The Bioscan 8(4): 1489-1492, 2013 (Supplement on Genetics & Plant Breeding)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

INDUCED MUTAGENESIS IN *CHRYSANTHEMUM MORIFOLIUM* VARIETY 'OTOME PINK' THROUGH GAMMA IRRADIATION

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KEYWORDS
Chrysanthemum
Gamma irradiation
Induced mutagenesis.

Received on :
13.08.2013

Accepted on :
27.10.2013

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ABSTRACT

Rooted cuttings of chrysanthemum variety 'Otome Pink' were treated with 0, 10, 15 and 20 Gy of gamma rays and evaluated for various morphological, palynological and anatomical characters. Plant survival, plant height, number of flower heads, stems per plant, stem diameter and leaves per plant reduced after gamma irradiation. The delayed flowering and plant in vegetative stage were observed at 20 Gy gamma irradiation dose. Pollen fertility, number of chloroplasts per guard cell, flower head size and fresh weight also decreased as the dose increased. The leaf abnormalities were observed in terms of changes in leaf shape, leaf size, leaf margin and leaf apex. Flower head fasciation and asymmetrical development of flower heads increased with increased dose of gamma irradiation. Various changes in flower colour and shape were recorded after treatment in the form of chimeras. Two variants, one at 10 Gy having yellow colour and other at 15 Gy having quilled petals were obtained and further multiplied vegetatively.

INTRODUCTION

Chrysanthemum, one of the top 10 cut flowers of the world, is member of the family Asteraceae. It is native to Northern hemisphere, chiefly Europe and Asia, (Anderson, 1987). The basic chromosome number of chrysanthemum is $x=9$, however a ploidy level ranges from $2n=36$ to 45, 47, 51-57 (Nazeer and Khoshoo, 1983). Being a cross pollinated species many new chrysanthemum varieties were developed by hybridization. High degree of heterozygosity coupled with frequent polyploidy cause a complex inheritance of genetic factors, which pose serious handicap in conventional breeding. Chrysanthemum is easy to multiply through vegetative means and its higher ploidy level is suitable for inducing mutation through chemical as well as physical means. Induced mutations consequently have a high potential for bringing about further genetic improvement. Although a large number of varieties are available in market but due to ever increasing demand of new types, there is still a scope for the development of new varieties. The induction of mutation in chrysanthemum has attracted considerable attention as any mutation in the dominant gene is easily expressed in the first generation. Thus, the selection of mutants of directly perceptible characters like flower colour, shape and size etc. is generally very easy and can be directly put to commercial use. More mutations have been documented in the pink cultivars as compared to others (Datta and Banerji, 1993). The present study aimed to induce mutations for flower colour and form in chrysanthemum variety 'Otome Pink' through gamma irradiation and to study the different morphological and anatomical changes associated with gamma ray irradiation.

MATERIALS AND METHODS

The present investigation was carried out at the experimental farm of the Department of Floriculture and Landscaping, Punjab Agricultural University Ludhiana during 2010-11. Rooted cuttings of chrysanthemum variety 'Otome Pink' (Korean Double type) were irradiated with 0, 10, 15 and 20 Gy of gamma rays from a ^{60}Co source (Low Dose Irradiator 2000 ANSI-N4333.1) and immediately planted in the pots under open field condition. The experiment was laid out in CRD design with 3 replications for each treatment. Data were recorded on different vegetative and floral characters in the field and final plant height, number of branches, number of leaves and flower size were recorded at full bloom stage.

To determine the pollen fertility (%), pollen grains collected soon after anthesis were stained with drop of acetocarmine and viewed under microscope (Leica Bright Field Research Microscope) at 200X. Uniformly stained and round pollens were considered as viable whereas unstained and broken as non-viable and percent pollen fertility was worked out. Size of well-stained pollen grains (in μm) was recorded under microscope. Size of guard cells (μm) and size of stomatal pores was measured by microscopic observation of the replica of the leaf epidermal surface. A drop of an adhesive (Quick fix) was applied to the abaxial (lower) surface of leaf and spread thoroughly over an area of 1-2 cm^2 during mid day (11:00 - 13:00 pm) while they are still attach to plant. When solidified, the replica containing impressions of epidermis and stomata was stripped off the leaf and studied under microscope.

To determine the number of chloroplasts per guard cell, a drop of I-KI was placed on the peel, removed from abaxial surface of leaf and allows to react for >5 min. The peel was then washed with deionised water. Starch containing regions (chloroplasts) get stained. After washing, the peel was observed under microscope at 400X.

Any change in stem and flower colour due to gamma rays treatment was observed visually and marked with the help of Royal Horticulture Society Colour Chart. To find out the change in flower form, chimera was critically observed and compared with the normal plants. Data were subjected to statistical analysis by using CPCS-1 software developed by department of Mathematics and Statistics, Punjab Agricultural University, Ludhiana. The data was analyzed as advocated by Singh (1998).

RESULTS AND DISCUSSION

Radiation effect on vegetative characters

It is evident from the data presented in Table 1, that gamma rays had significant influence on plant survival and growth. Gamma irradiation significantly reduced the per cent plant survival and reduction in survival increased with increase in dose of gamma rays. Gamma irradiation significantly reduced final plant height. Significant reduction in plant survival was also observed by Banerji and Datta (1992) in variety 'Jaya' after irradiation. The reduction in survival after exposure to gamma rays has been explained due to disturbances of auxin synthesis (Gordon, 1957). Reduction in plant height after 2 kR gamma rays exposure of chrysanthemum varieties was also observed by Dilta *et al.* (2003).

Among the different gamma rays treatments, maximum number of stems was recorded in control (5.70) while minimum number of stems per plant (1.80) was recorded at 20 Gy gamma rays. The less number of branches may be due to inhibitory effect of higher mutagenic doses of gamma rays. These results are in close conformity with results of Banerji and Datta (2002) in varieties 'Lalima'.

In the present study it has been observed that all the treatments of gamma rays significantly reduced the number of leaves and stem diameter and this reduction was increased with the increased doses. This decrease in number of leaves is mainly due to the decrease in number of branches per plant and poor growth of plant. Irradiation of cuttings significantly reduced the leaf length and width. The reduction in leaf size in terms of length and width of plants treated with higher doses of gamma rays may be due to inactivation or decrease in auxin content or disturbances in auxin synthesis (Gorden, 1957).

Plant abnormalities increased after irradiation and among the different gamma rays treatment, maximum abnormal plants (89.96 %) recorded with 20 Gy gamma rays treatment whereas minimum (0.57%) with 0 Gy. The leaves abnormalities also increased after treatment. The different types of leaf abnormalities included change in leaf shape and size, margins, apex, fission and fusion were recorded after irradiation (Plate 1). There was no dose specific abnormality in leaves. No leaf variegation was observed after irradiation with any dose. The leaf abnormalities in irradiated plants may be due to chromosomal aberrations, disturbance in the production and/ or distribution of growth substances, breakdown of phosphate metabolism and accumulation of free amino acids (Gunckel and Sparrow, 1961).

Radiation effect on floral characters

Floral characters of chrysanthemum variety 'Otome Pink' were significantly influenced by different gamma rays irradiation (Table 2). The results indicate that flower bud formation was earlier in untreated plants (60.21 days) while those irradiated with 20 Gy of gamma rays took longer time (84.34 days) for bud initiation. The delay in flower bud initiation may be due to reduction in the rate of various physiological processes with gamma irradiation. The delay in bud initiation ultimately resulted in late blooming. These results corroborate the finding of Datta and Gupta (1981). As a result of irradiation many biosynthetic pathways are altered which are directly and indirectly associated with the flowering physiology (Mahure *et al.*, 2010). The increase in doses of gamma rays had significantly reduced the number of flower heads per plant. The decrease in number of flower heads per plant may be due to decrease in number of branches per plant. The size of flower heads was also influenced significantly by irradiation. The maximum flower head size (8.91cm) was recorded with untreated plants which were statistically at par with result obtained at 10 Gy gamma rays treatment. Smaller flower head size (6.40 cm) was recorded at 20 Gy gamma rays treatment. The decrease in flower head size could be attributed to the poor growth of plant on the irradiated plants due to radiation damage. Earlier Gupta and Jugran (1978) also recorded smaller sized flower heads in chrysanthemum and concluded that the reduced flower head size could be due to physiological, morphological and cytological disturbance caused by gamma

Table 1: Effect of gamma irradiation on vegetative characters of chrysanthemum variety 'Otome Pink'

Observations	Dose of gamma rays (Gy)				CD(p=0.05)
	0 Gy	10 Gy	15 Gy	20 Gy	
Plant survival (%)	91.70 [73.40]	80.00 [63.55]	53.40 [46.95]	45.00 [42.12]	7.76
Plant height (cm)	55.27	51.07	43.72	35.27	5.38
Branches per plant	5.70	3.74	2.70	1.80	0.58
Leaves per plant	68.84	54.84	37.40	26.04	9.65
Stem diameter (cm)	0.674	0.529	0.480	0.387	0.059
Leaf length (cm)	6.35	6.24	5.51	4.70	0.45
Leaf width (cm)	4.10	3.85	3.85	3.53	3.51
Abnormal plants (%)	0.00 [0.00]	31.00 [33.82]	66.70 [54.78]	100.00 [90.00]	3.61
Abnormal leaves per plant (%)	0.01 [0.57]	11.85 [20.12]	22.31 [28.18]	36.79 [37.34]	0.27

Values in parenthesis are angular transformed.

Table 2: Effect of gamma irradiation on floral characters of chrysanthemum variety 'Otome Pink'

Observations	Dose of gamma rays (Gy)				CD(p=0.05)
	0 Gy	10 Gy	15 Gy	20 Gy	
Days to bud initiation	60.21	62.80	73.77	84.40	4.06
Days to full bloom	115.2	116.55	125.55	133.51	4.80
Number of Flower heads	8.54	7.17	5.11	3.49	0.79
Flower head size (cm)	8.91	8.34	7.70	6.40	0.74
Flower head weight (g)	10.36	8.06	7.07	6.12	0.62
Flower head height (cm)	3.34	2.84	2.54	2.23	0.40
Plants with flower head fasciation (%)	0.00 [0.00]	1.67 [24.05]	14.40 [39.15]	25.72 [36.92]	6.22
Plants with asymmetrical heads (%)	0.00 [0.00]	16.67 [4.31]	40.00 [22.27]	36.11 [30.46]	7.37

Values in parenthesis are angular transformed

Table 3: Effect of gamma irradiation on palynological and anatomical characters of chrysanthemum variety 'Otome Pink'

Observations	Dose of gamma rays (Gy)				CD (p=0.05)
	0 Gy	10 Gy	15 Gy	20 Gy	
Pollen fertility (%)	91.83 [73.46]	91.52 [73.10]	84.86 [67.13]	72.78 [58.57]	2.97
Pollen size (μm)	24.60	23.70	23.03	22.78	NS
Chloroplasts/ guard cell	9.26	9.10	9.67	7.40	0.64
Guard cell length (μm)	35.12	28.99	28.30	27.04	3.52
Guard cell width (μm)	8.76	6.60	7.11	7.14	0.68
Stomata pore length (μm)	25.49	21.46	20.52	17.13	3.48
Stomata pore width (μm)	7.54	7.67	6.80	7.60	NS

Values in parenthesis are angular transformed; NS- Non-significant

radiation. Decrease in the flower head weight with increase in gamma rays treatments was found with maximum flower head weight (10.36g) at 0 Gy and minimum (6.00 g) at 20 Gy gamma rays irradiation. Reduction in flower head weight was after irradiation may be due to reduced size of the flower head and reduced number of petals in flower head. The maximum floral abnormalities in the form of head fasciation and asymmetrical heads were recorded at 20 Gy. The number of plants with asymmetrical flower heads was increased with gamma rays treatment. In the present work it was reported that at higher dose of gamma rays (20 Gy) some plants even failed to produce bud and flowers and remained in vegetative stage. Significant increase was found in per cent plants with flower head fasciation due to irradiation of plants with different doses of gamma rays. Flower heads became fasciated in different forms (Plate 4). This abnormality might be due to chromosomal aberrations, disturbance in the production and/or distribution of growth substances caused by gamma rays. The formation of fascinated heads after irradiation are in close conformity with the findings of Misra *et al.* (2009).

The change in flower colour was observed in the form of chimeras. After irradiation with different doses of gamma rays change in form of flower was observed. The results showed a wide range of flower colour from yellow, orange, light pink to

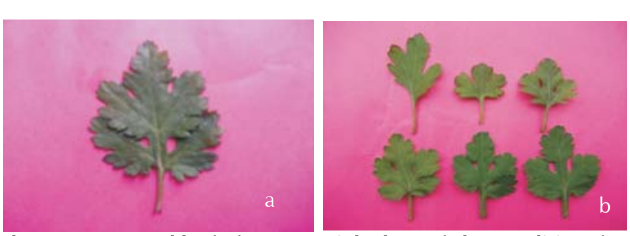

Plate 1: (a) Normal leaf of 'Otome Pink' (b) Leaf abnormalities after irradiation

dark purple at 10 and 15 Gy. It is also pertinent to mention here that changed colour was only in one flower or in all flowers on one branch or only in one or two petals of a flower (Plate 2). A mutant with all flowers having quilled form (tubular florets) was obtained at 15 Gy gamma rays treatment (Plate 3 a). At 10 and 15 Gy half quilled flowers were also observed. These types of chimeras can only be stabilized by the tissue culture techniques. A mutant with all yellow flowers was obtained at 10 Gy gamma rays irradiation and matched with Yellow Orange Group 15 C Royal Horticulture Colour Chart (Plate 3b).

Radiation effect on Palynological and anatomical characters

The appraisal of Table 3 elucidate that gamma irradiation have significantly affected the pollen fertility. At higher doses of gamma rays more pollens grains were remained unstained and shrivelled which shows reduction in fertility. Maximum pollen fertility (91.84 %) was recorded in untreated plants and decreased significantly at 20 Gy gamma rays irradiation (72.78 %). Datta (1988) also obtained similar palynological investigation of mutants developed after gamma irradiation and colchicines treatment. The results on pollen size (μm) reveals that doses of gamma irradiation had no significant effect on pollen size.

A significant difference was recorded in number of chloroplasts per guard cell, after gamma irradiation. The maximum number of chloroplasts (9.67) was recorded at 15 Gy gamma rays treatment whereas minimum number of chloroplasts (7.40) was recorded at 20 Gy. The chloroplasts were more round and bold in untreated plants and were in proper line whereas at higher dose (20 Gy) they were not in proper shape and were broken or fused. Gamma rays irradiation had significant effect on length of guard cell. Maximum guard cell length (35.12 μm) was recorded in untreated plants whereas minimum guard cell length (27.04 μm) was recorded at 20

Plate 2: Original variety 'Otome Pink' (top left) and chimeras in different colours

Plate 3: (a) Quill type variant obtained at 15 Gy (b) Flower colour variant obtained at 10 Gy

Plate 4: (a) Flower head fasciation (b) Asymmetrical flower head

Gy. At 10 Gy gamma rays treatment guard cell length was 28.99 μm which was statistically at par with length at 15 Gy (20.52 μm). The width of guard cell was also significantly affected with gamma irradiation and maximum guard cell width (8.76 μm) was recorded at 0 Gy gamma rays whereas minimum (7.11 μm) at 15 Gy. The pore length of stomata is significantly affected by gamma irradiation whereas change in pore width was not significant.

In the present study 10 Gy and 15 Gy gamma rays dose was found good in induction of useful mutations for flower colour and shape. At higher dose of 20 Gy abnormalities were very high and survival was also very less. Most of the mutations were in the form of chimeras so use of tissue culture techniques may be useful for establishing these chimeras.

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