proceedings

MDPI

Proceedings

Congenial In Vitro γ-Ray Induced Mutagenesis Underlying the Diverse Array of Petal Colours in Chrysanthemum (*Dendranthemum grandiflorum kitam*) cv. "Candid"

Ambreena Din [1,*], Z. A. Qadri [1], Muneeb Ahmad Wani [1], Z. A. Rather [1], Shameen Iqbal [1], Sajid Ali Malik [1], Peerzada R. Hussain [2], Sadaf Rafiq [1] and I. T. Nazki [1]

[1] Division of Floriculture and Landscape Architecture, Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir, India; drzahoorqadri@gmail.com (Z.A.Q.); wanimuneeb05@gmail.com (M.A.W.); drzahoorrather@gmail.com (Z.A.R.); shameeniqbal32@gmail.com (S.I.); maliksajidali92@gmail.com (S.A.M.); sadaf.rbhat@gmail.com (S.R.); mitnazki@gmail.com (I.T.N.)

[2] Astrophysical Sciences Division, Nuclear Research Laboratory, Bhabha Atomic Research Centre, Srinagar, India; mutteebar@gmail.com

* Correspondence: dinambreena@gmail.com

† Presented at the 1st International Electronic Conference on Plant Science, 1–15 December 2020; Available online: https://iecps2020.sciforum.net/.

Published: 1 December 2020

Abstract: Chrysanthemum (*Dendranthemum grandiflorum kitam.*) is a leading flower with applied value worldwide. Flower color is an important trait that influences the commercial value of chrysanthemum cultivars. Developing new chrysanthemum cultivars with novel characteristics such as new flower colors in a time- and cost-efficient manner is the ultimate goal for breeders. Understanding the molecular mechanisms that regulate flower pigmentation may provide important implications for the rationale manipulation of flower color. To generate 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 decreased with the increase in irradiation dose from 0 Gy to 40 Gy, while as, 10 Gy dose proved to record minimum decline as compared to the control. Survival, leaf size and number of leaves plant^{-1} after 8th week interval also decreased with the increasing trend of gamma irradiation dose but recorded minimum decline in plants developed from shoots irradiated with 10 Gy gamma irradiation dose with respect to the control. Apparently minimum delay in number of days to floral bud appearance took under 10 Gy as compared to control. Highest number of flower colour mutants were recorded under 10 Gy (light pink, orange pink,white and yellow). Amountable mutation frequency on the basis of flower colour was desirable in plants irradiated with least dose of 10 Gy.

Keywords: Chrysanthemum; mutagenesis; gamma irradiation; mutants

1. Introduction

Chrysanthemum is very popular and important cut flower crop grown all over the world in Japan, China, USA, France, UK, and India. It is a major horticultural crop and it is the second largest in terms of cut flowers after rose, among the ornamental plants traded in the global flower market [1]. The complex genetic hetrozygosity make the cultivated chrysanthemum an unlimited source of new flower form and cultivars. The common garden chrysanthemum is hexaploid with 54 chromosomes [2]. It is propagated vegetatively and has a strong self incompatibility system [3], hence new cultivars are difficult to obtain by crossing. Traditionally, new cultivars have been obtained from spontaneous mutations in vegetative reproduction, sports, being some variations more stable than others [4]. In the last few years, induced mutations and somaclonal variations derived from the tissue

culture process have been employed as a new source of variability [5–10]. Although extensive work has been carried out to develop novelties in chrysanthemum through induced mutations using physical and chemical mutagens [11], there is always a need to explore the possibility of new variety for floriculture trade. Mutation breeding by radiation has been widely used to upgrade well-adapted plant varieties and also to develop new variations within improved agricultural characteristics. Since most cultivated chrysanthemum cultivars are polyploids with high genetic heterogeneity, mutants with allied flower colour, shape, floral size and shape are often recovered. Allied flower colours with chimeric tissue can be easily induced by radiation and can be isolated using in vitro tools [1]. Mutation techniques are used because chrysanthemum is hexaploid plant and vegetative propagated which make it difficult to conduct the hybridization [12]. Genetic variation is essential in any plant breeding programme for crop improvement. Mutation breeding is efficient way to produce heritable change particular for the flower colour. Increasing demand to new form of chrysanthemum lead to research for obtaining new varieties. Mutation breeding by radiation, an agricultural application of nuclear technology has been widely utilized to improve the well-adapted plant varieties by one or few important traits [1,7,10,13]. Commercially important traits in horticulture plants have been altered in as positive way by the various physical mutagens. Among the physical mutagens, gamma rays are widely used for inducing mutations in flowering plants due to their easy application and high efficiency. The physical irradiations have been used effectively for induction of mutation in chrysanthemum and the optimum dose range from 1.0 to 3.0 Krads depending upon the genotypes [14]. 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, 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. Thus the genetic variability created by mutation was studied for development of new cultivar in chrysanthemum having significant consumer preference. Therefore, with consideration to above factors the present investigation entitled "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 diverse array of flower colour mutants through mutagenesis**.**

2. Experiments

2.1. Materials and Methods

Tissue culture developed micro shootlets of *chrysanthemum* cv. 'Candid' (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 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 was recorded at the initiation of flower bud appearance. Plant height was recorded at the end of full flower bloom. Flower colour was recorded in terms of difference between the parent flower and mutants obtained and the frequency of mutation was calculated on the basis of flower colour, as the ratio between such desired or undesired colour mutant and total plants irradiated with each gamma irradiation dose.

Dendranthemum morifolium L. cv. "Candid"

Figure 1. Chrysanthemum cultivar selected for the investigation.

2.2. Statistical Analysis

Statistical analysis of the data collected for different parameters during the present investigation was subjected to analysis of variance for completely randomized design with four replications [15]. To satisfy model assumptions for analysis of variance, percentage data was subjected to square root transformation as suggested by [16]. The means were separated by Duncan multiple range test.

3. Results and Discussion

3.1. Influence of γ-rays on Rhizogenesis and Percent Survival

The irradiation doses had enervating effect on all the parameters of rooting in comparison to control. Significant decrease in mean rooting per cent and number of roots shoot^{-1} in all the irradiation treatments in comparison to control was observed. Among irradiation treatments 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 number of roots were registered with 40 Gy dose (Figure 3a,b). Rhizogenesis is a process of dedifferentiation of speciific pre determined cells near the vascular bundles. Any damage to cell division ability will have a negative effect on dedifferentiation of cells and subsequent reorganization into root primordia. This may result in failure of rooting or delayed emergence of roots. Ref. [17] also reported that increased doses of gamma irradiation (from 20 to 50 Gy) decreased rooting percentage of carnation cv. 'Espana'. Radiation treatments also delayed root initiation significantly in comparison to control. Ref. [18], 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 significant decline in root number per shoot under 10 to 30 Gy treatments. Ref. [19], 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 seems closer with the findings recorded in the present study. Survival of rooted shoots at the end of 4 week was significantly minimum by the shoots treated with 40 Gy dose as against control, followed by 30 and 20 Gy dose (Table 2). Under minimum dose of 10 Gy, there was a minimum decline in survival of shoots at the end of the 4 week over control. At the end of 8 week, shoots treated with 10 Gy dose recorded maximum survival, followed by 20 and 30 Gy dose (Figure 3c,d). Whileas, lowest survival per cent was recorded in 40 Gy irradiated shootlets corresponding to heavy decline in comparison to control. Ref. [20] 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 formation of low or reduced wax component on the post irradiation plants. As the wax component determines the rate of water loss through the cuticle and the susceptibility of tissue-cultured plants to desiccation attributed to a reduction or absence of wax acting as antitranspirant. The epicuticular wax is reduced or absent on

the carnation leaves of in vitro cultured plants compared to glasshouse or field-grown plants [21], but during acclimatization, the density of waxes increases as the humidity is lowered [22]. Since the irradiation impairs the epidermal skin of the plants leading to low wax formation even during the acclimatization process and hence leads to mortality.

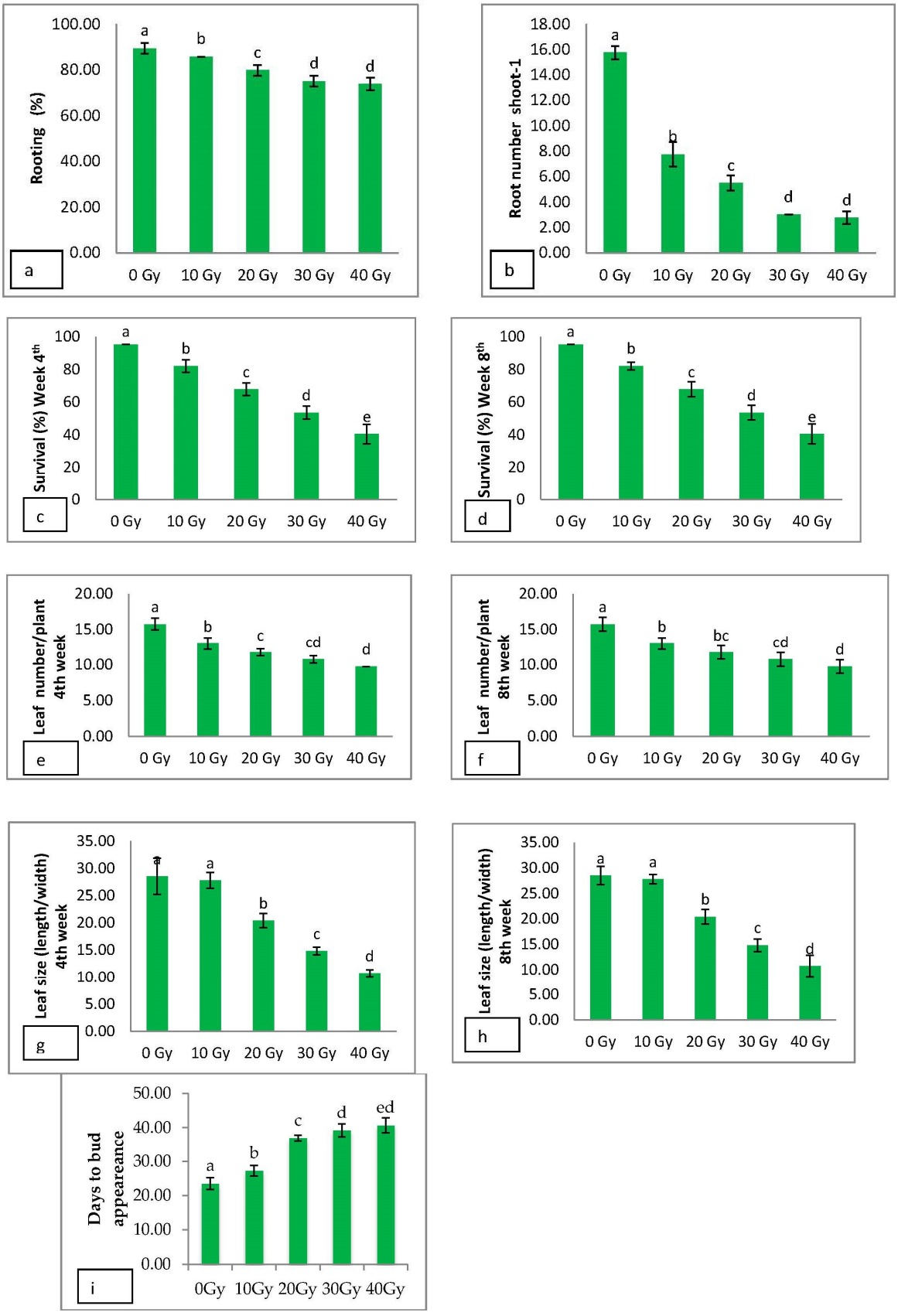

Figure 3. Influence of γ-rays on (**a**) rooting percentage (**b**) root number per shoot (**c**) survival percentage of rooted shoots at 4th week (**d**) survival percentage of rooted shoots at 8th week (**e**) leaf number per plant at 4th week (**f**) leaf number per plant at 8th week (**g**) leaf size per plant at 4th week (**h**) leaf size per plant at 8th week (**i**) days to flower bud appearance.

3.2. Influence of γ-rays on Number of Leaves and Leaf Area

Gamma irradiation treatments significantly recorded a decline in leaf number plant^{-1} and leaf size in both the intervals i.e., 4 and 8 week as compared to control. At the end of 4 week, significantly minimum leaf number plant^{-1} and size was registered under highest dose of 40 Gy, followed by 30 and 20 Gy and the lowest gamma irradiation dose 10 Gy recorded a minimum decrease in leaf number and size, as compared to the control. At the end of 8 week, both leaf number as well as leaf size improved in all the gamma irradiation doses including the control plants but recorded the similar trend of decline in both the parameters as in the 4 week interval with the successive gamma irradiation doses (Figure 3e–h). Leaf area increment is a result of 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. Refs. [23,24], recorded biological damage in carnation on increasing the dose of radiation. Ref. [25] in tuberose; Ref. [26] in gladiolus; Ref. [27] in costus; Ref. [28]; Ref. [29] in chrysanthemum; Ref. [30] in gladiolus; Ref. [31] in chrysanthemum and Ref. [32] in rose also reported the decrease in number of leaves with the increase in dosage of gamma irradiation whereas, [33], reported reduction 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. Ref. [34], recorded that lower doses like 10 and 20 Gy increased leaf area but 30 Gy decreased leaf area over control. In yet another study by [35], reduction in leaf number was reported in *Dendranthemum grandiflorum kitam* cv. "Gulmohar" under gamma irradiation dose range of 1.0–3.0 kR.

3.3. Influence of Gamma Irradiation on Days to Floral Bud Appearance

With the increment of each dose of irradiation (Figure 3i), there was a significant delay in days to bud appearance in comparison to control plants (23.50). Under 10, 20 and 30 Gy doses days to bud appearance was recorded 27.25, 37.00 and 39.25, respectively. Whereas, days to bud appearance under last dose of 40 Gy was recorded significantly highest 40.75, which represented maximum delay as compared to control. The results in the present study may be due to the disturbances in biochemical pathway which assists in synthesis of flower inducting substances and hence delay in flowering. The results in the present study are in concurrence with the findings of [36], who observed delayed flowering behaviour after irradiating rooted cutting of small decorative type chrysanthemum cv. "Kalyani Mauve". In another study [14], also observed significant delay in days to bud formation, buds showing colour and days for full bloom in the treated plants of ten chrysanthemum cultivar as compared to control. Similar were the results obtained by [31] in chrysanthemum cv. "Pooja".

3.4. Influence of Gamma Irradiation on Flower Colour and Mutation Frequency

Regarding 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 same as control i.e., showing original red colour (Figure 2a–d). Higher doses of 20, 30 or 40 Gy produced either distorted red buds or distorted red (Figure 2e,f). Colour mutants under 20, 30 and 40 Gy were undesirable. The results in the present study may be due to physiological changes which occur in plant, hence, delayed flowering occur at higher doses due to inhibitory effect. This can be attributed to the fact that no chimeric growth was developed in shoot as result of mutagenesis. Shoot or tissue without chimeric growth lead to non-formation, different colour variation in petals reported by [37] in chrysanthemum. This quoted observation is in close conformity to the present study. Data regarding the mutation frequency in chrysanthemum flowers on the basis of flower colour, there was 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, but produced undesirable mutants. The results obtained in the present study are in accordance to the finding of [38], who reported increase in mutation frequency when plants were UV irradiated.

(**a**) Light pink (**b**) Orange pink

(**c**) White (**d**) Light yellow

(**e**) Distorted red bud (**f**) Distorted red floret

Figure 2. Mutants of ^{60}Co gamma irradiation doses.

4. Conclusions

The study concludes that irradiation 40 Gy dose resulted in significant decrease in days to floral bud appearance and mutation frequency. Highest number of desired mutants with respect to flower colour (Light pink, Orange-pink, White and yellow) and highest mutation frequency were recorded in shoots irradiated with 10 Gy. Hence, 10 Gy gamma irradiation treatment is congenial for mutagenesis in Chrysanthemum Cv. "Candid".

Acknowledgments: I am highly thankful to Maulana Azad National Fellowship for financial assistance during the whole programme.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kumar, S.; Prasad, K.V.; Choudhary, M.L. Detection of genetic variability among chrysanthemum radiomutants using RAPD markers. *Curr. Sci.* **2006**, *90*, 1108–1113.
2. Wolff, K. RAPD analysis of sporting and chimerism in chrysanthemum. *Euphytica* **1996**, *89*, 159–164.
3. Richards, A.J. *Plant Breeding Systems*; George Allen and Unwin: London, UK, 1986.
4. Miñano, H.S.; González-Benito, M.E.; Martin, C. Molecular characterization and analysis of somaclonal variation in chrysanthemum cultivars using RAPD markers. *Sci. Hort.* **2009**, *122*, 238–243.
5. Schum, A.R. Mutation breeding in ornamentals: An efficient breeding method. *Acta Hortic.* **2003**, *612*, 47–60.
6. Datta, S.K.; Misra, P.; Mandal, A.K.A. In-vitro mutagenesis a quick method for establishment of solid mutant in chrysanthemum. *Curr. Sci.* **2005**, *88*, 155–158.
7. Jain, S.M.; Spencer, M.M. Biotechnology and mutagenesis in improving ornamental plants. In *Floriculture, Ornamental and Plant Biotechnology*: *Advances and Topical Issues*; Teixeira da Silva, J.A., Ed.; Global Science Books: Isleworth, UK, 2006; Volume 1, pp. 589–600.
8. Zalewska, M.; Lema-Rumińska, J.; Miler, N. In vitro propagation using adventitious bud techniques as a source of new variability *in* Chrysanthemum. *Sci. Hort.* **2007**, *113*, 70–73.
9. Jain, S.M. Mutagenesis in crop improvement under the climate change. *Rom. Biotechnol. Lett.* **2010**, *15*, 88–106.
10. Barakat, M.N.; Abdel, F.R.S.; Badr, M.; El-Torky, M.G. In-vitro culture and plant regeneration derived from ray florets of *Chrysanth. Morifolium. Afr. J. Biotechnol.* **2010,** *9*, 1151–1158.
11. Broertjes, C.; Van-Harten, A.M. Application of mutation breeding methods in the improvement of vegetatively propagated crops. An interpretive literature review. In *Developments in Crop Science (2)*; Elsevier Scientific Publishing Company: Amsterdam, Switzerland, 1978; p. 316.
12. Dwimahyani, I.; Widiarsih, S. The effects of gamma irradiation on the growth and propagation of in vitro chrysanthemum shoot explants (cv. Yellow Puma). *Atom Indones.* **2010**, *36*, 45–49.
13. Chatterjee, J.; Mandal, A.K.A.; Ranade, S.A.; Teixeira-da-Silva, J.A.; Datta, S.K. Molecular systematics in *Chrysanthemum x grandiflorum* (Ramat) Kitamura. *Sci. Hortic.* **2006**, *110*, 373–378.
14. Dilta, B.S.; Sharma, Y.D.; Gupta, Y.C.; Bhalla, R.; Sharma, B.P. Effect of gamma rays on vegetative and flowering parameters of chrysanthemum. *J. Ornam. Hortic.* **2003**, *6*, 328–334.38.
15. Gomez, K.A.; Gomez, A.A. *Statistical Procedures for Agricultural Research*; Wiley-Interscience Publications: New York, NY, USA, 1983; p. 84.
16. Steel, R.G.D.; Torrie, J.H. *Principles and Procedures of Statistical Analysis*; McGraw Hill Book Co. Inc.: New York, NY, USA, 1980; pp. 232–251.
17. Singh, K.P.; Singh, B.; Raghava, S.P.S.; Misra, R.L.; Kalia, C.S. In vitro induction of mutation in carnation through gamma irradiation. *J. Ornam. Hortic.* **1999**, *2*, 107–110.
18. Sooch, M.; Arora, J.S.; Singh, K.; Gosal, S.S. Effect of gamma ray irradiation on in vitro multiple shoot formation and establishment of carnation plants. *J. Ornam. Hortic.* **2000**, *3*, 118–119.
19. El-Sharnouby, M.E.; El-Khateeb, M.A.A. Effect of BA, NAA and gamma irradiation on the production of three cultivars of carnation (*Dianthus caryophyllus* L.) through tissue culture. *Ann. Agric. Sci. Moshtohor* **2005**, *43*, 1937–1948.
20. Broertjes, C.; De-Jong, J. Radiation induced male sterility in daisy type of *Chrysanthemum morifolium* Ramat. *Euphytica* **1984**, *33*, 433–434.
21. Sutter, E.; Langhans, R.W. Epicuticular wax formation on carnation plantlets regenerated from shoot-tip culture. *J. Am. Soc. Hortic. Sci.* **1979**, *104*, 493–496.
22. Wardle, K.; Dobbs, E.B.; Short, K.C. In vitro acclimatization of aseptically cultured plants to humidity. *J. Am. Soc. Hortic. Sci.* **1983**, *108*, 386–389.
23. Simard, M.H.; Ferriere, N.M.; Silvy, A. Variants of carnation (*Dianthus caryophyllus* L.) obtained by organogenesis from irradiated petals. *Plant CellTissue Organ Cult.* **1992**, *29*, 37–42.
24. Cassels, A.C.; Walsh, C.; Periappuram, C. 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* **1993**, *70*, 167–174.

25. Gupta, M.N.; Sumiran, R.; Shukla, R. Mutation breeding of tuberose Polianthus tuberosa L. In Proceedings of the Symposium on Use of Radiations and Radio Isotopes in Studies of Plant Productivity, 1974; Volume 12–14, pp. 169–179.
26. Misra, R.L.; Bajpai, P.N. Mutational studies in gladioli (Gladiolus) I. Effect of physical and chemical mutagens on sprouting and survival of corms. *Haryana J. Hortic. Sci.* **1983**, *12*, 1–6.
27. Gupta, M.N.; Laxmi, V.; Dixit, B.S.; Srivastava, S.N. Gamma rays induced variability in *Costus Speciosus. Progress. Hortic.* **1982**, *14*, 193–197.
28. Acharya, N.N.; Tiwari, D.S. Effect of MMS and gamma rays on seed germination, survival and pollen fertility of *Hamatocactus setispinus* in M_1 generation. *Mysore J. Agric. Sci.* **1996**, *3*, 10–13.
29. Siranut, L.; Peeranuch, J.; Arunee, W.; Surin, D.; Prapanpongse, K. Gamma ray induced morphological changes in Chrysanthemum. *Kasetsart J. Nat. Sci.* **2000**, *34*, 417–422.
30. Srivastava, P.; Singh, R.P.; Tripathi, V.K. Response of gamma radiation (60^{o}C) on vegetative and floral characters of gladiolus. *J. Ornam. Hortic.* **2007**, *10*, 135–136.
31. Misra, P.; Banerji, B.K.; Kumari, A. Effect of gamma irradiation on chrysanthemum cultivar 'Pooja with particular reference to induction of somatic mutation in flower colour and form. *J. Ornam. Hortic.* **2009**, *12*, 213–216.
32. Kahrizi, Z.A.; Kermani, M.J.; Amiri, M.E.; Vedadi, S. Identifying the correct dose of gamma-rays for in vitro mutation of rose cultivars. In *Acta Horticulturae, 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*; Fabbri, A., Rugini, E., Eds.; 2011; Volume 923, pp. 121–127.
33. Kumari, K.; Dhatt, K.; Kapoor, M. Induced mutagenesis in Chrysanthemum morifolium variety 'Otome Pink'through gamma irradiation. *Bioscan* **2013**, *8*, 1489–1492.
34. Mahure, H.R.; Choudhry, M.L.; Prasad, K.V.; Singh, S.K. Mutation in chrysanthemum through gamma irradiation. *Indian J. Hortic.* **2010**, *67*, 356–358.
35. Dilta, B.S.; Sharma, Y.D.; Dhiman, S.R.; Verma, V.K. Induction of somatic mutations in chrysanthemum by gamma irradiation. *Int. J. Agric. Sci.* **2006**, *2*, 77–81.
36. Datta, S.K.; Banerji, B.K. Gamma ray induced somatic mutation in chrysanthemum cv. 'Kalyani Mauve'. *J. Nucl. Agric. Biol.* **1993**, *22*, 58–61.
37. Langton, F.A. Chimerical structure and carotenoid inheritance in *Chrysanthemum morifolium* (Ramat.). *Euphytica* **1980**, *29*, 807–812.
38. Siavash, H.A.; Jirair, C.; Jalil, K. The effects of UV radiation on some structural and ultrastructural parameters in pepper (*Capsicum longum*). *Turk. J. Biol.* **2009**, *34*, 122–125.