Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

**E-ISSN:** 2278-4136
**P-ISSN:** 2349-8234
JPP 2019; 8(4): 871-874
Received: 16-05-2019
Accepted: 18-06-2019

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# Gamma ray induced foliage variegation and anatomical aberrations in *Chrysanthemum* (*Dendranthema grandiflora* T.) cv. Maghi

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**Abstract**
Rooted cuttings of (*Guldaudi*) *Chrysanthemum* cv. Maghi, were subjected to radiation 0, 100, 150, 200, 250, 300, 350, 400 and 450 Grays by using physical mutagen with the objective of developing novel variegated leaf mutants as well as variation in flower color etc. The present experiment was laid out in RBD with nine treatment three replication. The quantitative and qualitative characters studied and revealed variegation in foliage and also in colour of flowers in plants grayed with 100 and 150 Gy irradiation. Maximum number of variegated plants (6), number of branches (31), number of flowers per plant (285) diameter of flower (3.7 cm.) and number of ray florets/flower (443) were observed with 100 Gy. A significant adverse effect of irradiation was observed with increasing doses of gamma rays, in chimeric generation. Delayed flowering were also observed with higher doses of irradiation. Size of stomata viz., length of Guard cell (30 micron), width of Guard cell (17 micron), length of pore (26 micron) and width of pore (7 micron) was recorded for the lower radiation doses (100 and 150 Gy). Higher radiation doses of 300 to 450 Gy were ineffective since they adversely affected the plant performance in terms of its survival, vegetative as well as flower growth. Chimeric plants were produced which could be multiplied for obtaining mutants in pure form.

**Keywords:** *Chrysanthemum*, Maghi, gamma radiation, variants, scanning electron microscopy

## Introduction
*Chrysanthemum* (*Dendranthema grandiflora* T.) is a herbaceous, short day flowering plant widely grown worldwide. It is a perennial flowering herb having beautiful bloom with excellent (vase life) flower life flowering observed in the early winter. It is a member Asteraceae family and native of China (Bajpay and Dwivedi, 2017 and 2018) [2, 1]. *Chrysanthemum* is one of the most popular commercial cut flower in the world flower trade. Gamma irradiation is a relevant tool for genetic improvement in plants those are difficult to sexually propagate especially ornamental plants (Banerji, 2014) [4]. Mutation breeding through physical mutagenesis for development of new and novel cultivars in *Chrysanthemum* is best way, due to self-incompatibility. Mutagenesis may affect different characters of morphology, anatomy, biochemistry and physiology, depending upon the doses of irradiation as is observed in enhanced rooting in crops like bougainvillea spp., hibiscus, gladiolus, rose and Lantana (Datta, 2009) [9] and in reduction in size and number of stomata in *Glycine max* (Celik *et al*., 2014) [7], *Chrysanthemum* (Banerji, 1989) [3] and *Jatropha* (Pandey and Datta, 1995) [13] at higher doses of physical mutagen.

## Materials and methods
Rooted cuttings (10 cm long) of small flowered *chrysanthemum* cv. Maghi were subjected to gamma radiation 0, 100, 150, 200, 250, 300, 350, 400 and 450 Grays gamma rays (source $Co^{60}$) with the objective of inducing mutations for developing novel variegated mutants as well as variation in flower color. Irradiated cutting were planted immediately after irradiation along with equal number control cuttings, each treatment consisted of 27 cuttings in experimental field. The field trial was conducted in Randomize Block Design with 9 treatments replicated thrice at a spacing of 30x30 cm (plants x row). Pants were planted in the field and observations were recorded for vegetative and floral traits, at field of Horticulture Research Farm, Babasaheb Bhimrao Ambedkar University Lucknow. To study the effect of gamma radiation on plant morphology of *Chrysanthemum* cv Maghi, Vegetative and floral traits viz., plant height, number of leaves, branch, suckers, percent abnormal plants, number of flowers per plant, ray florets/flower, diameter of flower, duration of flowering, induced colour variegation in leaves and flowers were studied. Colour variegation in leaves and flowers were observed and matched with royal horticulture color chart.

For stomatal study, Full mature leaves were collected and chopped (2-4 mm)→ fixed in 2.5% Glutaraldehyde Karnovsky's fixative for 2-6 hours at $4^0$C, → sample was washed in 0.1 M Phosphate buffer solution for three changes each for 15 minutes at 4 0 C for removing unreacted fixative → dehydrated with acetone to remove water from leaves → sample was mounted on to the Aluminum Stubs with Carbon tape and coated by using Sputter coater to make sample conductive → samples were observed under Scanning Electron Microscope at Babasaheb Bhimrao Ambedkar University Lucknow.

**Statistical Analysis**

The data was analysis through the statistical software OPSTAT, 1998. One way ANOVA was applied for data analysis. The calculated 'F' value is compared with table F values at 5% level of significance for field data. If the calculated 'F' value is greater than the table value the difference is said to be significant and critical difference is calculated for further comparison, (Sheoran *et al*., 1898) [14].

**Result and discussion**

The quantitative and qualitative characters were studied after physical mutagenesis with gamma rays and revealed that the variegation in foliage and also in color and shape of flowers in plants treated with 100 and 150 Gy gamma radiation over control. Maximum number of variegated plants (6), number of branches (31), Height of plant (65 cm), sucker per plant (14.2), diameter of flower (3.7 cm) and size of stomata (Length of Guard cell 30 micron, width 17 micron, length of pore 26 micron and width of pore 7 micron) was recorded for the lower radiation doses (100 and 150 Gy). Higher radiation doses of 300 to 450 Gy were ineffective since they adversely affected the plant performance in terms of its survival, vegetative as well as flower growth. Maximum Chimeric plants were recorded (10.33 % flower chimera and 22.66 % foliage chimera) at 150 Gy treatment followed by 100 Gy (5.22% flower chimera and 18.66 % foliage chimera). Colour of chimeric plants were identified and recorded with the help of colour chart of Royal Horticulture Society, London (Figure 1 and Table 1, 2 and 3). Chimeric plants were produced which could be multiplied for obtaining mutants in pure form. Similar work was reported by other authors. The various growth and floral parameter were reduced after gamma radiation. However, increasing dose of irradiation increased abnormalities in growth and floral traits viz., leaf shape, leaf size, leaf apex and leaf margin, flower head shape and size and also changes in flower color (Datta and Banerji, 1994; Banerji *et al*., 1996; Kumari *et al*. 2013 and Banerji and Datta, 2002) [8, 6, 12, 5]. Floral and foliage abnormalities were observed in Dendrobium Orchid cv Sonia-28. Lower doses of gamma radiation were found effective in terms of improvement in flowering traits, while higher doses of gamma radiation adversely affected plant growth and flowering (Dehgahi and Joniyasa, 2017) [10].

**Table 1:** Effect of gamma radiation on vegetative traits of *Chrysanthemum* cv. Mahi in $M_1$ generation.

| Treatments | Survival % | Plant height (cm) | Percent abnormal leaves | Number of branches | Percent abnormal plants | Number of suckers |
|---|---|---|---|---|---|---|
| Control | 99.2 | 67.4 | 1.4 | 38.1 | 0.0 | 20.4 |
| 100 Gy | 85.9 | 65.8 | 22.2 | 31.0 | 8.0 | 14.2 |
| 150 Gy | 73.3 | 59.0 | 33.8 | 19.1 | 17.0 | 16.1 |
| 200 Gy | 61.0 | 53.0 | 42.2 | 8.6 | 40.4 | 12.6 |
| 250 Gy | 42.8 | 40.4 | 56.2 | 5.7 | 61.7 | 11.1 |
| 300 Gy | 16.9 | 32.6 | 71.6 | 3.8 | 88.9 | 3.4 |
| 350 Gy | 10.0 | 16.8 | 86.1 | 2.5 | 95.4 | 1.0 |
| 400 Gy | 00.0 | 00.0 | 00.0 | 00.0 | 00.0 | 00.0 |
| C.D. | 7.1 | 4.3 | 7.4 | 7.9 | 6.3 | 3.2 |
| SE(m) | 2.3 | 1.4 | 2.4 | 2.6 | 2.1 | 1.0 |
| SE(d) | 3.3 | 2.0 | 3.4 | 3.7 | 2.9 | 1.5 |

**Table 2:** Effect of gamma radiation on floral traits of *Chrysanthemum* cv. Maghi in $M_1$ generation

| Treatments | Days to first color shown | Days to full bloom | Number of flowers | Diameter of flower | Number of ray florets/ flower | Number of disc floret/flower |
|---|---|---|---|---|---|---|
| Control | 81.5 | 101.2 | 321.7 | 3.8 | 434.4 | 8.1 |
| 100 Gy | 84.2 | 108.0 | 285.5 | 3.7 | 443.5 | 5.3 |
| 150 Gy | 85.7 | 102.0 | 235.0 | 3.1 | 384.5 | 4.4 |
| 200 Gy | 85.1 | 110.3 | 115.1 | 2.7 | 216.6 | 4.0 |
| 250 Gy | 84.7 | 110.4 | 59.4 | 2.5 | 119.3 | 1.6 |
| 300 Gy | 85.1 | 118.4 | 18.3 | 2.5 | 43.6 | 1.6 |
| 350 Gy | 91.0 | 154.3 | 7.3 | 00.0 | 12.3 | 00.0 |
| 400 Gy | 00.0 | 00.0 | 00.0 | 00.0 | 00.0 | 00.0 |
| C.D. | 4.1 | 34.9 | 21.8 | 0.27 | 32.8 | 1.0 |
| SE(m) | 1.3 | 11.5 | 7.2 | 0.09 | 10.8 | 0.3 |
| SE(d) | 1.9 | 16.3 | 10.2 | 1.3 | 15.3 | 0.4 |

**Table 3:** Percent chimera observed in *Chrysanthemum* cultivar Maghi in $M_1$ generation

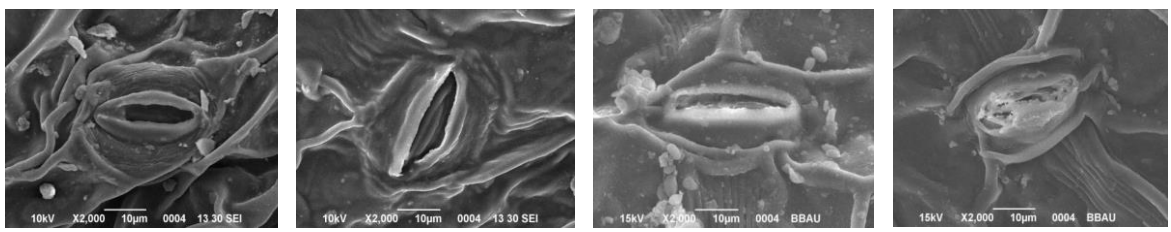
| Treatments | Percent chimeric plants observed (%) | |
|---|---|---|
| | Flower chimera | Foliage chimera |
| Control | 0.00 % | 0.00 % |
| 100 Gy | 5.22 % | 18.66 % |
| 150 Gy | 10.33 % | 22.66 % |
| 200 Gy | 4.77 % | 16 % |
| 250 Gy | 2 % | 10% |

**Fig 1:** Induced foliage variegation in *Chrysanthemum* cv. Maghi at 150 Gy and 200 Gy

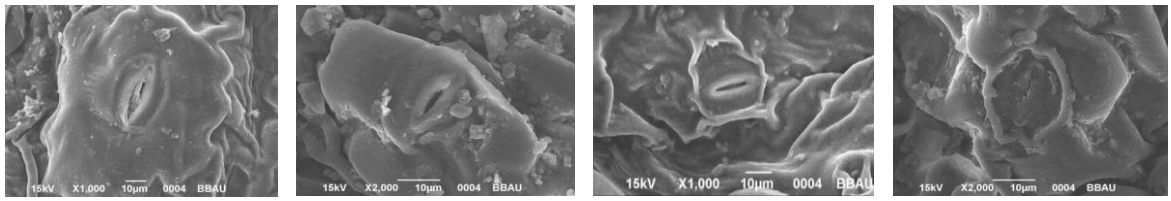
Gamma radiation significantly influenced stomatal morphology of *Chrysanthemum* cv Maghi. Stomatal size of leaf epidermis was observed under scanning electron microscope, with the help of micrograph. Vigorous size of stomata viz., length of Guard cell (30.36 micron), width of Guard cell (20.10 micron), length of pore (27.10 micron) and width of pore (7.33 micron) was recorded for the lower radiation doses (100 and 150 Gy). Higher radiation doses (300 to 450 Gy) were adversely affected the morphology of stomata, Due to higher irradiation treatments stomata size were significantly decreased, so that stomata were not able to gaseous exchange, and plant survival was also decreased (Table.4 and Figure 2.). Similar findings were reported by many authors. Gamma radiation treatments have significantly affected the number of stomata and size of stomata in *Gladiolus* cultivars. Number and size of stomata was decreased as the increased dose of gamma rays (Dogra *et al*., 2013) [11]. Higher doses of gamma radiation have significant variation on size, shape and stomata number in *Chrysanthemum* (Kumari *et al*., 2013) [12] and in *Jatropha* (Pandey and Datta, 1995) [13].

**Table 4:** Effect of gamma radiation on anatomical (stomata) aberrations in *Chrysanthemum* cv. 'Maghi'

| Treatments | Length of pore (Micron) | Width of pore (Micron) | Length of guard cell (Micron) | Width of guard cell (Micron) |
|---|---|---|---|---|
| Control | 21.53 | 5.83 | 29.73 | 17.43 |
| 100 Gy | 25.23 | 7.33 | 30.17 | 20.10 |
| 150 Gy | 27.10 | 4.66 | 30.36 | 17.67 |
| 200 Gy | 23.02 | 4.20 | 29.40 | 12.00 |
| 250 Gy | 16.66 | 4.56 | 23.00 | 11.33 |
| 300 Gy | 16.00 | 3.66 | 19.40 | 14.00 |
| 350 Gy | 10.66 | 3.00 | 22.00 | 9.50 |
| 400 Gy | 8.00 | 2.26 | 18.00 | 6.00 |
| C.D. | 0.00 | 0.00 | 0.00 | 0.00 |
| SE(m) | 2.987 | 0.743 | 1.15 | 3.65 |
| SE(d) | 0.988 | 0.246 | 0.38 | 1.20 |




Control 100 Gy 150 Gy 200 Gy



250 Gy 300 Gy 350 Gy 400 Gy

**Fig 2:** Stomatal abnormalities in cv Maghi at varying doses of gamma radiation

## Conclusion

The results revealed that physical mutagenesis on rooted cuttings of *Chrysanthemum* cv Maghi treated with lower gamma radiation doses induced maximum percent of foliage mutation and, also stomatal aberrations as compared to control. Morphological and anatomical aberrations were indicated distinctness of induced mutation to parent plant. While, higher radiation doses of gamma rays increased aberrations in morphological and anatomical traits. It may conclude that lower dose of physical mutagenesis is useful for inducing foliage variegation in ornamental plants.

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