

## 学位論文要旨 Dissertation Abstract

氏名： タナポーン ラオジュンタ  
Name Tanapoom LAOJUNTA

学位論文題目： トレニア遺伝資源の評価，創出ならびに保存に関する研究  
Title of Dissertation Evaluation, creation and preservation of *Torenia* genetic resources

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*Torenia* is a popular summer bedding plant belonging to family Linderniaceae. Recently the number of commercially available cultivars including seed propagated (SP) and vegetative propagated (VP) genotypes has increased. To provide an overview of the *Torenia* cultivars sold in Japan, twenty-five *Torenia* cultivars were grown in a greenhouse under 50% shading. The cultivars could be divided into two groups; with or without yellow blotch on the lower petal. A wide range of petal color was observed; deep to light blue, deep to light reddish purple, yellow and white. Flower size varied 20 to 32 mm in width and 25 to 37 mm in length. Leaf size varied 8 to 30 mm in width and 10 to 40 mm in length. Leaf shape also varied depending on the cultivar; round to narrow round shape (length/width ratio, 0.95 to 1.62). Flow cytometry analysis revealed that the VP cultivars included diploids, triploids and tetraploids. The VP cultivars are assumed to be interspecific hybrids derived from crossing of *T. fournieri*, *T. concolor* and *T. baillonii*. The putative cross combination of each VP cultivar was determined by RFI. *Torenia* cultivars could be divided into three groups by plant shape, erect (*T. fournieri*), semi-erect (Interspecific hybrid between *T. fournieri* and *T. concolor* or *T. baillonii*) and creep (*T. concolor*, *T. baillonii* and Interspecific hybrid between *T. concolor* and *T. baillonii*). Flower of *T. fournieri* had six anthocyanins (Mv 3,5 dG, Pn 3,5 dG, Pt 3,5 dG, Dp 3,5 dG, Cy 3,5 dG and Pg 3,5 dG). The pigment component varied depending on petal color.

The narrow genetic source from only three species brings some limitations. To produce new novel flower type of interspecific hybrid, new species are required to use as parents. In this study, three species *T. bicolor*, *T. siamensis* and *T. hirsutissima* from Thailand were selected and crossed with *T. fournieri*, *T. concolor* and *T. baillonii*. The pollen germination percentages of all species were high enough for a reciprocal crossing experiment. Chromosome number of *Torenia* varied from  $2n=16$  to  $2n=34$ . Fully developed mature seeds were obtained from only three of 30 cross combinations. When ovule culture was applied, new 14 cross combinations produced progeny. The results show that ovule culture is very effective in expanding crossing potential in the interspecific hybridization of *Torenia*. Newly produced interspecific hybrids showed intermediate chromosome numbers between parents. Interspecific hybrids derived from cross combinations of distant related species often showed sterile because of the unbalanced chromosome number and different gene components, resulting in

reduced chromosome pairing during meiosis. Chromosome doubling is an efficient way to restore the pollen fertility of interspecific hybrids. Efficient chromosome doubling by chemical treatment in vitro requires efficient regeneration system. Leaf explants with scarification cultured on MS medium supplemented with 2 mg L<sup>-1</sup> BA showed high adventitious shoot regeneration. The present result showed that 20 days of 1500 mg L<sup>-1</sup> colchicine treatment is optimal. Hybrids from two way cross of *Torenia* species often showed limited variation of flower color and plant form. The backcrossed hybrids ('Crown Violet' × *T. baillonii*) × 'Crown Violet' had 2n = 18 and showed pollen fertility. Only one hybrid had pink color but other seven hybrids had violet petals indicating that all hybrids accumulated anthocyanin on all upper, lateral and lower petal. On the other hand forty-eight hybrids were obtained from cultured 250 ovules of ('Crown Violet' × *T. baillonii*) × *T. baillonii*. Ten of the backcrossed hybrids had 2n=16, other 38 had 2n =17. Only the hybrids had 2n=16 showed pollen fertility. Serrated petal appeared in the backcross of both ('Crown Violet'× *T. baillonii*) × 'Crown Violet' No. 3 and ('Crown Violet'× *T. baillonii*) × *T. baillonii*. Self-pollination of ('Crown Violet'× *T. baillonii*) × 'Crown Violet' No. 3 having serrated petal produce 100 % serrated petal progenies. The progenies showed wider range of flower color variations. The results demonstrated that backcross and selfing are effective in expansion of petal color variation in *Torenia*. In case of tri-parental cross, the progenies showed rather narrow variation in flower color as same as other interspecific hybrids.

Market demand of *Torenia* young plants concentrates in short period of spring. To catch up the demands efficient vegetative propagation is required. Leaf cutting of *Torenia* was investigated. Explants taken from the upper and middle positions of the shoots showed higher regeneration than lower positions with a higher number of shoot and longer shoots. Whole leaf explants without petiole from the middle position showed the highest shoot regeneration with a larger number of shoots.

Vegetative propagated cultivars require a stable method for mother plant maintenance. Cryopreservation of shoot tips is one of the most reliable methods for long-term storage of genetic resources. The small explants with 2 days of pre-culturing showed 50% regrowth after cryopreservation. On the other hand, the large explants with 2 days of pre-culturing and the small size explants without pre-culturing browned after thawing and no regrowth was observed. Regrowth percentages of non-cryopreserved shoot tips decreased with the increase in exposure time to PVS2 from 0 to 50 min at room temperature. Ten-minute exposure to PVS2 at room temperature gave the highest regrowth of cryopreserved shoot tips. The results confirmed that both explant size and conditions of PVS2 treatment are critical for successful of cryopreservation of *Torenia* shoot tips.