Effects of dose and dose rate of gamma ray irradiation on mutation induction and nuclear DNA content in chrysanthemum

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We investigated the effect of total irradiation dose and dose rate on flower color mutation and nuclear DNA content as an index of radiation damage in chrysanthemum. *Chrysanthemum morifolium* cv. ‘Taihei’ plants grown by *in vitro* culture were gamma-irradiated with a total dose of 15, 30 and 60 Gy at a rate of 0.5, 1, 2 and 5 Gy/h. Leaf explants cut from the irradiated plants were tissue cultured, and the regeneration rates and frequency of flower color mutation were investigated. Nuclear DNA content was measured by flow cytometric analysis. The regeneration rate decreased with increase in the total dose and dose rate of irradiation. Mutation frequency did not differ significantly among dose rates, indicating that mutation frequency was independent of dose rate and was dependent mainly on total dose. Comparison of the average of the nuclear DNA content with each treatment revealed that it was influenced by both dose rate and total dose, and that the reduction in nuclear DNA content was less at low dose rates, even when total doses were high. It appears that same mutation frequencies were obtained without large reduction in nuclear DNA content by 0.5 Gy/h, when compared with 2 Gy/h. Consequently, we conclude that gamma ray irradiations of high total doses at low dose rates efficiently induce mutations with less radiation damage in chrysanthemum.

Key Words: chrysanthemum, dose, dose rate, gamma ray irradiation, mutation breeding, nuclear DNA content, radiation damage.

Introduction

Chrysanthemum is one of the most widely cultivated ornamental plants. Mutation is an important tool for breeding new cultivars, and many cultivars have been produced using spontaneous (sports) and induced mutations. Gamma rays and X-rays are widely used for mutation induction in chrysanthemum. However, in addition to mutations, radiation treatment induces radiation damage, such as chromosomal aberrations, in plants. Reduction in chromosome number is one such chromosomal aberration reported in chrysanthemum irradiated with gamma rays (Dowrick and El-Bayoumi 1966, Ichikawa et al. 1970) or X-rays (Dowrick and El-Bayoumi 1966). Furthermore, reduction in chromosome number is correlated with reduced diameter of inflorescence in chrysanthemum (Ichikawa et al. 1970). Therefore, reduction of chromosome number is an undesirable effect of radiation treatments aimed at obtaining mutants with improved desirable traits. Consequently, the radiation method with less radiation damage for chrysanthemum is desired.

Dose rate is one important factor of radiation treatment, and its effect has been investigated by evaluating various traits, including lethality (Broertijes 1968, Killion and Constantin 1971, Sripichtt et al. 1988), growth (Bottino et al. 1975, Killion and Constantin 1971, Killion et al. 1971, Yamashita 1964), and fertility (Killion and Constantin 1971, Yamashita 1964). Therefore, it is believed that the dose rate also influences radiation damage in chrysanthemum.

Dose rate also affects somatic mutation. It was reported that on irradiating at the same dose, higher dose rates were more effective in inducing mutations than lower dose rates in oats (Nishiyama et al. 1966) and in maize (Mabuchi and Matsumura 1964). Moreover, in saintpaulia (Broertijes 1968) and Tradescantia (Nauman et al. 1975), it was observed that the dose rate effect occurred at higher doses, while little dose rate effect was observed at lower doses. In chrysanthemum, Nagatomi et al. (2000) reported that flower color mutants were efficiently obtained with chronic gamma ray irradiation. However, no data is available regarding the
effect of total irradiation dose and dose rate on mutation induction in chrysanthemum.

Information on the effect of dose rate on radiation damage and mutation induction and the interaction between dose and dose rate is useful for radiation breeding in chrysanthemum. We investigated the effect of dose and dose rate on flower color mutation and nuclear DNA content as an index of radiation damage in chrysanthemum, using a combined method of gamma ray irradiation and tissue culture.

Materials and Methods

Irradiation treatment and tissue culture

Chrysanthemum plants (Chrysanthemum morifolium cv. ‘Taihei’) maintained on MS medium (Murashige and Skoog 1962) supplemented with 1% sucrose and 0.9% agar in 100-ml conical beakers were propagated vegetatively by herbaraceous cuttings. Plants with 7–10 leaves in the 100-ml conical beakers were irradiated with total doses of 15, 30 and 60 Gy of gamma rays at dose rates of 0.5, 1, 2 and 5 Gy/h in the gamma-room (Institute of Radiation Breeding, National Institute of Agrobiological Sciences, Hitachi-omiya, Japan). Leaf explants (10 × 5 mm) were cut from the irradiated plants and cultured in MS medium supplemented with 1 mg/l 6-benzylaminopurine (BA), 0.2 mg/l 1-naphthylacetic acid (NAA), 2% sucrose, and 0.9% agar in 100-ml conical beakers for callus induction. After 3 weeks, the explants were cultured in MS medium supplemented with 1 mg/l BA, 0.1 mg/l NAA, 2% sucrose, and 0.9% agar in 100-ml conical beakers for regeneration.

The regeneration rate was investigated using 150–300 leaf explants from 3–7 irradiated plants per radiation treatment. The experiments were replicated four times for each radiation treatment. The regeneration rates were determined as the ratio of the number of leaf explants developing a minimum of one regeneratd shoot to the number of investigated explants. Investigation of regeneration was carried out 6 weeks after transferring the explants to the regeneration medium.

Flower color mutation

To eliminate duplication of mutated shoots originating from a single mutated cell, only one shoot was taken from each leaf explant (callus). They were cultured on MS medium supplemented with 1% sucrose and 0.9% agar. The rooted shoots were habituated and transplanted to a field. Flower color mutation was investigated using 100–363 plants per radiation treatment. The mutation frequencies were determined as the ratio of the number of flower color mutants to the number of investigated plants. Chi-square analysis for independence was performed to determine significant differences in the mutation frequencies.

Flow cytometric analysis

Nuclear DNA content was measured by flow cytometric analysis. For each sample, about 0.5 cm² of leaf was chopped with a razor blade in 1 ml of a staining solution containing 10 mM Tris, 50 mM sodium citrate, 2 mM MgCl₂, 0.1% (v/v) Triton X-100, and 2 mg/l 4, 6-diamidino-2-phenylindole at pH 7.5. After filtration though a 30-μm nylon mesh, the filtrate was incubated on ice for 5 min, and analysed using flow cytometer (Partec PA, Partec, Münster, Germany). Leaves of the garden pea (Pisum sativum cv. Narikoma Sanjunichi) were used as an internal reference standard, and the nuclear DNA content of the sample was determined by comparing the peak position of nuclei of the garden pea with that of the sample. Each plant was measured twice using two leaves from each plant on two different days. A plant maintained by vegetative propagation but not irradiated was used as control, and was measured after approximately every 10 samples and at the beginning and end of each day. Relative DNA content was expressed as the ratio of the nuclear DNA content of the investigated plants divided by that of the control plants maintained by vegetative propagation; the data for both control and experimental plants were collected on the same day. Fifty regenerated plants at each total dose and dose rate were used for measurement. Hartley’s test and Mann–Whitney test were performed for comparison of the variance and the average of relative DNA content among each radiation treatment, respectively.

Results and Discussion

Callus formation occurred in all leaf explants derived from irradiated plants at every radiation as well as non-irradiation treatment. However, regeneration from callus decreased with increasing dose rate at the 3 radiation intensities tested (15, 30 and 60 Gy) (Fig. 1). The influence of dose rate on the regeneration rate differed with the dose, that is, the differences in the regeneration rate between dose rates were small at 15 and 60 Gy, whereas regeneration rate varied markedly with dose rate at 30 Gy. These results indicate that the regeneration rate can be controlled by altering both dose and dose rate.

The frequencies of flower color mutation increased significantly (p < 0.05 chi-square test) when the total dose increased from 15 Gy to 30 Gy in each dose rate, though similar differences were not observed when the dose increased from 30 to 60 Gy (Table 1). On the other hand, the mutation frequencies did not differ significantly among dose rates in each total dose; the mutation frequencies at all dose rates were similar when the total dose was 15 Gy. At the total dose of 30 Gy, mutation frequencies did not differ significantly among the dose rates according to chi-square tests for independence, although the mutation frequencies at 0.5 and 2 Gy/h were somewhat lower than that at 1 Gy/h. Similarly, at the total dose of 60 Gy, mutation frequencies did not differ significantly among the dose rates, although mutation frequencies at 0.5 and 2 Gy/h were somewhat higher than that at 1 Gy/h. These results show that mutation frequency was independent of dose rate and was mainly dependent on the total radiation dose. The frequency of spontaneous mutation
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was 0.6%, as estimated by examining 937 plants that originated from non-irradiated leaf explants.

More than 8 types of flower color mutants were obtained from the pink flower color of the donor cultivar ‘Taihei’ and the number of each type obtained with each treatment is shown in Table 2. The spectrum did not appear to differ among radiation treatments, but we were unable to perform statistical analysis to verify this hypothesis because the number of each type of flower color mutant was small.

The nuclear DNA content was affected by gamma ray irradiation (Fig. 2). The relative DNA content in plants regenerated from non-irradiated leaf ranged from 0.97 to 1.03, while in the plants regenerated from the irradiated leaf, there were plants which had less than 97% of the nuclear DNA content as compared to the control plant. The greatest decline was 10% in some of the plants that received 60 Gy at the dose rate of 2 Gy/h. Variance in the nuclear DNA content increased significantly (p < 0.05 Hartley’s test) among the doses at dose rates of 0.5 and 2 Gy/h.

Comparison of the averages of relative DNA content revealed significant decreases in the nuclear DNA content (p < 0.01 Mann–Whitney test) in every radiation treatment. For treatments at the dose rates of 1 or 2 Gy/h, the nuclear DNA content decreased significantly (p < 0.05 Mann–Whitney test) as the dose increased from 15 Gy to 30 Gy; similar trend was observed as the dose increased from 30 Gy to 60 Gy. In contrast, at the dose rate of 0.5 Gy/h, the nuclear DNA content was significantly less (p < 0.05 Mann–Whitney test) when irradiated with 30 Gy than that with 15 Gy, but was approximately equal to that with 60 Gy. In addition, at a total dose of 60 Gy, the nuclear DNA content was significantly higher (p < 0.05 Mann–Whitney test) when the dose rate was 0.5 Gy/h than that when it was 1 Gy/h; these differences were not observed when the total dose was 15 and 30 Gy. These results indicated that the reduction in nuclear DNA content was small at low dose rates, even when the total doses were high.

The relationship between reduction in relative nuclear DNA content and mutation frequency is shown in Figure 3. During irradiation at the dose rate of 2 Gy/h, there was a

### Table 1. Effects of total dose and dose rate of gamma ray irradiation on mutation induction

<table>
<thead>
<tr>
<th>Total dose (Gy)</th>
<th>Dose rate (Gy/h)</th>
<th>Frequency of flower color mutation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0.5</td>
<td>4.6 (15/325)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>5.0 (18/363)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.3 (9/210)</td>
</tr>
<tr>
<td>30</td>
<td>0.5</td>
<td>8.9 (22/248)</td>
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<tr>
<td></td>
<td>1</td>
<td>12.1 (29/240)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9.6 (26/272)</td>
</tr>
<tr>
<td>60</td>
<td>0.5</td>
<td>12.2 (40/326)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>8.4 (22/262)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12.0 (12/100)</td>
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</tbody>
</table>

Number of flower color mutants/number of plants evaluated are shown in parentheses. The frequency of spontaneous mutation was 0.6%, as estimated using 937 plants that originated from non-irradiated leaf explants.

### Table 2. Number of flower color mutants resulting from treatment with different total dose and dose rate of gamma rays

<table>
<thead>
<tr>
<th>Total dose (Gy)</th>
<th>Dose rate (Gy/h)</th>
<th>No. of plant investigated</th>
<th>Pale pink</th>
<th>Rather pale</th>
<th>Deep pink</th>
<th>White</th>
<th>Pinkish white</th>
<th>Pale yellow</th>
<th>Orange</th>
<th>Deep orange</th>
<th>Other</th>
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<tr>
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<td>325</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
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</table>

The flower color of original cultivar ‘Taihei’ is pink.
significant correlation between the reduction in nuclear DNA content and mutation frequency. Similar relationship was observed at the dose rate 0.5 Gy/h. The same mutation frequencies were obtained at 0.5 Gy/h without a large reduction in nuclear DNA content compared to that at 2 Gy/h. Although the correlation of the dose rate 1 Gy/h with reduction in nuclear DNA content and mutation frequency is not clear, its effect seems to be intermediate between that of 0.5 Gy/h and 2 Gy/h. These results suggest that gamma ray irradiation with low dose rate induces mutation with small reduction in nuclear DNA content in chrysanthemum.

It has been found that there is a significant correlation of diameter of an inflorescence and the length and width of a leaf to the nuclear DNA content, as well as chromosome number, in chrysanthemum cv. ‘Taihei’ used in this study (Yamaguchi, unpublished data). Similarly, a significant correlation has been reported between the nuclear DNA content and the length and diameter of stem in sugar cane (Degi et al. 2001). Thus, the decrease in the nuclear DNA content by gamma ray irradiation is undesirable to obtain useful mutants for commercial varieties unless obtaining smaller flowers is the objective.

It was reported that the dose rate affected mutation

![Figure 2](image-url)  
**Fig. 2.** Effects of total dose and dose rate of gamma ray irradiation on nuclear DNA content. Relative DNA content is expressed as the ratio of the nuclear DNA content of investigated plants divided by that of control plants maintained by vegetative propagation. Nuclear DNA content was measured in 50 regenerated plants per treatment.

![Figure 3](image-url)  
**Fig. 3.** Relationship between reduction of nuclear DNA content and mutation induction. Relative DNA content is expressed as the ratio of the nuclear DNA content of investigated plants divided by that of control plants maintained by vegetative propagation. Nuclear DNA content was measured in 50 regenerated plants per treatment.
frequency in saintpaulia (Broertjes 1968) and oats (Nishiyama et al. 1966). However, these results are not in agreement with those obtained in our study. The effect of dose rate on mutation frequency might differ among species. Therefore, it seems that the effects of specific doses and dose rates need to be evaluated for each type of plant material.

Our results show that gamma ray irradiation at lower dose rates leads to less radiation damage in plants during mutant induction. This indicated that the irradiation at lower dose rate is useful especially for radiation breeding of vegetatively propagated crops because the obtained mutants would be directly used as new cultivars. It has been hypothesized that chronic radiation with a gamma field is useful for mutation breeding of vegetatively propagated crops and some mutant varieties have been produced. In chronic radiation with a gamma field, dose rates are lower, and the exposure period is generally longer; therefore, the total dose is higher than that obtained in the present study. However, we consider that the results of the present study are applicable to chronic radiation in a gamma field and support the usefulness of chronic radiation.

**Literature Cited**


