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BRIEF COMMUNICATION

# Mutation in flower colour and shape of *Chrysanthemum morifolium* induced by γ-radiation

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## Abstract

Flowers of *Chrysanthemum morifolium* Ramat cv. Lalima were greyed red and florets were flat spoon shaped. Ray florets after inoculation on the Murashige and Skoog's medium supplemented with 1.07 μM α-naphthaleneacetic acid and 8.87 μM benzyladenine were irradiated with γ-radiation (0.5 Gy and 1 Gy). All the regenerated shoots either from control or from γ-irradiated florets were isolated, rooted and transplanted in the field after hardening. Two mutants were obtained in the γ-irradiated plants (0.5 Gy). Both the mutants were yellow coloured but one having flat spoon shaped ray florets similar to the original cultivar, while the other having tubular florets. Both the mutants were propagated vegetatively and have produced true-to-type flowers.

*Additional key words*: chimera, *in vitro* mutagenesis, ray florets.

Treatment of propagules (suckers/cuttings) of chrysanthemums with mutagens develop chimera, which was the main bottleneck in creating new flower colour/ shape mutants through induced mutation. Conventional propagation methods can help only to isolate the chimeric branches.

Recently a novel technique has been developed for management of floral chimeric sector in chrysanthemum through direct regeneration of mutated individual florets (Chakrabarty *et al.* 1999). Induced mutation breeding has been succesfully utilized for the development of new flower colour/shape mutants and they are being commercially exploited (Broertjes and van Harten 1988, Datta 1988, 1998).

An attempt has been made in the present experiment to treat ray florets of chrysanthemum with γ-radiation just after culturing them on regeneration medium and to isolate induced mutated cells through direct regeneration without normal chimeric competition.

Ray florets were collected from the outer whorl of the chrysanthemum (*Chrysanthemum morifolium* Ramat cv. Lalima), of a half bloom flower head after approximately 110 d of planting, from field-grown plants. The explants were washed in running tap water for 15 min, in 5 % liquid detergent solution for 5 min and then washed with distilled water. Surface sterilization was done by a quick dip in 70 % alcohol and then in 0.1 % $HgCl_2$ solution for 2 min. Finally the explants were washed thoroughly 3 - 4 times with sterile double distilled water. Murashige and Skoog's (1962) medium along with 3 % sucrose and 0.8 % bacto-agar was used as the basal medium, and NAA and BA in different concentrations and combinations were used as the growth regulators. 25 explants were used for each treatment. The pH of all the media used was adjusted to 5.8 before autoclaving at 1.08 kg $cm^{-2}$ for 15 min. The cultures were incubated at temperature of 26 ± 2 °C, irradiance of 50 - 60 μmol $m^{-2}s^{-1}$, 16-h photoperiod and relative humidity 55 - 60 %.

Ray florets immediately after plating in Petri plates were given two doses of 0.5 Gy and 1 Gy of γ-radiation ($^{60}Co$, 60 s). After 30 to 40 d of culture initiation (Table 1), approximately 2 cm long shoots were excised and rooted in half strength MS + 0.54 μM NAA + 1.5 % sucrose and 0.8 % bacto-agar. Rooted control or treated

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*Abbreviations*: BA - benzyladenine; MS medium - Murashige and Skoog's medium; NAA - α-naphthaleneacetic acid.

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plantlets were transplanted in the small plastic pots having a mixture of leaf mould:soil (3:1). The shoots were hardened within 15 d, by gradually increasing the temperature from 25 to 30 °C and decreasing the humidity from 80 to 50 % in the hardening chamber. After hardening, the tissue culture raised plants were transferred to the field. Data was recorded on different vegetative and floral characters of the regenerated shoots (Table 2).

Table 1. Effect of γ-radiation treatment on shoot bud differentiation in *C. morifolium* cv. Lalima, in MS + 1.07 μM NAA + 8.87 μM BA, after 4 weeks of culture initiation.

| Treatment | Shoot bud differentiation [d] | Response [%] | Number of shoot buds [responding explant]$^{-1}$ |
|---|---|---|---|
| Control | 15 | 75 | 8.24 ± 0.57 |
| 0.5 Gy | 20 | 56 | 6.50 ± 0.48 |
| 1.0 Gy | 23 | 44 | 4.35 ± 0.37 |

In a preliminary experiment, three concentrations of NAA (0.54, 1.07 or 2.69 μM) and BA (4.44, 8.87 or 22.19 μM) were used in all possible combinations. Out of which 1.07 μM NAA + 8.87 μM BA proved to be the best combination for direct shoot organogenesis which started after two weeks of culture incubation (Fig. 1*A*). Direct regeneration of shoots is required because regeneration through lengthy callus phase resulted in loss of genetic uniformity in chrysanthemum (Malaure *et al.* 1991). The

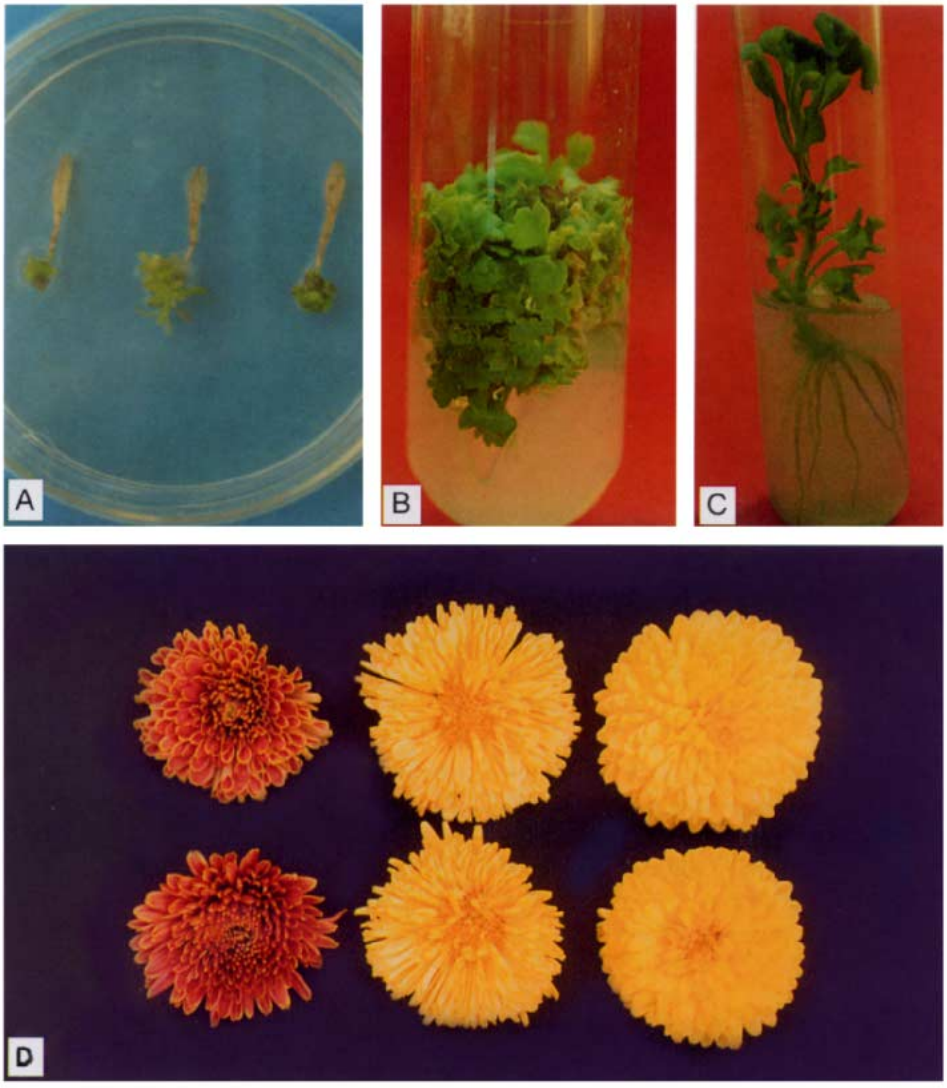


Fig. 1. *A* - Direct shoot organogenesis from ray floret of chrysanthemum. *B* - Proliferation of shoots. *C* - Rooting in isolated shoot. *D* - Flowers from tissue-raised plants of chrysanthemum (from left to right: control; Mut 1, yellow coloured with tubular petals; Mut 2, yellow coloured with spoon shaped petals).

Table 2. Effect of γ-radiation on morphological characters of plants regenerated from ray florets of *C. morifolium* cv. Lalima (+ - $P < 0.02$ ; * - $P < 0.05$; ** - $P < 0.01$).

| Characters | Control | 0.5 Gy | 1.0 Gy | Mut 1 (0.5 Gy) | Mut 2 (0.5 Gy) |
|---|---|---|---|---|---|
| Plant height [cm] | 40 ± 1.24 | 39 ± 1.11 | 38 ± 0.98 | 38 ± 1.21 | 38 ± 1.32 |
| Number of leaves [plant$^{-1}$] | 166 ± 2.01 | 150 ± 3.22 | 135 ± 1.82+ | 162 ± 2.12 | 160 ± 1.98 |
| Leaf length [cm] | 5.0 ± 0.52 | 5.2 ± 0.62 | 5.5 ± 0.43 | 4.9 ± 0.38 | 4.9 ± 0.38 |
| Leaf width [cm] | 3.45 ± 0.32 | 4.05 ± 0.43 | 4.60 ± 0.48 | 3.5 ± 0.32 | 3.5 ± 0.35 |
| Petiole length [cm] | 1.33 ± 0.18 | 1.40 ± 0.20 | 1.45 ± 0.21 | 1.36 ± 0.18 | 1.35 ± 0.19 |
| Number of flowers [plant$^{-1}$] | 82 ± 1.56 | 66 ± 1.24* | 50 ± 1.10** | 78 ± 1.42 | 75 ± 1.32 |
| Flower diameter [cm] | 3.93 ± 0.80 | 3.84 ± 0.91 | 3.50 ± 0.76 | 3.83 ± 0.78 | 3.90 ± 0.86 |
| Ray floret length [cm] | 2.05 ± 0.12 | 1.75 ± 0.10 | 1.70 ± 0.98 | 2.08 ± 0.15 | 2.02 ± 0.13 |
| Ray floret width [cm] | 0.27 ± 0.08 | 0.30 ± 0.09 | 0.30 ± 0.06 | 0.15 ± 0.05 | 0.27 ± 0.05 |
| Flower bud initiation [d] | 75 ± 1.56 | 80 ± 1.62 | 86 ± 1.82 | 75 ± 1.45 | 76 ± 1.42 |
| First colour shown [d] | 105 ± 2.24 | 111 ± 2.30 | 120 ± 2.73 | 107 ± 1.87 | 106 ± 1.98 |
| Full bloom [d] | 120 ± 2.11 | 133 ± 2.24 | 155 ± 2.56 | 123 ± 2.08 | 121 ± 2.17 |

time taken for shoot bud differentiation was delayed with increase in exposure to γ-radiation. Percentage of responded explants and the number of regenerated shoots per explant decreased with the increase in γ-radiation dose (Table 1) in contrast to the findings of Chakravarty and Sen (2001), where regeneration was enhanced by increase in γ-radiation dose. However, low doses of gamma irradiation were used to enhance microtuber production in potato (Al-Safadi *et al.* 2000). The regenerated shoots were further proliferated and grown by subculturing in the same medium (Fig. 1*B*). 100 % rooting occurred within one week of incubation (Fig. 1*C*). Rooted plants were acclimatized in the hardening chamber and transferred in the field, where all the plants survived and flowered. There was no significant difference in plant height, number of leaves, leaf size and flower and floret size among the control and γ-radiation treated regenerated plants except the number of leaves and number of flower heads which were significantly reduced after 1 Gy γ-radiation. Days to flower bud initiation, first colour showing and full bloom were delayed after treatment and with increase in exposure (Table 2).

Somatic mutations in flower colour and shape were detected in 0.5 Gy treated population. First type of mutation (Mut 1) was observed only in 10 plants out of the population of 100 regenerated plants (10 %), having mutation in both flower colour and shape, while another mutation (Mut 2) was in 30 plants in the same population of 100 plants (30 %), which was in flower colour (Fig. 1*D*) only. Both the mutations were solid in nature. The floret colour was matched with the Royal Horticultural Colour Chart. The original floret colour of Lalima was greyed red (Group 180A, Fan4) and spoon shaped. The flower colour of both the mutant was bright yellow (Group 6A, Fan1). The floret shape of Mut 1 was tubular, while that of Mut 2 spoon shaped (Fig. 1*D*). All other vegetative and floral characters of both the mutants were similar to the original (Table 2). It was interesting to note that no morphological abnormalities were found in leaves and flower heads of the treated population, which is a normal phenomenon in the first generation of mutagen treated plants. It may be assumed that the factors responsible for leaf/flower abnormalities were eliminated during rege-neration or abnormal cells could not take part in regeneration. The main constraint in mutation breeding of vegetatively propagated crops is the formation of chimera after mutagen treatments. Isolation of chimera is possible through available conventional techniques when the entire branch is mutated but it is almost impossible when a sector (one or few florets) of a flower is mutated. In the present experiment both the mutants developed through ray floret regeneration were solid in nature, having same colour but different shapes indicating no chimera formation (Datta *et al.* 2001, Mandal *et al.* 2000).

Present experiment indicates that solid flower colour/shape mutations can be developed through direct *in vitro* mutagenesis by avoiding chimeric phase.

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