Effects of Gamma-ray Irradiation in Plant Morphology of Interspecific Hybrids between *Torenia fournieri* and *Torenia baillonii*

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ABSTRACT

Gamma (γ) -ray irradiation is a conventional technique to produce mutants in plant breeding. To investigate the effect of γ rays to produce flower color mutants in the genus *Torenia* (Linderniaceae), interspecific hybrids were exposed to acute (Cs-137) or chronic (Co-60) γ-ray irradiation combined with a detached-leaf-technique. Application of the detached-leaf technique provided irradiated plants that could be used for selection for 90 d. Although the different striking procedures produced different survival rates, the mutation efficiencies of the acute and chronic levels of irradiation for flower color showed no differences. The mutation rate was 1% for explants. The production of mutants with yellow-colored petals suggested disruption of anthocyanin synthesis.

Keywords: adventitious bud induction, γ-ray irradiation, *Torenia*, mutation

INTRODUCTION

Torenia, or wishbone flower, is a dicotylodeon in the family Linderniaceae that is native to Southeast Asia, Africa and Madagascar. Most species are found in the tropics and subtropics, in rocky, humid, mountainous areas at elevations of 300–1,200 m above sea level (Yamazaki, 1985). *Torenia* hybrids have been used as experimental plants for the study of phenotypic floral color characters in ion beam mutation studies (Miyazaki *et al*., 2006) because their relatively small genome of 2*n*=18, and 171 Mbp (Kikuchi *et al*., 2007) makes it easy to induce mutations.

The use of γ-ray radiation to induce mutations is a method that has been applied in plant breeding to increase genetic variations (Brunner, 1995). In ornamental horticulture, radiation has been used to alter flower color, flower form, type of inflorescence, fertility or leaf color/variegation depending on the objectives of the breeder. Many instances have been reported of the use of radiation-induced mutations to produce novel flower colors, such as the use of ion beams to change the color, form and number of petals in chrysanthemums (Matsumura *et al*., 2010). X-rays were used to create begonias with different flower colors and forms as well as variegated leaves (Roest *et al*., 1981).

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The detached-leaf technique is often used in radiation breeding studies in order to avoid the occurrence of chimera. The adventitious bud technique has been shown to produce 95% solid mutations as opposed to chimera (Van Harten *et al.*, 1981). The detached-leaf technique makes it easier to isolate the mutated sectors in a plant and reduces the time required for the selection of mutants. The technique makes use of different propagation methods to obtain solid mutants. New plants are derived from a single epidermal cell at the base of the petiole. Adventitious buds that arise from leaves that have been exposed to radiation may be solid mutants or may not have mutated (Roest *et al.*, 1981), but the detached leaf makes it more likely that the mutated cell will grow into a new plant, forming a solid mutant (Broertjes and Van Harten, 1978). The detached-leaf technique has been used together with radiation breeding in many genera, including *Kalanchoe*, *Saintpaulia*, *Achimenes*, *Streptocarpus* (Broertjes and Van Harten, 1978), *Begonia* (Roest *et al.*, 1981), *Torenia* (Takeuchi *et al.*, 1985), *Chrysantheumum* (Broertjes *et al.*, 1976) and in sweet potato (Van Harten *et al.*, 1981).

In *Torenia*, Miyazaki *et al.* (2006) demonstrated that heavy-ion beams provide production of flower color mutation at an average mutation rate of 1.06% for acclimated plants. The mutation rate of ion beams combined with transgenic plants may increase, and a novel type of flower form may appear (Sasaki *et al.*, 2008). However, compared with the wide variety of mutagens, there are no reports that have studied the effect of more conventional low-linear Energy Transfer (LET) radiation in *Torenia*. For the *Torenia* breeding program, information about the efficiency of γ-ray irradiation is necessary.

In the present study, *Torenia* hybrids were sterile and could not be improved further; thus the investigation involved the effects of acute gamma irradiation combined with the detached-leaf technique and chronic gamma irradiation combined with the detached-leaf technique to induce mutation in the *Torenia* interspecific hybrid. The characters of flower color mutants obtained in this study were also considered.

MATERIALS AND METHODS

Plant materials

The interspecific hybrid between *Torenia fournieri* as the female and *T. baillonii* was used in this study.

The M0 and M1 plants were maintained in a greenhouse at a day temperature of 33–35 °C and 60–65% relative humidity and a night temperature of 29–33 °C and 65–70% relative humidity.

γ-ray irradiation

Acute and chronic gamma irradiation can induce mutations in the flower color and other characteristics. For example, acute and chronic gamma irradiation induced mutations in the flower color of *Portulaca* spp. (Tangsombatvichit, 2008); thus both acute and chronic irradiation was used to induce mutation in interspecific hybrids of *Torenia*.

For acute γ-ray irradiation, detached leaves were irradiated at a dose of 0, 50, 100, 150, and 200 gray using a gamma irradiator Mark I machine (J.L. Shepherd & Associates, San Fernando, CA) with a Cs-137 source and then planted in soil.

For chronic irradiation, detached leaves were planted in soil for 7 d until rooting commenced and then these leaves were placed at a distance of 1.5 m from a Co-60 source in a gamma radiation room. The exposure doses were 0, 50, 70, 90, and 110 gray.

In total, 100 leaves per exposure dose for the acute and chronic levels were irradiated. Regenerated shoots appeared by day 21. The surviving plants had matured 30 d after irradiation.

The experiments were conducted at the

Horticulture Department Test Field, Kasetsart University School of Agriculture and the Kasetsart University Gamma Radiation and Nuclear Technology Research Service Center at the Bangkhen campus, Bangkok. The research was conducted from December 2009 to December 2010.

Phenotype evaluation and cytological analysis

The plant height, plant spread and flower size of irradiated plant 30 d after exposure were measured with a ruler.

At least 10 mitotic chromosome samples of root tip cells were prepared by the standard squash method as described by Kikuchi *et al.* (2008). The slides were mounted with Vecta Shield (Vector) containing 5 mg/mL 4', 6-diamidino-2-phenylindole (DAPI) for staining of chromosomes. A Leica DM RXA2 phase contrast light microscope was used for observation of the chromosomes.

Statistical analysis

Statistical differences were tested using Duncan's new multiple range test at the $P < 0.05$ level.

RESULTS AND DISCUSSION

Effects of γ-ray irradiation on plant growth

Torenia plants are known to be easily propagated by tissue culture *in vitro* (Takeuchi *et al.*, 1985). In the present study, leaves were placed into soil and regenerated shoots were produced from the leaf vein at the basal position (Figure 1). This adventitious bud induction technique omits several *in vitro* works and reduces the regeneration time and acclimatization time in the production of M_1 plants. After γ-ray irradiation, the regenerated shoots were grown for 30 d. At 30 d, the survival rate was recorded as 100, 95, 24, 1 and 0% for the leaves exposed to 0, 50, 100, 150 and 200 gray of radiation, respectively (Table 1). Thus, the 200 gray dose appeared to be fatal. After the regenerated plants had grown for another 45 d, the treatment group that was exposed to 150 gray of acute radiation also had a survival rate of 0%. The half lethal dose (LD_{50}) of acute γ-rays was 83 gray. The effect of γ-rays on plant survival was gradual depending on the exposure dose, irrespective of the irradiation method. A similar gradual reduction in the survival rate has been observed in many species, for example, X-ray irradiated wheat (Kikuchi *et al.*, 2009), though this

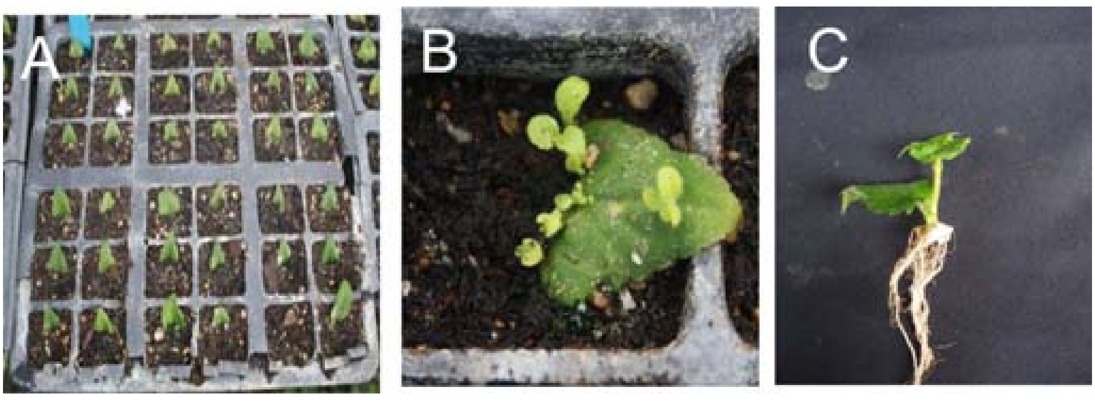

Figure 1 Detached-leaf technique: (A) Detached-leaf at day 1; (B) Regenerated shoots from leaf after irradiation with g rays 30 d after exposure; and (C) Regenerated shoot from the base of the petiole.

feature of low-LET irradiation is not linked to any beneficial use. Table 2 shows the plant height, crown spread, number of branches and number and size of flowers in regenerated plants from irradiated leaves. The crown spread was measured as the longest plant radius. The mean height and crown spread of the 100 gray group were significantly less than those of the control group and the 50 gray group and there was no statistically significant difference in the number of branches

Table 1 Survival rate, number of leaves, number of floral color mutants and mutation rate for different irradiations levels.

Irradiation dose (gray)	Number of leaves	Number of survival leaves	Survival rate (%)	Number of floral color mutant	Number of tetraploid mutants	Mutation rate (%)
Acute irradiation						
0	100	100	100	0	0	0
50	100	95	95	0	1	0.01
100	100	24	24	0	0	0
150	100	1[1)]	1[1)]	-	-	-
200	100	0	0	-	-	-
Chronic irradiation						
0	100	100	100	0	0	0
50	100	92	92	0	0	0
70	100	87	87	0	0	0
90	100	73	73	1	0	0.01
110	100	62	62	0	0	0

[1)] Plant was dead 45 d after irradiation.

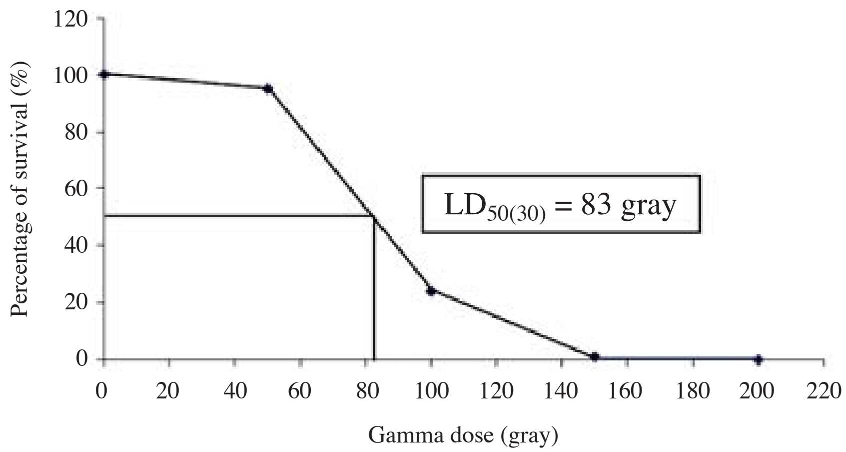

Figure 2 Percentage of surviving hybrid *Torenia* plants 90 d after exposure to varying doses of acute gamma radiation compared to the control, showing the LD50 level.

between the control and the treatment group that received 50 gray, but there was a statistically significant difference in the number of branches and the number of flowers between the treatment group that received 100 gray of radiation compared to the groups that received 0 and 50 gray of radiation. Higher radiation doses led to less branching.

In the chronic γ-ray treatment, 30 d after the hybrid *Torenia* leaves were exposed to 0, 50, 70, 90, and 110 gray of chronic gamma radiation. With chronic radiation, any radiation damage to the cells was less severe and the plants were able to repair themselves, resulting in lower fatality levels compared to acute gamma radiation. The survival rates of the leaves exposed to 0, 50, 70, 90 and 110 gray of chronic gamma radiation were 100, 92, 87, 73 and 62%, respectively. No statistically significant differences were observed in crown spread, number of branches and number and size of flowers at exposure levels of 0, 50, 70 and 90 gray, but plant height for the plants that were subjected to 50, 70, 90 and 110 gray were significantly different from the control (0 gray), especially the *Torenia* plants subjected to 110 gray that had the least height when compared to plants subjected to 0, 50, 70 and 90 gray.

Isolation of color mutation

Allotetraploid *Torenia* plants (Figure 3C) were found in the acute radiation dose of 50 gray, with thicker leaves, stems, petals and greater flower size than the control. Cytological analysis confirmed that the allotetraploid mutant had chromosome number 2n=34 (Figure 3E), whereas the normal chromosome number for *Torenia* hybrids was 2n=17 (Figure 3D). Allotetraploid mutation may have occurred at the nucleotide level, as ion beams were thought to be more effective at producing chromosome rearrangement in wheat (Kikuchi *et al.*, 2008).

Table 2 Mean plant height, spread, number of branches and number of flowers and flower size 90 d after exposure to acute and chronic gamma radiation at different doses.

Irradiation dose (gray)	Number of plants	Plant height (cm)	Crown spread (cm)	Number of branches	Number of flowers	Flower size (cm)
Acute irradiation						
0	100	10.8^{b}	15.8^{b}	13.8^{a}	16.00^{a}	2.00
50	95	12.7^{a}	20.7^{a}	14.3^{a}	16.00^{a}	2.00
100	24	5.90^{c}	8.50^{c}	5.90^{b}	3.00^{b}	2.00
F-test		*	*	*	*	ns
Chronic irradiation						
0	100	15.5^{a}	22.00	13.50	12.00	2.00
50	92	13.5^{c}	23.00	13.00	12.00	2.00
70	87	14.5^{b}	22.50	13.00	10.50	2.00
90	73	14.5^{b}	23.50	14.00	12.00	2.00
110	62	10.5^{abc}	23.50	12.00	9.00	2.00
F-test		*	ns	ns	ns	ns

Note: In each column, values with different superscript letters are statistically significantly different using Duncan's new multiple range test at the $P < 0.05$ level.
* = Means are statistically significantly different at the 95% confidence level.

One mutant with a yellow petal color was produced with 90 gray of chronic γ-ray irradiation (Table 2; Figures 3A and 3B), whereas the original color was a dull yellow on the top and bottom, and dark purple on the sides. Plants subjected to no irradiation (0 gray) did not show any mutation. The mutation rates seemed to be significantly lower than those in other ornamental plants, for example, 4.3–12.2% in chrysanthemum (Yamaguchi *et al.*, 2008). Ion beam irradiation also

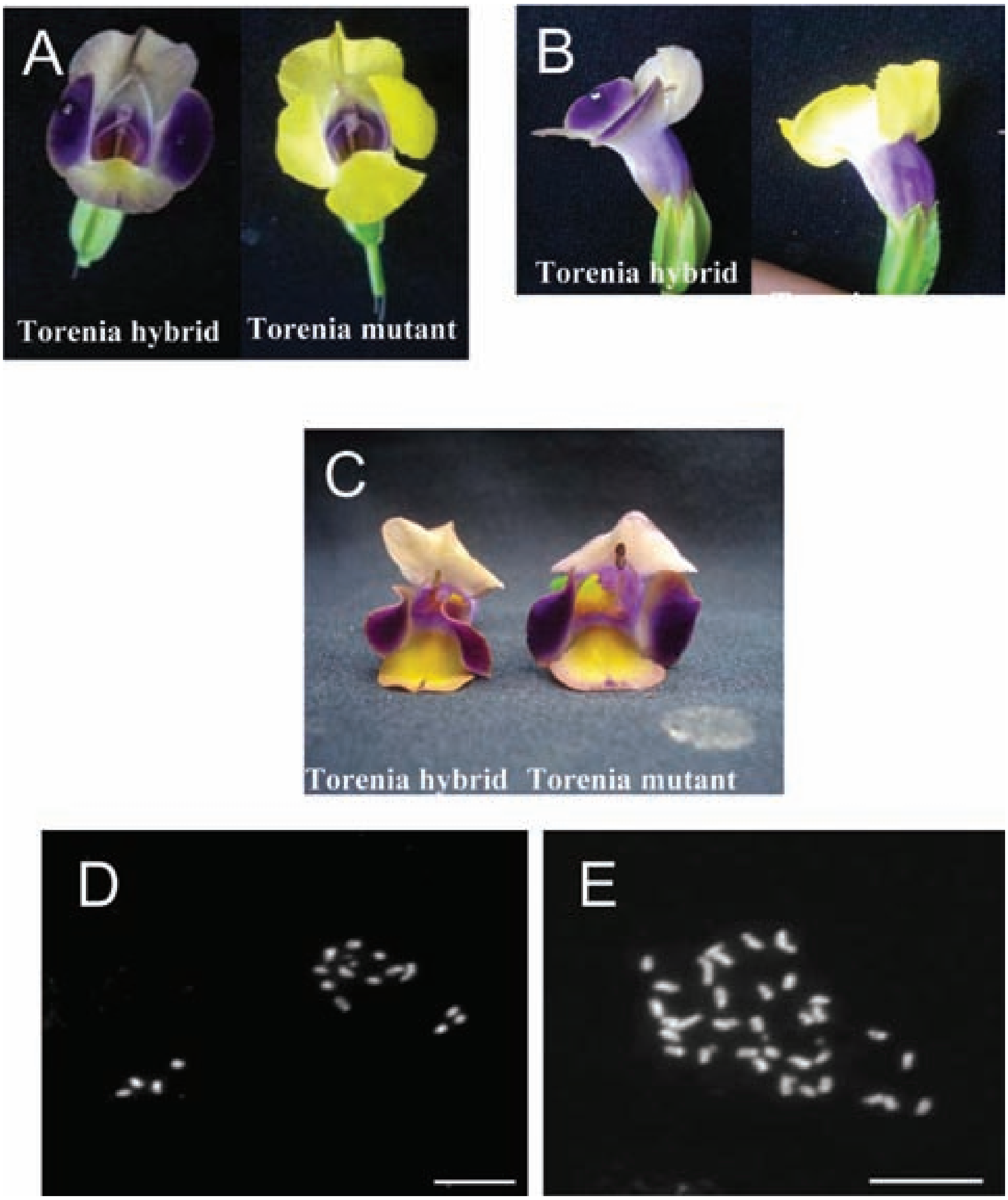

Figure 3 (A) Top side view; and (B) Lateral side view of yellow mutant of chronic 90 gray; (C) Tetraploid plant mutant after acute irradiation at 50 gray; (D) Normal chromosome number of *Torenia* hybrid; and (E) Chromosome number of tetraploid *Torenia* mutant. (Scale bars = 10 μm.)

showed similar frequency levels, that is, 0.61–1.89% per acclimated plant (Miyazaki *et al*., 2006). This fact indicates that *Torenia* tends to resist irradiation by γ-ray and ion beams, although the factor or mechanism is totally unknown. The changes in the petal color in the mutants are probably due to mutation in several dominant genes that control anthocyanin synthesis. Suzuki *et al*. (2000) produced yellow flower plants from transgenic lines of *Torenia hybrida* (*T. fourenieri* × *T. concolor*) by cosuppression of chalcone synthase (CHS) or dihydroflavonol 4-reductase (DFR) genes, involving anthocyanin biosynthesis.

The mutants obtained in the present study did not show other phenotypic changes. These mutants could maintain the character of the mutation by vegetative cutting without any reverse mutation back to the original color.

CONCLUSION

Irradiation levels of 50-100 Gy could be used as a valuable parameter in the production of the *Torenia* flower color mutants by gamma-ray radiation because these levels produced inhibition of plant growth. However, the mutation rate is limited, suggesting a high tolerance to a wide range of radiation levels in *Torenia*. To produce genetically modified *Torenia* plants efficiently, a combination of radiation and transformation described by Sasaki *et al*. (2008) or other new technique is necessary.

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