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Gamma rays induced variations in seed germination, growth and phenotypic characteristics of *Zinnia elegans* var. Dreamland

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: *Zinnia elegans* is a herbaceous annual with diverse flower colours, flower types and plant height. *Zinnia elegans* are popular as pot plants and also for landscape gardening. The commercial value of *Zinnia* can be increased with novel traits such as flower colour and form. One of the techniques to develop plant varieties with superior traits is to induce mutations using gamma radiation. Hence, three doses of gamma radiation (75Gy, 100Gy and 125Gy) were utilised to obtain new and novel varieties of *Zinnia elegans* var. Dreamland and to study the effect of gamma rays on germination of seeds, growth and survival of the seedlings, height of the plants. All the three gamma ray doses were found to decrease the germination and survival of seeds of *Zinnia elegans*. The higher doses of gamma rays were found to be detrimental for the germination and survival of seeds and height of the seedlings. Phenotypical variations such as plant height, the number of flowers and flower diameter of the third generation mutants were highly significant as compared to the control. Eight floral variations could be obtained with novel form and colour.

1. Introduction

In recent years floriculture has become a flourishing industry. One of the requirements of the floricultural industry is diversity in order to introduce new ornamental plants in the market. *Zinnias* are important ornamental plants on the world floral market. *Zinnia* has been reported to be the first flower to be grown in space stations (Loff, 2016). *Zinnia elegans* offers a wide range of flower forms and colours which have an immense ornamental value. However there is a continuous demand for ornamental cultivars with new/novel forms and colours in modern and industrialized horticulture (Yunus *et al.,* 2013). Induced mutations offer a possibility of obtaining ornamentals with novel forms and colours to meet this demand (Tiwari and Kumar, 2011). More than 2,200 mutant varieties of crop plants have been released using induced mutagenesis and among them, 566 represent ornamental plants (Jain, 2005; Barakat and El-Sammak, 2011). 76 new mutant ornamental varieties with changed flower colour/shape and chlorophyll variegation in leaves have been developed using gamma rays and released (Datta *et al.*, 2009; Barakat and El-Sammak, 2011). But, no reports of registration of mutant varieties of *Zinnia* under mutant variety database of International Atomic Energy Agency are found. There is only one report of production of four new varieties in *Zinnia* by Venkatachalam and Jayabalan (1997). Radiation with Gamma rays has been reported to give rise to a large number of novel mutants in several ornamental species (Chrysanthemums, orchids, rose, pelargonium, canna, and carnations). The present study was carried out to utilize the mutagenic effect of gamma rays on *Zinnia elegans* to obtain dwarf varieties and flowers with novel architecture and colours.

2. Materials and Methods

Plant materials

Seeds of *Zinnia elegans* var. Dreamland with pink flowers which were certified to be pure breeding were procured from the Indo American Hybrid Seeds, Bangalore.

Gamma irradiation

Zinnia elegans var. Dreamland seeds were treated with gamma radiation at the Bhabha Atomic Research Center, Mumbai, India. Three gamma ray doses (75GY, 100GY and 125GY) were selected after studying the radiosensitivity and lethal dose (LD50) of *Zinnia* seeds (Pallavi *et al*., 2017). The gamma ray doses were given at the rate of 1.7Gy/min using ^{60}Co source.

Evaluation of germination of seeds, survivability and growth of seedlings

For the evaluation of germination of the irradiated seeds, survivability and growth of the seedlings, a total of 90 seeds were treated in triplicates. 30 non-irradiated seeds were established as controls. The irradiated seeds were maintained as axenic cultures. The germination of the seeds was recorded for the first seven days and the percentage of survival of seedlings on the 15th day after planting. The seedling growth parameters such as plant height and root length were recorded at an interval of 5 days for 15 days.

The data obtained was used to calculate the percentage of germination and the percentage of survival for each treatment as follows:

Germination % = No. of germinated seeds × 100/No. of irradiated seeds planted;

Survival % = No. of survived seedlings × 100/No. of irradiated seeds.

Evaluation of phenotypic variations observed in M_3 generation

To determine the effect of gamma rays on phenotypic characteristics, 500 seeds were irradiated with 75Gy, 100Gy and 125Gy doses of gamma radiation in each treatment. 500 non irradiated seeds were established as control. The irradiated seeds were sown in the green house of St. Aloysius College, Mangalore and the seedlings were grown up to the flowering stage and self-pollinated. The seeds of self-pollinated flowers were collected and sown and the procedure was repeated till the third mutant generation. The irradiated populations were screened for mutants at the M_3 generation. The plant height was recorded after 60 days of germination. The floral characters such as flower diameter, the number of flowers and flower colour were recorded at the flowering stage.

Statistical analysis

The data was recorded and statistically analysed using IBM SPSS 20 software. One-way ANOVA test was performed to determine significant differences between the variations. Tuckey HSD was used to ascertain significant differences among treatments at p= 0.05.

3. Results

Effect of gamma rays on seed germination, survivability and seedling growth

The seeds started germinating within 3 to 4 days of sowing in all the treatments and the control. The percentage of germination of control seeds was found to be 68.89±2.22. The percentage of germination of seeds irradiated with 75Gy (55.55±2.22) was not significantly different from the control. Irradiation with 100Gy and 125Gy gamma ray doses significantly decreased the germination percentage of seeds as compared to the control (Fig. 1). Furthermore, the percentage of survival of seedlings obtained from seeds irradiated with 75Gy, 100Gy and 125Gy was significantly lower as compared to the control (Fig. 1). No significant difference was observed in the percentage of survival of seedlings obtained from seeds irradiated at 100Gy and 125Gy. There was no significant variation in plant height and root length of the seedlings up to 10 days as compared to the control. From the 10th day onwards there was a significant decrease in plant height and root length in seedlings obtained from seeds irradiated at 100Gy and 125Gy as compared to the control (Figs. 2 and 3).

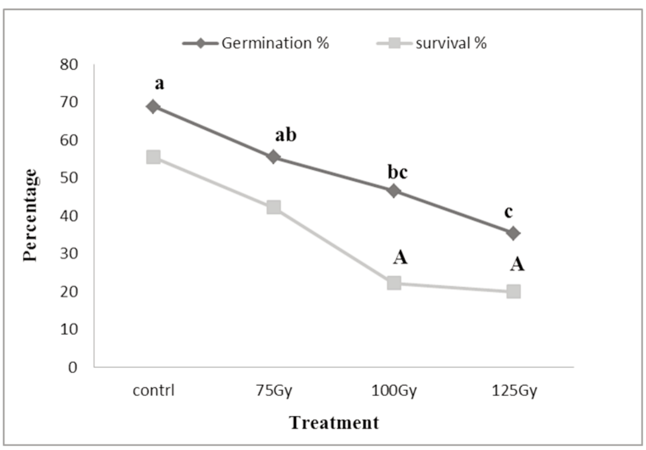

Fig. 1 - Effect of gamma radiation on percentage of seed germination and percentage of plant survival in *Zinnia elegans* var. Dreamland. The mean values with same alphabets do not differ significantly at P<0.05 level.

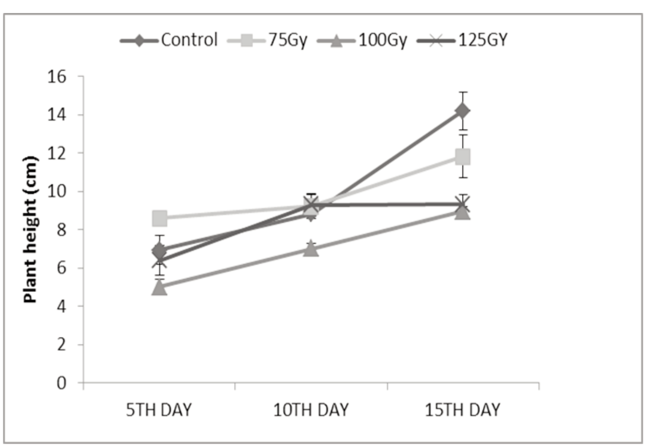

Fig. 2 - Effect of gamma radiation on plant height of the seedlings at 5 days interval.

Mutants obtained at M3 generation

Variation in plant height. Variation in plant height was observed in third generation plants obtained from seeds irradiated at all three levels (Fig. 4). The lowest plant height was seen in variant V1 (18.33±2.028 cm) obtained from seeds irradiated at

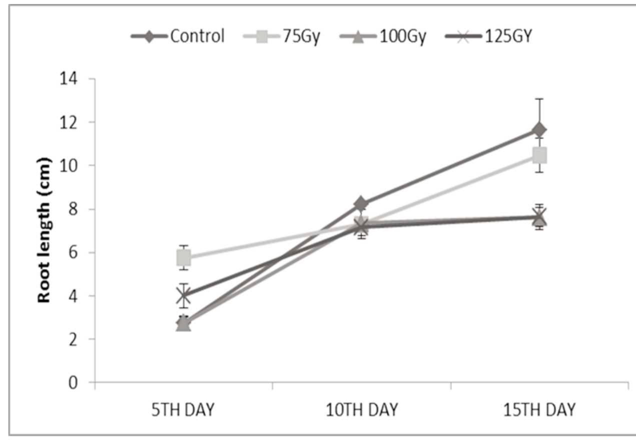

Fig. 3 - Effect of gamma radiation on the root length of seedlings at 5 days interval.

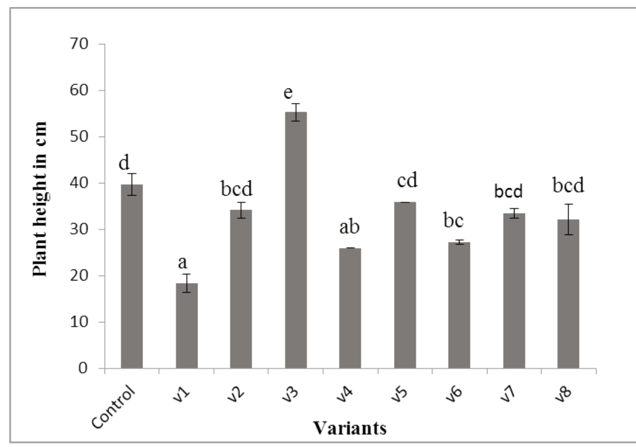

Fig. 4 - Comparison of plant heights of the variants and control plants of *Zinnia elegans* var. Dreamland. The mean values with same alphabets do not differ significantly at P<0.05 level.

the 75Gy dose. The variant V3 obtained from seeds irradiated at 100Gy dose had the highest mean value of plant height (55.33±1.85 cm).

Variation in number of flowers. The number of flowers found in the variants is shown in figure 5. The variant V7 gave the highest mean number of flowers

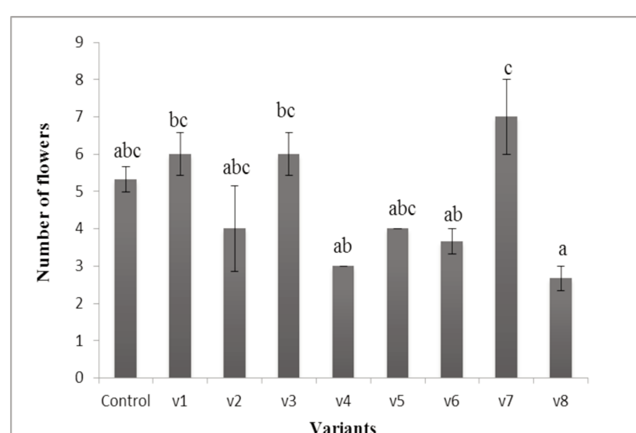

Fig. 5 - Comparison of number of flowers in control and variants of *Zinnia elegans* var. Dreamland. The mean values with same alphabets do not differ significantly at P<0.05 level.

(7±0.57) at 100Gy dose. The lowest number of flowers was given by variant V8 (2.66±0.33) at 125Gy as compared to the control (5.33±0.33). The variation found in the number of flowers in all the variants is statistically highly significant.

Variation in flower diameter. Variation in the diameter was found in the flowers of plants of seeds radiated at all three doses (Fig. 6). Except variant V3 most of the flowers were smaller than the control. The variations in the smaller flowers are not significant except for the variant V7.

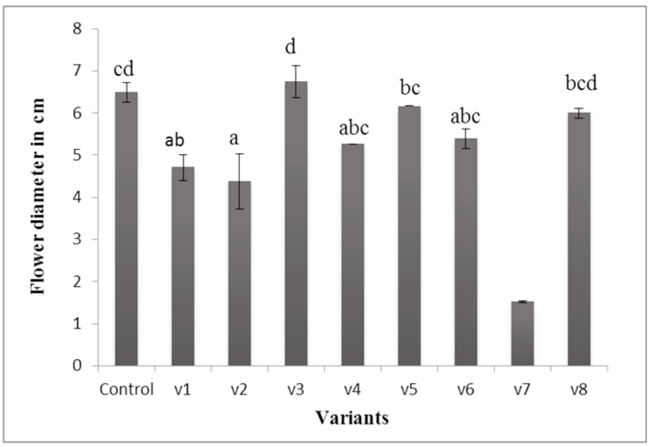

Fig. 6 - Comparison of flower diameter in control and variants of *Zinnia elegans* var. Dreamland. The mean values with same alphabets do not differ significantly at P<0.05 level.

Variation in form and colour of flowers. Variations in flower form and colour were observed after treatment with gamma rays of different doses as shown in Table 1 and figure 7. In plants irradiated with 75GY, two variants V1 with white coloured flowers and V2 with single whorled light yellow coloured flowers were obtained. In plants of seeds irradiated with 100GY, 5 flower colour variants were observed. V4 and V5 plants showed light yellow coloured ray florets with pink coloured tips. V3 showed single whorl pink flowers, V6 plants had orange yellow coloured flowers. The V7 had only yellowish disc florets without any ray florets. In plants irradiated with 125GY, only one variant with yellow flowers was obtained.

Fig. 7 - Effect of gamma radiation on flower colour and form of *Zinnia elegans* var. Dreamland. C= control, V1-V8 = Variants.

4. Discussion and Conclusions

Higher doses of gamma radiation were found in our study to reduce seed germination and survival of seedlings. This is confirmed by Hanafiah *et al.* (2010), who found that higher doses of radiation have an adverse effect on seed germination and survival of soybean seedlings. Similarly, Kumari *et al*. (2013) reported that Gamma rays significantly reduced plant survival and growth of *Chrysanthemum morifolium* variety 'otome pink'; reduction in survival increased with increase in dose. Significant reduction in survival of plants obtained from seeds irradiated with gamma rays was also observed by Jala and Bodhipadma (2011) in *Celosia argentea* var. cristata. According to

Table 1 - Effect of gamma radiation on flower colour and form

Plant variants	Gamma ray doses (Gy)	Flower characteristics
Control	-	Pink, double whorled
V1	75	White, double whorled
V2	75	Light yellow, single whorled
V3	100	Pink, single whorled
V4	100	Light yellow, double whorled ray florets with pink shaded tips
V5	100	Light yellow, double whorled ray florets with light pink shaded tips
V6	100	Orange yellow, double whorled
V7	100	No ray florets
V8	125	Yellow, double whorled

Datta and Gupta (1982), Banerji and Datta (2002), and Khan (2003), the decreased survival percentage of plants obtained from seeds treated with higher gamma radiation doses is due to chromosomal aberrations and gene mutation after irradiation treatment. According to Tiwari and Kumar (2011) many mutations can be lethal due to the inhibition of cell division and induction of cell death. In our study higher doses of radiation were found to reduce height of the plants in *Zinnia*. Significant reduction in plant height is also reported with higher doses of radiation in *Chrysanthemum morifolium* variety 'otome pink' (Kumari *et al*., 2013). The decrease in plant height and root length observed in our study has also been reported in a number of other crops (Thimmaiah *et al*., 1998; Yaqoob and Ahmad, 2003; Al-Salhi *et al.,* 2004; Toker *et al.,* 2005; Kon *et al*., 2007; El Sheriff *et al*., 2011). The plant height and root length of seedlings from the seeds treated with 100Gy and 125Gy was found to be similar to the control seedlings until 10th day in our study but both decreased after the 10th day. Similar results have been reported by Khalil *et al*. (1986) in barley and El Sheriff *et al*. (2011) in *Hibiscus sabdariffa*. According to them the probable cause for the reduction in plant height and root length after the 10th day, could be the decrease in activity of mitotic division of meristematic tissues and decrease in the moisture content of the seeds. Induction of mutations based on ionising radiations has played a major role in the development of many new and novel flower colour and shape mutants in ornamentals (Datta *et al.,* 2005). Schum and Preil (1998) reported that 55% of the records on induced mutation in ornamental plants concerned changes in flower colour and 15% in flower morphology. Yamaguchi *et al*. (2008) obtained more than 8 types of flower colour mutants from *Chrysanthemum* plants bearing pink flowers. Datta and Chakrabarty (2009) developed four mutants with flower colour and floret shape variation by irradiating the ray florets of five decorative type *Chrysanthemums*. Venkatachalam and Jayabalan (1997) induced four types of new flower colour mutations: majenta, yellow, red and red with white spots in *Zinnia elegans* Jacq. cv. crimson red using gamma irradiation. In our study, eight *Zinnia* mutants with novel variations in form and colour of flowers were obtained with gamma rays. Studies by Datta *et al*. (2009); Zalewska *et al*. (2011) on pigment analysis of florets of chrysanthemum flower colour mutants indicated that changes in flower colour were due to qualitative and quantitative changes in the pigments as a result of mutations induced by gamma rays. Flower pigments are composed of flavonoids including anthocyanins, flavones and flavonols (Harborne and Grayer, 1988; Hattori, 1992). Mutations in the core structural genes or regulatory loci of Anthocyanine biosynthesis pathway result in changes in flower colour according to Streisfeld *et al.* (2013) and Nakatsuka *et al.* (2005). The blockage in the early steps of anthocyanin synthesis leads to the loss of floral anthocyanine pigments ultimately resulting in the formation of white flowers whereas a blockage in the later steps leads to flower colour changes from blue to red because of the accumulation of a particular anthocyanin (Mato *et al.,* 2000; Lee *et al.,* 2008; Tanaka *et al.,* 2008; Casimiro-Soriguer *et al*., 2016).

A higher frequency of flower colour mutation was observed in 100Gy. This could be a suitable dose for obtaining higher number of flower colour mutants in *Zinnia elegans* var Dreamland. Our studies have resulted in obtaining one dwarf variety (18.33±2.028 cm) and eight varieties with varying floral colours from which desirable variants can be selected and commercially exploited.

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