# Induction of Flower Color Mutations by Gamma Irradiation and Its Modification through Tissue Culture in Chrysanthemum

Yoshiro ONOZAWA

## Introduction

Artificially induced somatic mutation has long been a significant role on the improvement of vegetatively propagated plants, especially of ornamental crops, such as chrysanthemum, saintpaulia, achimenes, rose, streptocarpus and so on.[3), 4), 10—16), 24)]

As well known, most of somatic mutations are found in the chimeric structure of mutated and non-mutated cell layers, which especially in the sectorial chimera, have a possibility to sprout the reversional types of the characters concern during their vegetative propagation. Therefore, it is requested for some desirable mutations to eliminate the chimera or to stabilize the mutated traits if they might be brought to practical or commercial use.

In the efforts to escape from chimera or eliminate it, several methods or principles have been proposed. For example, the repeated cutting back following mutagenic treatment has proved to be an effective way for increasing mutation frequency and eliminating chimera in some ornamentals and fruit trees.[1), 6), 20)] Broertjes et al[2), 4), 5)] have emphasized usefulness of the adventitious bud techniques for obtainning the solid mutations directly from mutagen treated materials and they developed new commercial varieties in streptocarpus, achmenes, saintpaulia and other ornamentals, applying this method.

At any rate, it is primalily important that mutated sectors appeared in the original plants would be large enough to allow escaping from the chimera easily in the succeeding procedures including the cutting back method or the adventitious bud techniques.

In this respect, the tissue culture of mutagen treated materials would be an idea for enlarging initial mutated sector. Devreux[7)] has explained about the role of in vitro culture for mutation breeding in a broad sense. In most plants, tissue culture has both dedifferentiation (callus formation) and redifferentiation process under reasonable culture condition. Since redifferen-

*tiation of shoots or buds on the callus should be originated from single cell* or a small number of cells, it is quite probable that if a small tip of mutated sector induced primalily in a highly differentiated tissue of organ were involved in this process, it would be expected in a highly expanded one of the newly developed plant.

An experiment carried out along with the idea mentioned above will be described here.

## Materials and Methods

A chrysanthemum cultivar, Delaware which has kindly been given by the Chiba Regional Experiment Station of Horticulture was used as a source of materials. Following two experimental schemes which differ in the time of irradiation were employed.

**Scheme－1**

Gamma irradiation of cuttings of vegetative shoots

↓

Inoculating explants from the shoot apices on culture media

↓

Callus formation and redifferentiating shoots

**Scheme－2**

Inoculating explants from the non-irradiated shoot apices on culture media

↓

Callus formation

↓

Gamma irradiation onto the calluses

↓

Continuation of the culture for redifferentiation

The total gamma exposures ranging from 1.0 to 3.5 KR from the Gesium 137 source were given in the intensities of 1,460 R/h for scheme-1 and 2.251 R/h for scheme-2. Explants excised from shoot apices being smaller than 1 mm were sterilized with immersing in 1 per cent sodium hypochlorite solution for about 10 minutes and cultured on agar medium which has been suggested by Earle and Langhans[8), 9)] composed of Murashige-Skoog's basal medium, 2 mg/l kinetin, 0.02 mg/l NAA, 3 per cent sucrose and 0.8 per cent agar. The calluses with plantlets were once subcultured on the similar medium, then the

plantlets with several leaves were transplanted to pots with sterilized soil in a green house. Finally, they were grown in the field and cut back one time before the flower buds formation in order to increase the nember of flowers. An experiment of the conventional method was also accompanied for a comparison, in which rooted cuttings were chronically exposed to gamma-ray from Cobalt 60 source for 44 days at gamma field of the National Institute of Agricutural Science, Ohmia. The exposure rates and total exposures are shown in table 2.

## Results and Discussion

### 1. Tissue culture

Calluses were formed in average 85 per cent of explants within one week after their inoculations and most of calluses continued to develop steadyly up to 1.5-2.0cm in their diameters during another two weeks, followed by beginning of redifferentiation. Although callus formation per cent varied in the case, there seemed to be no relationship with irradiation dose.

Beginning of rediferentiation was noticed clearly by the protrusion of greenish leafy shoots on the callus which, though not all, developed to form plantlets (Fig. 1 ). A patern of plantlet generation in the tissue culture and final yield of plants, in relation with irradiation were summarized in table 1. Number of plantlets per callus varied from 0 to 5, but average value in

Table 1. Plantlet generation in the tissue culture of shoot apices and their final survivals in relation with gamma irradiations.

| Experimental scheme | Total exposure KR | Calluses observed (A) | Calluses with plantlet | | Plantlets /callus | Plantlet height % of control | Plants to flowring (B) | Final survivals (A)/(B)×100 |
|---|---|---|---|---|---|---|---|---|
| | | | No. | % | | | | |
| Scheme-1 | 0 | 20 | 12 | 85.0 | 1.41 | 100 | 7 | 35.0 |
| | 1.0 | 19 | 10 | 84.2 | 1.60 | 104 | 10 | 52.6 |
| | 2.0 | 18 | 15 | 83.3 | 1.60 | 85* | 10 | 55.6 |
| | 3.5 | 24 | 14 | 83.3 | 1.21 | 45* | 13 | 54.2 |
| Scheme-2 | 0. | 14 | 10 | 71.4 | 1.40 | 100 | 7 | 50.0 |
| | 1.5 | 14 | 12 | 85.7 | 1.41 | 132 | 2 | 14.3 |
| | 3.0 | 14 | 11 | 78.6 | 1.18 | 117 | 5 | 35.7 |

* : Significant difference from the control (no irradiation ) at 5 % level

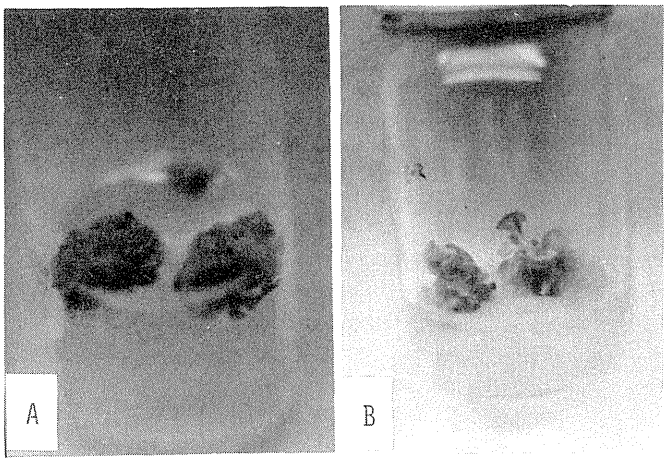


Fig. 1 Callus developed from shoot apices explant of chrysanthemum. A: Calluses about three weeks after inoculation. B: Calluses redifferentiating mutiple plantlets.

no irradiation was quite the same with what had been reported by Earle and Langhans.[8), 9)] On the whole, not much effects of the irradiation were found. However, when irradiated explants were brought into tissue culture as denoted for the Scheme-1, it was observed that the number of plantlets produced on a callus slightly increased at lower dose and decreased at higher dose, and growth of plantlets was inhibited depending on the given dose. While, when calluses were irradiated as denoted for the Scheme-2, only increasing of number of plantlets per callus and of plant height at lower dose were found. Shama Rao and Narayanaswamy[23)] have found the stimulative effect of irradiation in tissue culture of pigeon pea, in which low dose irradiation was rather essential for callus growth and redifferentiation. Some positive effects that have been found in the present experiment as increasing of plantlets per callus and height of plantlet at lower dose are possibly due to a stimulation.

Mabuchi and Kuwada[19)] cultured the meristem of chrysanthemum, and found a proprotional decreasing of the survivals to the given doses, However, it seemed to be difficult to find out any relationship between irradiation and survivals in the present experiment. A majority of young plants derived from tisse culture died due to the failure of water uptake and the suffering from microbial pests after their transplanting to the open air and resulted

42 per cent of final survivals which was much lower in comparison with 90 per cent reported by Earle and Langhans.[8),9)]

Iizuka et al and Takahashi et al[17),25)] have suggested avairability of the tublar floret culture in chrysanthemum. Iizuka et al[18)] have also described that various floral organs of cruciferous plants such as petal, filament, pistil and floral disc would be all good inoculum for in vitro culture expecting callus formation and organ differentiation. Excision of explants from shoot apices must be usually a time-consuming and laborious work. Thus, using any of floral organs as inoculum in stead of shoot apices may be a great help for further development of the experiment.

## 2. Flower color mutations

Only a change from original red to yellow has been found. The results are shown in table 2 for the conventional method and in table 3 for the method through tissue culture.

Table 2. Flower color mutations induced by conventional gamma irradiation

| Dose rate | Total dose | Materials observed | | Mutations in different sector size | | | | Frequency per | |
|---|---|---|---|---|---|---|---|---|---|
| (R/day) | (KR) | Plants | Heads | Whole head | Floret (s) | Streak | Total | plant | Head |
| 0 | 0 | 39 | 2,873 | 0 | 3 | 12 | 15 | 0.4 | 0.005 |
| 50 | 4.31 | 29 | 398 | 0 | 8 | 32 | 40 | 1.4 | 0.101 |
| 100 | 8.62 | 18 | 336 | 0 | 11 | 138 | 194 | 10.2 | 0.545 |

Table 3. Flower color mutations induced in the plants derived through tissue culture

| Experimental scheme | Dose (KR) | Materials observed | | Mutations in different sector size | | | | Frequency per | |
|---|---|---|---|---|---|---|---|---|---|
| | | Plants | Heads | Whole head | Floret (s) | Streak | Total | Plant | Head |
| No irradiation[1)] | 0 | 14 | 675 | 0 | 0 | 0 | 0 | 0 | 0 |
| Scheme-1 | 1.0 | 10 | 649 | 0 | 1 | 2 | 3 | 0.33 | 0.005 |
| | 2.0 | 10 | 512 | 0 | 0 | 6 | 6 | 0.66 | 0.012 |
| | 3.5 | 13 | 922 | 2 | 0 | 4 | 6 | 0.46 | 0.007 |
| Scheme-2 | 1.5 | 2 | 107 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 3.5 | 5 | 218 | 0 | 1 | 1 | 2 | 0.40 | 0.009 |

1) : No irradiation for scheme-1 and scheme-2 are combined.

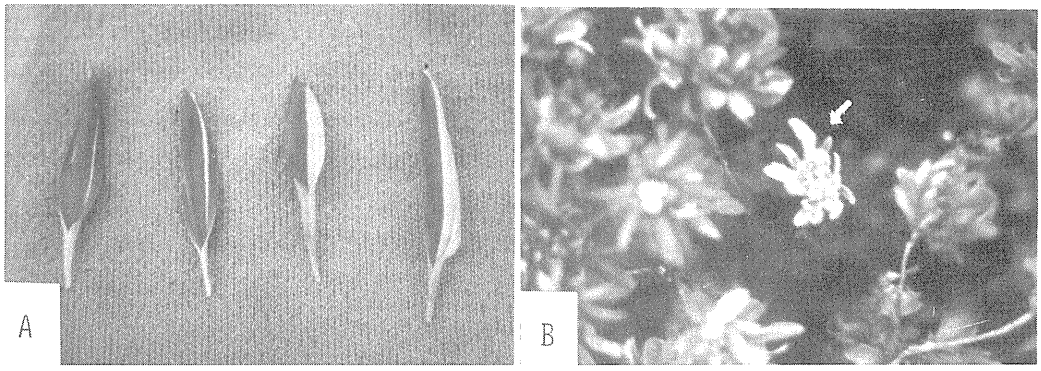


Fig. 2 Examples of yellow flower color mutations. A: Some small mutated sectors occured as a streak in a floret. B: A whole flower head change (Arrow).

The conventional method can be characterized by giving the higher mutation frequency and the smaller size of mutated sectors, where the frequency increaased exponentially with increasing of irradiation dose and a great number of mutations were induced, but of total 249 mutations, 227, a majority of them, were expressed as a streak within floret and no one covered a whole flower head. On the other hand, the method through tissue culture gave very low mutation frequency that estimated at about one twentieth to that of conventional method and being not responsible to the dose. However, of total 17 mutations, two were completly covered a whole flower head. It is very interesting that both cases have been observed in identical 3.5 KR of the experimental scheme-1 (Fig. 2).

The results seem to be supporting the idea mentioned previously. Higher mutation frequency, wider spectrum and larger size of mutated sector would be desirable factors in the inducing mutations for breeding. In the vegetatively propagated crops, however, it is quite reasonable of saying that how to get large mutated sector must be of particular importance, while mutation frequency may be not necessarily so. Thus, the tissue culture of mutagen treated materials may have a possibility providing a useful means for mutation breeding in the vegetatively propagated plants.

**Summary**

The present experiment carried out in order to see whether tissus culture of irradiated materials is avairable for enlarging mutated secter size or not.

Explants or calluses from vegetative shoot apices of chrysanthmum were cultured to redifferentiate the plantlets. The plantlets were further grown in the field to flowering.

There was no irradiation effect on the callus formation, but some effects were observed on the redifferentiation of plantlets and their growth including a stimulative effect at lower dose. The frequencies of flower color mutations and their sector size were observed in comparison with that of the conventional method. The method through tissue culture seems to be characterized resulting very low mutation frequency with larger size of mutated sectors in contrast to the result from the conventional method which gave high mutation frequency with smaller size of mutated sectors.

The result of the experiment is suggesting that dedifferentiation (callus formation) and redifferentiation (plantlet generation) process in tissue culture has a role for expanding mutated sector size.

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# ガンマー線照射によるキクの花色突然変異の誘発と組織培養による修飾

小野沢　芳　郎

突然変異セクターの大きさが，組織培養によって拡大するか否かをキクを用いて検討した。

あらかじめガンマー線で処理したキク（品種デラウエア）の茎頂端分裂組織を培養し，カルス形成を経て幼植物を再分化させた。また，無処理のものを培養してできたカルスにガンマー線を照射した後再び培養を続けて同様に幼植物を再分化させた。培養過程でのガンマー線照射の影響は著しくはなかったが，高線量区において再分化幼植物の生育抑制がみられ，低線量区では幼植物の発生率及び生育において，逆に刺激効果が認められた。

幼植物を圃場栽培して開花させ，判定が明瞭な黄色花突然変異についてその変異頻度ならびに変異セクターの大きさを調べた。一方，比較対照とするため，通常行なわれている分技系の緩照射による突然変異誘発実験も併行して行ない同様に調査した。その結果，慣行法では変異誘発頻度は線量が増すにつれて指数関数的に増大し，数多くの突然変異が得られたが，大形の変異セクターは皆無であった。一方組織培養を経過した場合は，変異誘発頻度は慣行法にくらべて著しく低下したが，1頭花を超える大きな変異セクターが2ケース含まれていた。

以上の結果から誘発原処理と組織培養とを組合せることにより大形の突然変異セクターが得られる可能性のあることが推定された。

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