. Dilta BS, Sharma YD, Dhiman SR, Verma VK (2006) Induction of somatic mutations in chrysanthemum by gamma irradiation. International Journal of Agricultural Sciences 2(1):77–81
13. Dilta BS, Sharma YD, Gupta YC, Bhalla R, Sharma BP (2003) Effect of gamma rays on vegetative and flowering parameters of chrysanthemum. Journal of Ornamental Horticulture 6:328–334
14. Dwimahyani I, Widiarsih S (2010) The effects of gamma irradiation on the growth and propagation of in vitro chrysanthemum shoot explants (cv. Yellow Puma). Atom Indonesia 36(2):,45–49
15. El-Sharnouby ME, El-Khateeb MAA (2005) Effect of BA, NAA and gamma irradiation on the production of three cultivars of carnation (*Dianthus caryophyllus* L.) through tissue culture. *Annals of Agricultural Science* Moshtohor 43(4):1937–1948
16. Gomez KA, Gomez AA (1983) Statistical Procedures for Agricultural Research. Wiley-Interscience Publications, New York pp. 84
17. Gupta MN, Laxmi V, Dixit BS, Srivastava SN (1982) Gamma rays induced variability in. Costus speciosus Progressive Horticulture 14:193–197
18. Gupta MN, Sumiran R, Shukla R (1974) Mutation breeding of tuberose *Polianthus tuberosa* L. **In**: *Proceedings of the Symposium on Use of Radiations and Radio Isotopes in Studies of Plant Productivity.* 12–14, 169–179
19. Jain SM (2010) Mutagenesis in crop improvement under the climate change. Romanian Biotechnological Letters 15:88–106
20. Jain SM, Spencer MM (2006) Biotechnology and mutagenesis in improving ornamental plants. In: Teixeira da Silva JA (Ed) Floriculture, Ornamental and Plant Biotechnology:*Advances and Topical Issues (Vol I), Global Science Books, Isleworth, UK*. 589–600
21. Kahrizi ZA, Kermani MJ, Amiri ME, Vedadi S (2011) Identifying the correct dose of gamma-rays for *in vitro* mutation of rose cultivars. **In**: *Acta Horticulturae* (Eds. A. Fabbri and E. Rugini) *Proceedings of the XXVIIIth International Horticultural Congress on Science and Horticulture International Symposium on Micro and Macro Technologies for Plant Propagation and Breeding in Horticulture* 923, 121–127
22. Kole PC, Meher SK (2005) Effect of gamma rays of some quantitative and qualitative characters in zinnia (*Zinnia elegans* N. J Jacquin) in M_1 generation. Journal of Ornamental Horticulture 8(4):303–305
23. Kumar S, Prasad KV, Choudhary ML (2006) Detection of genetic variability among chrysanthemum radiomutants using RAPD markers. Current Science 90:1108–1113

24. Kumari K, Dhatt K, Kapoor M (2013) Induced mutagenesis in Chrysanthemum morifolium variety ‘Otome Pink’through gamma irradiation. Bioscan 8:1489–1492
25. Langton FA (1980) Chimerical structure and carotenoid inheritance in *Chrysanthemum morifolium* (Ramat.). Euphytica 29:807–812
26. Mahure HR, Choudhry ML, Prasad KV, Singh SK (2010) Mutation in chrysanthemum through gamma irradiation. Indian Journal of Horticultural 67:356–358
27. Miñano HS, González-Benito ME, Martin C (2009) Molecular characterization and analysis of somaclonal variation in chrysanthemum cultivars using RAPD markers. - Sci Hort 122:238–243
28. Misra P, Banerji BK, Kumari A (2009) Effect of gamma irradiation on chrysanthemum cultivar ‘Pooja with particular reference to induction of somatic mutation in flower colour and form. Journal of Ornamental Horticulture 12:213–216
29. Misra RL, Bajpai PN (1983a) Mutational studies in gladioli (Gladiolus) I. Effect of physical and chemical mutagens on sprouting and survival of corms. Haryana Journal of Horticultural Sciences 12(1/2):1–6
30. Richards AJ (1986) Plant Breeding Systems. George Allen and Unwin, London
31. Schum AR (2003) Mutation breeding in ornamentals: an efficient breeding method. - *Acta Horticulturae* 612:47–60
32. Siavash HA, Jirair C, Jalil K (2009) The effects of UV radiation on some structural and ultrastructural parameters in pepper (*Capsicum longum*). Turkish Journal of Biology 34:122–125
33. Simard MH, Ferriere NM, Silvy A (1992) Variants of carnation (*Dianthus caryophyllus* L.) obtained by organogenesis from irradiated petals. Plant Cell Tissue Organ Culture 29:37–42
34. Singh KP, Singh B, Raghava SPS, Misra RL, Kalia CS (1999) *In vitro* induction of mutation in carnation through gamma irradiation. Journal of Ornamental Horticulture New Series 2(2):107–110
35. Singh R, Goyal RK, Godara AK (2011) Effect of gamma radiation on growth, flowering and bulb production in tuberose (*Polianthes tuberosa* L.) cv. Double. Haryana Journal of Horticultural Science 40(3–4):128–130
36. Siranut L, Peeranuch J, Arunee W, Surin D, Prapanpongse K (2000) Gamma ray induced morphological changes in Chrysanthemum. Kasetsart Journal of Natural Sciences 34:417–422
37. Sooch M, Arora JS, Singh K, Gosal SS((2000) Effect of gamma ray irradiation on *in vitro* multiple shoot formation and establishment of carnation plants. Journal of Ornamental Horticulture New Series 3(2):118–119
38. Srivastava P, Singh RP, Tripathi VK (2007) Response of gamma radiation (60^{o}C) on vegetative and floral characters of gladiolus. Journal of Ornamental Horticulture 10(2):135–136
39. Steel RGD, Torrie JH (1980) Principles and Procedures of Statistical Analysis. McGraw Hill Book Co. Inc. Newyork, USA, pp 232–251
40. Sutter E, Langhans RW (1979) Epicuticular wax formation on carnation plantlets regenerated from shoot-tip culture. J Am Soc Hortic Sci 104:493–496

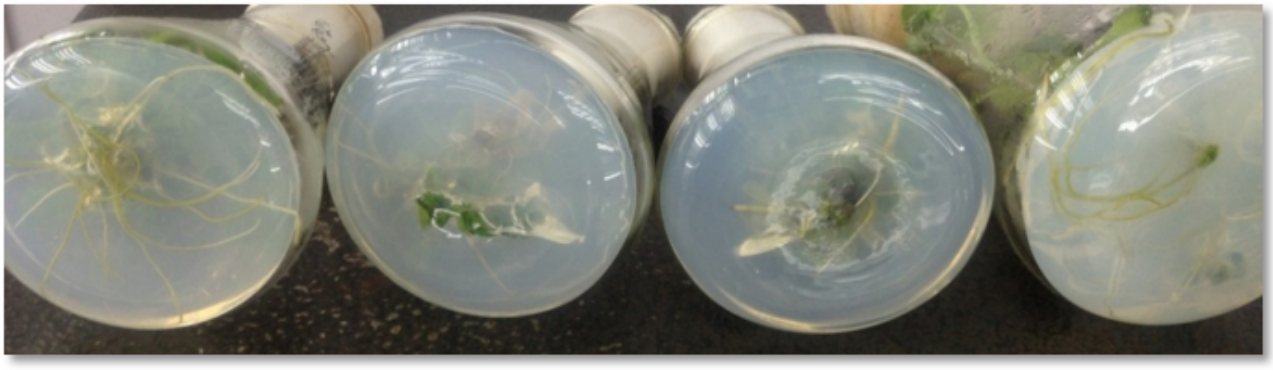
41. Wardle K, Dobbs EB, Short KC (1983) *In vitro* acclimatization of aseptically cultured plants to humidity. Journal of the American Society for Horticultural Science 108:386–389
42. Wolff K (1996) RAPD analysis of sporting and chimerism in chrysanthemum. Euphytica 89:159–164
43. Zalewskam rumin´ska LJ, Miller N (2007) “In vitro” *propagation using adventitious bud techniques as a source of new variability in* Chrysanthemum. - Sci Hort 113:70–73

Figures

Dendranthemum morifolium L. cv. “Candid”

Figure 1

Chrysanthemum cultivar selected for the investigation



(1) (2) (3) (4)

a) Mass view of rooting

b) Number of roots

^{60}Co gamma irradiation doses :

1) 10 Gy 3) 30 Gy

2) 20 Gy 4) 40 Gy

Figure 2

Rooting of 60Co gamma irradiated shoots

a) Light pink

b) Orange pink

c) White

d) Light yellow

e) Distorted red bud

f) Distorted red floret

Figure 3

Mutants of 60Co gamma irradiation doses

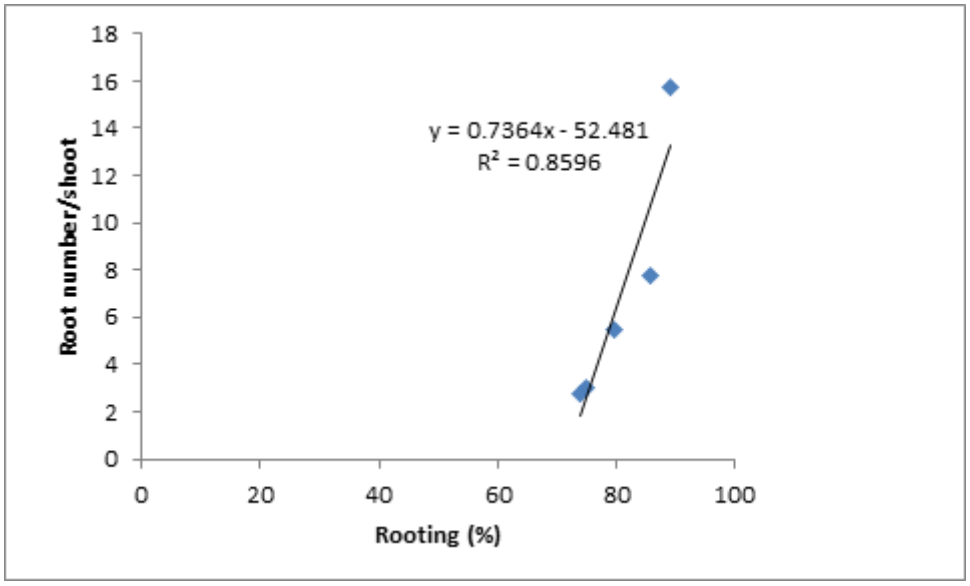


Figure 4

Correlation analysis of Root number/shoot vs Rooting (%)

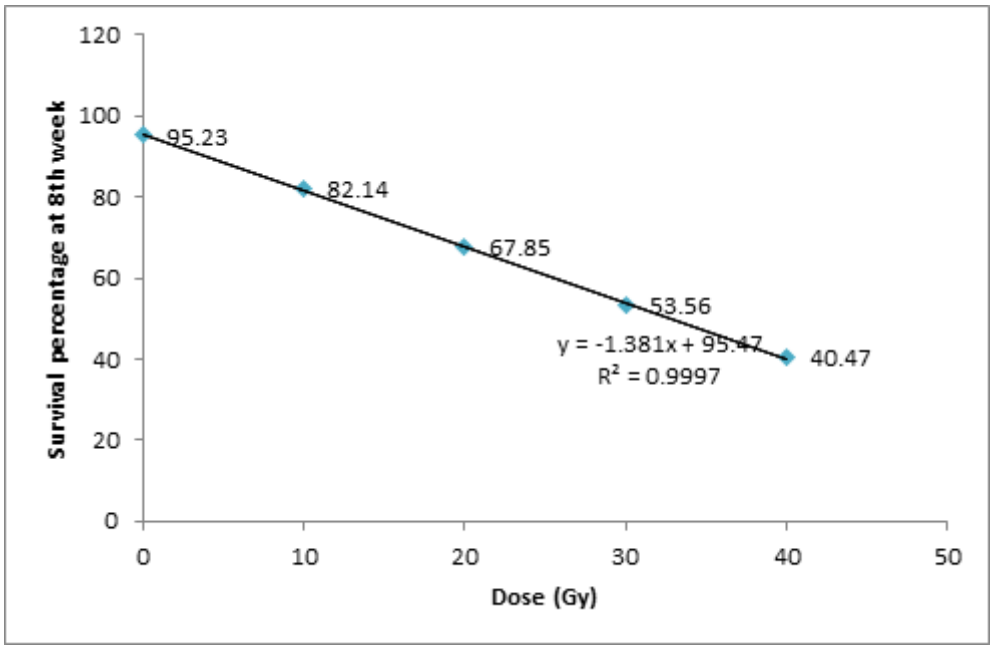


Figure 5

Correlation of survival percentage with γ irradiation dose.

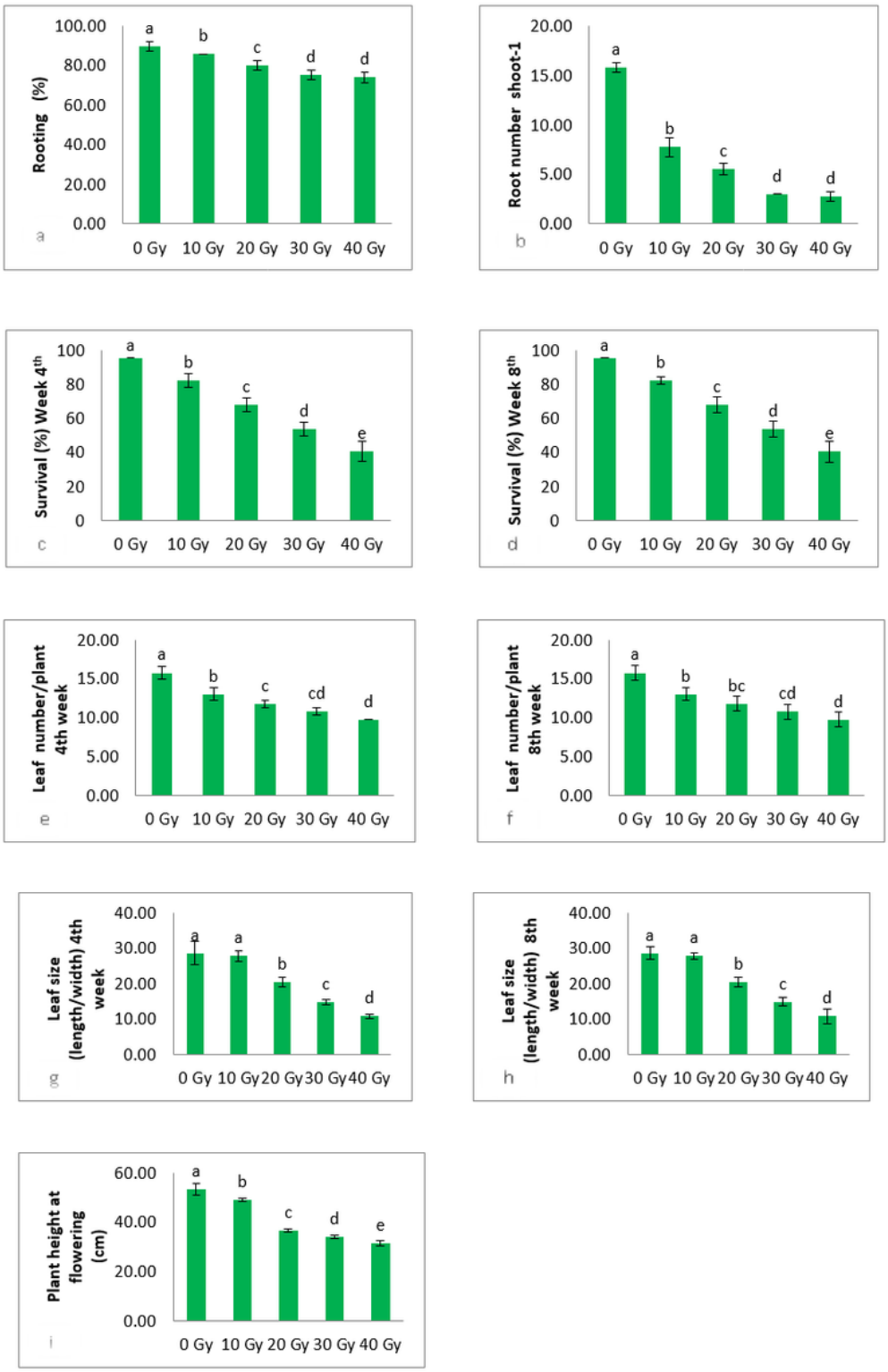


Figure 6

Influence of γ- rays on a) rooting percentage b) root number per shoot c) survival percentage of rooted shoots at 4th week d)survival percentage of rooted shoots at 8th week e) leaf number per plant at 4th week f) leaf number per plant at 8th week g) leaf size per plant at 4th week h) leaf size per plant at 8th week i) plant height at flowering.