



## Radiosensitivity of *In Vitro* Cultured *Torenia fournieri* Lind. from Thailand by $\gamma$ -ray Irradiation

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### ARTICLE INFO

#### Article history:

Received 01 April 2015  
Received in revised form  
15 May 2015  
Accepted 20 May 2015  
Available online  
21 May 2015

#### Keywords:

Axillary bud;  
Gamma irradiation;  
Tissue culture;  
Mutation breeding;  
Ornamental breeding.

### ABSTRACT

Gamma ray irradiation is a conventional technique used to produce mutants in plant breeding. The aim in this research was to develop new cultivars of *Torenia* (Linderniaceae) for the ornamental market. Leaves of *Torenia fournieri* Lind. were exposed to acute (Cs-137) gamma ray irradiation at 0, 20, 40, 60, 80, 100 and 120 grays. After culturing, the number of plantlets, plant height and root length of surviving plants was measured 60 d after irradiation. The results showed that the LD<sub>50</sub> was 63.65 grays in diploid plants and 72.00 grays in polyploid plants. Red colored leaves were observed in some plants after acute gamma irradiation treatment, for example in the 20-gray treatment group for diploid *Torenia* and in the 80-gray treatment group for polyploid *Torenia*. The plants with red leaves were selected for development in the future.

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## 1. Introduction

*Torenia*, or wish bone flower, is a dicotyledon in the family Linderniaceae that is native to Southeast Asia, Africa and Madagascar. Most species are found in the tropics and subtropics, in humid, rocky, mountainous areas at elevations of 300-1200 m above sea level Yamazaki (1985) reported a total of 50 species, 20 species from Cambodia, Laos, Vietnam, and 19 species from Thailand (Yamazaki, 1985) Other reports indicate 40 species of *Torenia* (Fisher, 2004; Spencer, 2006).

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Micropropagation has been proven to be an extremely useful technique for clonal propagation of many species, especially ornamental plants (Edwards, 2006). Propagation via shoot regeneration in tissue culture is useful for rapid and large-scale multiplication.

Mutation breeding has successfully been used over many decades to create new varieties of ornamental plants in many genera including *Dianthus*, *Chrysanthemum*, *Dahlia* and *Rosa* (Sigurbjornsson, 1974). A white variety of *Torenia* was reported as a result of mutation breeding using gamma radiation (Brand, 1989). Researchers in Japan reported a wide variety of flower color mutations after exposing *Torenia* varieties to heavy ion beam radiation (Miyazaki, 2006; Sasiki, 2008). Acute gamma irradiation can induce mutations in flower color and other characteristics. For example, acute gamma irradiation induced useful mutations in *Dahlia* spp., *Chrysanthemum* spp., *Rosa* spp., *Dianthus* spp., and *Rhododendron* spp. (Sigurbjornsson, 1974). Thus acute irradiation was used to induce mutation in *Torenia fournieri* Lind. in this research.

*Torenia fournieri* Lind., which is powdery mildew resistant, was selected for further improvement. Because it could not easily be improved by conventional breeding, thus the investigation focused on the effects of acute gamma irradiation combined with tissue culture technique to induce mutation in *Torenia fournieri* Lind. from Thailand.

## 2. Material and Methods

### 2.1 Plant material

This study focused on both diploid and polyploid lines of purple-flowered native Thai *Torenia* (*Torenia fournieri* Lind.) with a semi-recumbent, semi-erect habit. The 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.

### 2.2 Establishment of Aseptic Explants

Axillary buds of *Torenia fournieri* were used as explants. They were first surface cleaned by washing with Tepol® detergent (Jala, 2014), rinsed with tap water and dipped in 70 % alcohol for 1 minute. Next, the explants were surface sterilized by soaking in 20 % (v/v) Clorox® for 10 minutes, followed by 5 % (v/v) Clorox® for 10 minutes, then rinsed 3 times with sterile distilled water for 5 minutes each time. Explants were cultured on modified solid MS medium (Murashige, 1962) supplemented with BA 0.25 mg/l, activated charcoal and 3% sucrose. Cultures were incubated at 25±2 °C under a 16-hour photoperiod with illumination provided at 60 µmolm<sup>-2</sup>sec<sup>-1</sup>(TLD 18 w/18 lm Phillips, Holland). The leaves obtained after 8 weeks of culture were then used as the source of explants for the experiments.

### 2.3 γ-ray irradiation

For the acute γ-ray irradiation test, leaves were cultured on modified solid MS medium

(Murashige and Skoog, 1962) supplemented with BA at 0.25 mg/l, and 3% sucrose (in petri-dishes), and exposed to gamma ray irradiation at doses of 0, 20, 40, 60, 80, 100 and 120 grays using a gamma irradiator Mark I machine (J.L. Shepherd & Associates, San Fernando, CA) with a Cs-137 source, and then planted in new medium after irradiation.

One hundred leaves per replication were irradiated at each exposure dose, with 3 replications. Shoots were regenerated by culturing irradiated leaves on MS medium supplemented BA 0.25 mg/l, activated charcoal and 3% sucrose. The surviving plants, number of plants regenerated, plant height and length of root were measured 60 d after irradiation. The LD<sub>50</sub> was calculated based on the survival rate after 60 days.

The experiments were conducted at the Horticulture Department Test Field, Kasetsart University, School of Agriculture and the Kasetsart University Gamma Irradiation and Nuclear Technology Research Service Center at the Bangkhen Campus, Bangkok.

## 2.4 Statistical Analysis

Statistical differences were tested using Duncan's new multiple range test at the  $p < 0.01$  level.

## 3. Resultes and Discussion

### 3.1 Effect of Gamma Irradiation on Plant Growth

*Torenia* spp. are known to be easily propagated in tissue culture (Takeuchi, 1985) and 60 d after gamma irradiation, new shoots were regenerated at the surface of leaves cultured on MS medium supplemented BA 0.25 mg/l, activated charcoal and 3 % sucrose. In this study, a comparison was made between irradiation of diploid and polyploid *T. fournieri*. For diploid *T. fournieri*, the survival rate was recorded as 100.00, 86.67, 70.00, 56.67, 36.67, 20.00 and 1.11% for the leaves exposed to 0, 20, 40, 60, 80, 100 and 120 grays of irradiation, respectively (data not shown). The half lethal dose (LD<sub>50</sub>) was 63.65 grays (Figure 1). The effect of gamma rays on plant survival depends on the exposure dose, irrespective of the irradiation method. A similar reduction in the survival rate has been observed in many species, for example,  $\gamma$ -ray irradiation in *Torenia hybrida* (Sawangmee, 2011) and X-ray irradiation of wheat (Kikuchi, 2009). Table 1 shows the average number of shoots, plant height and root length 60 d after acute gamma irradiation. The plant height and root length were reduced with increasing doses of gamma ray irradiation (Figure 3). A similar result was observed in leaf explants after treatment with gamma rays in *Torenia fournieri* (Jala, 2011). For polyploid *Torenia fournieri*, the survival rate was recorded as 100.00, 83.33, 73.33, 60.00, 47.78, 33.33 and 11.11 % for the leaves exposed to 0,

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20, 40, 60, 80, 100 and 120 grays of irradiation, respectively (data not shown). The half lethal dose (LD<sub>50</sub>) was 72.00 grays (Figure 2). Table 2 shows the average of number of shoots, plant height and root length 60 d after treatment with gamma irradiation. The plant height and root length were reduced with increasing radiation dose (Figure 4). These results are consistent with those reported for *Curcuma alismatifolia* (Thohirah, 2009). The leaf color 60 d after treatment with acute gamma irradiation changed to red in some treatment groups, i.e. the 20 gray group for diploid *Torenia fournieri* (Figure 5B) and the 80 gray group for polyploid *T. fournieri* (Figure 5D). A similar result was observed in African violet, where leaf and flower color changed due to radiation treatment (Wongpiyasatid, 2007). Research comparing a radioresistant with a radiosensitive cultivar of *Cicer arietinum* L. revealed differences in the number of chromosome aberrations at the same dose. The 2 cultivars had already been shown by growth inhibition studies to be respectively sensitive and resistant to ionizing radiation. Chromosomal damage at the same doses, over a range from 20 to 150 kR, was determined by scoring percentages of anaphases with bridges at the first root-tip anaphase from dormancy and at anaphase I of meiosis. Mitotic delay with dose was also determined. The results indicated that more chromosomal damage at the same dose was produced in the cultivar which showed more growth-inhibition, lower survival and more dose-delay at mitosis. It could not be determined that a purely allelic difference accounted for sensitivity and resistance (Ahmad, 1981).

**Table 1** The average of number of shoots, plant height and root length of diploid *Torenia fournieri* Lind. 60 d after treatment with gamma irradiation.

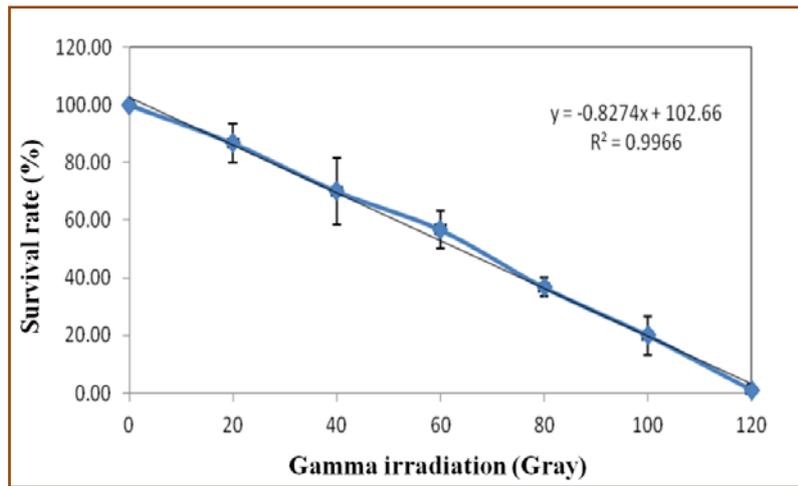
Dose (Gy)	Number of plants	Plant height (cm)	Root length (cm)
0	16.10c ± 3.87	5.82a ± 0.54	9.40a ± 0.87
20	16.20c ± 0.91	5.30b ± 0.58	6.75b ± 0.67
40	17.50c ± 1.64	4.30c ± 0.53	3.85c ± 0.78
60	37.90a ± 2.72	3.00d ± 0.52	0.40d ± 0.39
80	29.60b ± 1.89	0.70e ± 0.25	0.00d ± 0.00
100	12.90d ± 2.42	0.42ef ± 0.12	0.00d ± 0.00
120	0.10e ± 0.31	0.02f ± 0.06	0.00d ± 0.00
F-test	**	**	**
% C.V.	12.10	15.40	17.37

\*\*Means within the same column followed by different superscripts are significantly different using DMRT,  $p < 0.01$

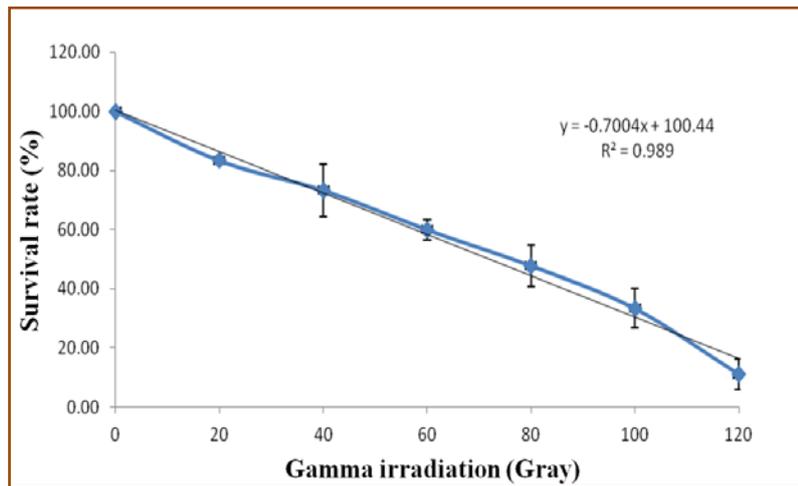
**Table 2** The average of number of shoots, plant height and root length in polyploid *Torenia fournieri* Lind. 60 d after treatment with gamma irradiation.

Dose (Gy)	Number of plants	Plant height (cm)	Root length (cm)
0	5.10b ± 0.73	5.60a ± 0.56	8.05a ± 0.76
20	5.40b ± 0.69	4.75b ± 0.58	5.25b ± 0.48
40	13.00a ± 1.82	3.20c ± 0.53	3.75c ± 0.42
60	14.40a ± 2.27	2.90c ± 0.45	3.70c ± 0.42
80	14.20a ± 1.75	2.00d ± 0.26	3.05d ± 0.92
100	5.40b ± 0.84	0.95e ± 0.38	0.15e ± 0.24
120	0.40c ± 0.51	0.20f ± 0.25	0.00e ± 0.00
F-test	**	**	**
% C.V.	16.83	18.81	15.97

\*\*Means within the same column followed by different superscripts are significantly different using DMRT,  $p < 0.01$



**Figure 1** Survival rate (%) of diploid *Torenia fournieri* Lind. 60 d after exposure to varying doses of acute gamma radiation compared to the control, showing the LD<sub>50</sub> level. (LD<sub>50</sub> = 63.65 Gy)



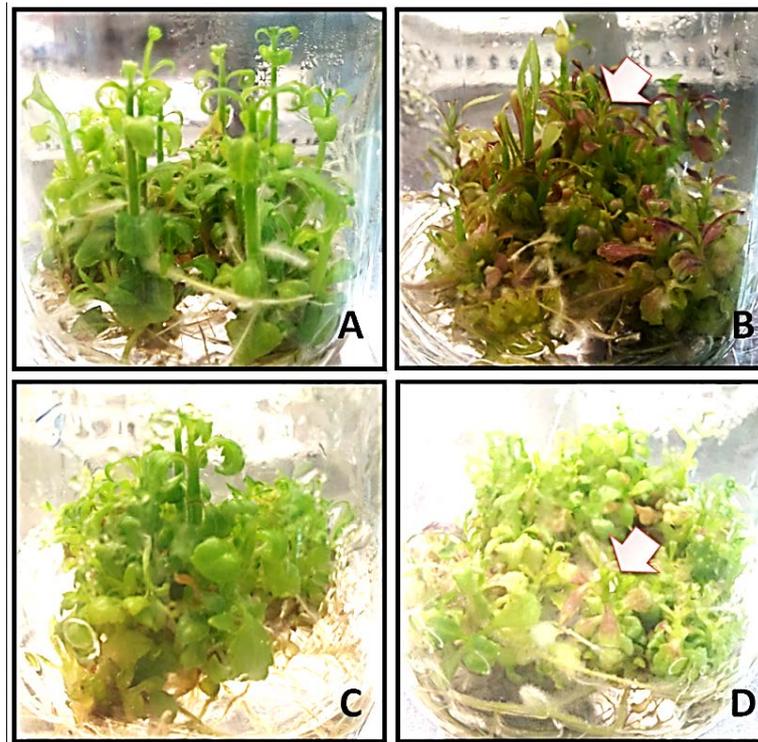
**Figure 2** Survival rate (%) of polyploid *Torenia fournieri* Lind. 60 d after exposure to varying doses of acute gamma radiation compared to the control, showing the LD<sub>50</sub> level. (LD<sub>50</sub> = 72.00 Gy).



**Figure 3** Morphology of diploid *Torenia fournieri* Lind. 60 d after exposure to varying doses of acute gamma radiation compared to the control (0, 20, 40, 60, 80, 100 and 120 Gy from left to right, respectively)



**Figure 4** Morphology of polyploid *Torenia fournieri* Lind. 60 d after exposure to varying doses of acute gamma radiation compared to the control (0, 20, 40, 60, 80, 100 and 120 Gy from left to right, respectively).



**Figure 5:** Red color of leaves in *Torenia fournieri* 60 d after treatment with acute gamma irradiation

- (A) Diploid control.
- (B) Diploid treated with 20 Gy of acute gamma irradiation.
- (C) Polyploid control.
- (D) Polyploid *T. fournieri* Lind. treated with 80 Gy of acute gamma irradiation.

#### 4. Conclusion

Both diploid and polyploid lines of purple-flowered native Thai *Torenia* were irradiated by acute gamma ray. The radiosensitivity of *Torenia fournieri* Lind. after  $\gamma$ -ray irradiation differs with ploidy level. Polyploid plants displayed lower sensitivity and could be exposed to higher radiation dosage. In *Torenia* wild type, the optimum radiation dose for acute gamma radiation was found to be LD<sub>50</sub> was 63.65 grays for diploid lines and 72.00 grays for polyploid lines. Red colored leaves were observed in some plants after acute gamma irradiation treatment, for example in the 20-gray treatment group for diploid *Torenia* and in the 80-gray treatment group for polyploid *Torenia*. The plants with red leaves were selected for development in the future.

## 5. Acknowledgments

This work was partially supported by the Center for Advanced Studies for Agriculture and Food, Institute for Advanced Studies, Kasetsart University Under the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, Ministry of Education, Thailand.

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