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Gamma-rays Induced Morphological Changes in Chrysanthemum. (*Chrysanthemum morifolium*)

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ABSTRACT

The purple color clone of spray type chrysanthemum available as pot plant in the market was used to study the effect of gamma radiation on *in vitro* culture of chrysanthemum (*Chrysanthemum morifolium*). Ray-florets were cultured on the MS medium containing 10 mg/l BA. Multiple shoots produced were irradiated with gamma rays at 0, 10, 30, 50, 70, 90 and 110 Gy. Subculturing was carried out three times from M_1V_1 to M_1V_4 after which M_1V_4 shoots were rooted and transplanted to the greenhouse. M_1V_4 shoots irradiated at 50 Gy and over died within 25-30 days. LD_{50} for this purple clone of chrysanthemum was 14 Gy. Only the controls and treated plants at 10 Gy were able to survive and gave rise to the full grown plants. After transplanting into the greenhouse for 60 days, control plants and treated ones were found to be different in four traits which were average height, average number of leaves, average number of nodes and % flowering. Plants were trimmed twice at three month intervals and allowed to produce flowers. Changes in flower characters were found in both controls and treated plants. However, the treated plants had much more variation than the controls and new flower color (yellow tinge) was only obtained from the treated ones.

Key words : chrysanthemum, mutation, gamma-rays, tissue culture

INTRODUCTION

Chrysanthemum (*C. morifolium* Ramat) is very much in demand and very popular in Thailand. More than 50 percent of local demand is imported from Malaysia and the need will have the tendency to increase every year. In order to reduce importation of the flowers into the country, farmers have brought foreign varieties to be planted in Thailand especially in the north. These varieties thrived very well in Thailand but their quality and quantity are insufficient for local consumption. It is therefore, very essential to produce new exotic varieties suitable for growing in the country and meet the demand of local market as well as the export. Induced mutation has been reported to be an efficient technique to achieve the desirable characters in flowers and ornamental plants (Maluszynski, 1995). The successful improvement of chrysanthemum through induced mutation and *in vitro* culture have been demonstrated (Nagatomi, *et. al*, 1996 ; Ahloowalia, 1992 ; Datta and Banerji, 1993 ;

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Matsumoto and Onozawa, 1990 ; Neto and Latado, 1996). The objective of this study was to use an *in vitro* mutation technique to improve our chrysanthemum in order to select the desirable characters such as flower shape, color, long life plus suitability for growth in adverse conditions.

MATERIALS AND METHODS

Ray florets of pale purple color clone (spray type) of chrysanthemum available as pot plant in the market were cultured in the MS medium (Murashige and Skoog,1962) supplemented with 10 mg/l N^6-Benzaladenine (BA). After organogenic calli followed by multiple shoots developed, the cultures were irradiated with gamma rays at, 0, 10, 30, 50, 70, 90 and 110 Gy. Following irradiation, M_1V_1 shoots were immediately cut into small pieces; each piece had 2 nodes with average length of 0.5 cm. They were cultured in the fresh medium of the same formula. Subculturing was then carried out at one month interval from M_1V_1 to M_1V_4. The M_1V_4 shoots were rooted in MS medium containing 10% coconut water. Rooted plants were then transplanted to the greenhouse for observation and selection of desirable characteristics.

RESULTS AND DISCUSSION

Effect of radiation on *in vitro* culture.

Table 1 shows the plant survival to which the controls are adjusted 100 percent, due to their being thrived normally i.e. leaves large and green, shoots erect with at least 2 cm in length. The shoots treated at 10 and 30 Gy had grades of 58.33% and 18.18% respectively. Abnormalities were found only in treated plants such as dwarf plant type, yellow and white – streak leaves.

The growth of treated shoots with 10 and 30 Gy of gamma rays was slower than that of the controls. Treatments at 50 Gy and over caused plant leaves to become yellow and wither and soon died within 30 days.

In order to obtain the LD_{50} (50% lethal dose) the data in Table 1 were plotted as shown in Figure 1. The LD_{50} obtained from the Figure 1 is 14 Gy.

Radiation effect on transplanted plants

When all rooted plants were transplanted to the greenhouse, it was observed that only the controls and those treated with 10 Gy were able to survive. Percent survival of the controls and treated plants were 98.8% and 95.2% respectively as shown in Table 2.

Growth of plants 60 days after transplanting

Growth of plants in the greenhouse was observed in four traits namely average height, average number of leaves, average number of nodes and % flowering as shown in Table 3. The average height of the controls and treated plants were 28 cm and 25 cm respectively. Average number of nodes of the controls was 21 and that of treated plants was 19.

Table 1 Survival (as % of control) of chrysanthemum grown on MS medium after 30 days of irradiation with different doses of gamma rays.

Dose (Gy)	Survival (% of control)
0	100
10	58.33
30	18.18
50	died
70	died
90	died
110	died

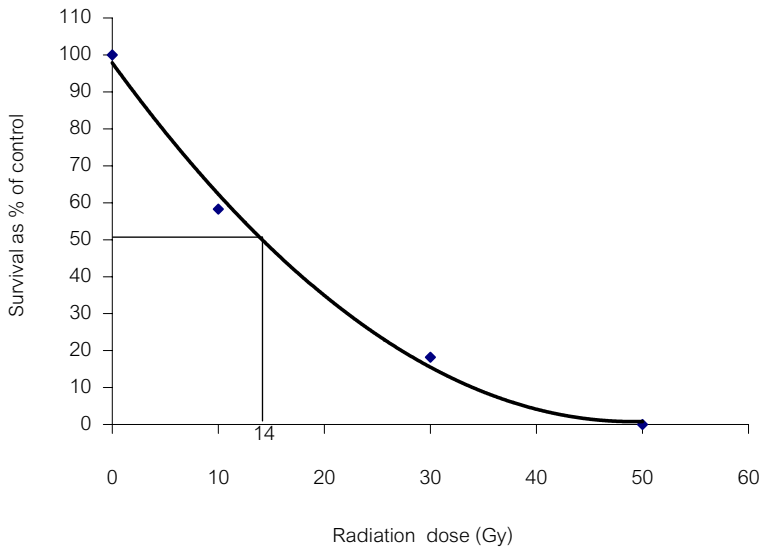

Figure 1 LD_{50} of 30 days old chrysanthemum plants treated with gamma rays at different doses.

Table 2 Percent plant survival 40 days after transplanting to the greenhouse.

Dose (Gy)	No. of transplants	Survival (%)
0	60	98.8
10	124	95.2

Table 3 Growth of plants 60 days after transplanting to the greenhouse.

Dose (Gy)	Average height (cm)	Average no. of leaves / plant	Average no. of nodes / plant	% flowering
0	28	25	21	12.7
10	25	25	19	5.9

The flowering rate of the controls was 12.7% while that of treated samples was only 5.9%.

Flower traits of control and treated plants

After the M_1V_4 plants grown in the greenhouse produced the first set of flowers, plants were cut back and trimmed for two times in order to allow new shoots to form. New shoots were observed for variations. In the control flower itself, there were differences in flower color, size and number of ray florets; the color varied from light purple to deep purple; the size varied from small to

large and the number of ray florets varied from semi double to double as shown in Figure 2.

However, variation in treated plants was more extensive than the controls as in Figure 3. The color ranged from deep purple to light purple and to almost yellow tinge. The size of inflorescence varied from plant to plant. The number of ray florets varied from semi double to double and from simple to compact and complex.

When three distinct types of flower color were compared as shown in Figure 4, they were grouped as deep purple, light purple and yellow tinge. The plants with these traits have been multiplied by tissue culture as well as by cutting to produce new clones and testing for market acceptance will be done later.

Form our experiment on the effect of gamma irradiation on *in vitro* culture of chrysanthemum, we found that irradiated shoots at 50 Gy and over were unable to produce shoots and soon die. This finding is consistent with the precious detailed study of Thin *et al.* (2000) They reported that by irradiating nodal explants of two cultivars of chrysanthemum with gamma rays at 50 Gy, none of nodal explants were able to produce shoots. Then the useful doses for mutation induction in chrysanthemum have been suggested as 10–20 Gy for *in vitro* cultures and 10–25 Gy for rooted cuttings (Thin *et al*. 2000; Broerties and Van Harten, 1988). There are numerous reports on alteration of flower colour of ornamental plants arising after mutagenic treatment. 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. To create flower colour variability by mutation in chrysanthemum, selection of an appropriate

Figure 2 Control plants with different flower color, size and number of ray florets in each inflorescence.

Figure 3 Treated plants with varying in flower color, size and number of ray florets in each inflorescence.

Figure 4 Three distinct flower color types of treated plants, varying from light purple, deep purple to yellow tinge.

genotype would be helpful. This would be seen from many reports on induced mutations in chrysanthemum. For example, Nagatomi (2000) demonstrated that pink genotype 'taihei' has given rise to many flower colour mutants ranging from white, light pink, dark pink, orange, yellow, bronze and striped. Moreover, pink genotypes gave rise to several sports that included most of the colours seen in chrysanthemum (Schum and Preil, 1998). In our experiment we were able to isolate only three distinct groups of mutants : deep purple, light purple and yellow tinge. Therefore, purple genotype of chrysanthemum that we used in our experiment might not be suitable for flower colour mutation induction because it gave rise to few mutants of interest.

From the study, it demonstrated that by using tissue culture technique in combination with gamma rays, flower color mutants could be isolated and multiplied *in vitro* as well as rooted cuttings to produce new varieties of chrysanthemum. However, this technique is to be improved to achieve more variations, not only in mutation induction step but also in separating the mutated sectors from those of normal tissues. The experience suggested that some useful variants might be lost during cutting back the treated shoots in the greenhouse, because they were discarded instead of being propagated to obtain variants.

CONCLUSION

Three distinct types of flower color mutants were isolated from the purple color clone of spray type of chrysanthemum after treating with gamma rays. Variations were also observed for size and shape of flowers. Flower mutants were propagated and multiplied to produce pot plant and will be further tested for market acceptance.

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