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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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学位論文要約：
Dissertation Summary

Torenia is a popular summer bedding plant (belonging to family Linderniaceae), known as the 'Figwort family'. The genus *Torenia* consists of 50 species distributed in tropical and subtropical Asia (Yamazaki, 1985). Only a few species, *T. fournieri*, *T. concolor* and *T. baillonii* have been used as ornamental plans. *Torenia fournieri* and *T. baillonii* are annual plants native to Southeast Asia. Original species of the seed propagated torenia is *T. fournieri*. Only erects type torenia with violet or white flower color had been available until the 'Crown' series, having pink, blue, and reddish-purple flower color, was released in 1988 from Pan American Seed (West Chicago, IL, USA). Now torenia has become popular horticultural plants in Japan and around the world after creeping type interspecific hybrids (*T. fournieri* × *T. concolor*), the 'Summerwave' series were released in 1995 from Suntory Ltd. (Osaka, Japan) (Aida, 1995).

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 divided 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 in to 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*).

Anthocyanin biosynthesis pathways have been well documented (Miyazaki et al, 2006, Nakamura et al, 2010, Tanaka et al, 2008). Aida et al. (2000) reported that five anthocyanins were detected in torenia 'Crown Violet', i.e. delphinidin 3,5-digucoside, cyaniding 3,5-digucoside, petunidin 3,5-digucoside, peonidin 3,5-digucoside and malvidin 3,5-digucoside. Three major of flavones were also reported as luteolin 7-gucoside, luteolin 7-glucuronide and apigenin 7-glucuronide in *Torenia* petals. In *Torenia concolor* three anthocyanins were reported, i.e. malvidin monorhamnosyl-dihexoside, peonidin monorhamnosyl-dihexoside and malvidin dihexoside (Iwashiwa and Kokubugata, 2014). *Torenia* 'Summer Wave Blue' which is an interspecific hybrids (*T. fournieri* × *T. concolor*) have malvidin 3,5 –diglucoside and peonidin 3,5 –diglucoside (Suzuki et al., 2000; Fukusaki et al., 2004). Present result showed that flower of *T. fournieri* had six anthocyanins, delphinidin 3,5-diglucoside, cyanidin 3,5-diglucoside, petunidin 3,5-diglucoside, pelargonidin 3,5-diglucoside, peonidin 3,5-diglucoside and malvidin 3,5-diglucoside. 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 was 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. The basic chromosome number is different depending on species; *Torenia fournieri* (2n=18), *Torenia baillonii* (2n=16) (Kikuchi et al., 2006). *Torenia concolor* (2n=34), *Torenia benthamiana* (2n=36) (Tsung-Hsin Hsieh and Yang, 2002, Kikuchi et al., 2007). It has been known that embryo development stopped at the globular stage resulting in seed abortion in interspecific crossing of *Torenia* (Kikuchi et al., 2007), which suggests that ovule cultures can be used to solve this problem. Ovule culture is a powerful tool to overcome post fertilization barriers and has been successfully applied in many plants: Lilium (Van Tuyl et al., 1991), Alstroemeria (De Jeu and Jacobsen, 1995), Lasquerella (Tomasi et al., 2002), and Vaccinium (Pathirana et al., 2015). Result of reciprocal crossings showed that 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. To clarify the hybridity of the obtained progenies in this study, PCR-RFLP of rRNA analysis was applied according to Haruki et al. (1997). The hybridity of all progenies from the 14 cross combinations was confirmed by the PCR-RFLP. No escape was observed in this study, suggesting ovule culture is a highly reliable method of obtaining interspecific hybrids in *Torenia*. Present study has produced 13 new interspecific hybrids, *T. fournieri* × *T. bicolor, T. fournieri* × *T. hirsutissima, T. baillonii* × *T. bicolor, T. baillonii* × *T. hirsutissima, T. baillonii* × *T. siamensis, T. bicolor* × *T. baillonii, T. bicolor* × *T. concolor, T. concolor* × *T. siamensis, T. concolor* × *T. bicolor, T. hirsutissima* × *T. fournieri, T. hirsutissima* × *T. baillonii, T. hirsutissima* × *T. bicolor* and *T. hirsutissima* × *T. concolor.*

Most of interspecific hybrids obtained in this study showed male sterile. Interspecific hybrids derived from cross combinations of distant related species were often sterile because of the unbalanced chromosome number and different gene components, resulting in reduced chromosome pairing during meiosis (Van Tuyl and De Jeu, 1997). 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^{-1} BA showed high adventitious shoot regeneration. The present result showed that 20 days of 1500 mg L^{-1} colchicine treatment is optimal in the combination of the high regeneration protocol. All hybrids tested produced chromosome double plants showed higher pollen fertility.

Hybrids from two way cross of *Torenia* species often showed limited variation in 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 petals. 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. The progenies showed wider range of flower color variations. 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 results demonstrated that backcross and selfing are effective in expansion of petal color variation in *Torenia*.

In case of tri-parental cross, pollen fertility of three hybrids consisting of *T. fournieri* × *T. baillonii*, *T. fournieri* × *T. bicolor* and *T. bicolor* × *T. baillonii* were tested. Only *T. bicolor* × *T. baillonii* was male fertile and other two hybrids *T. fournieri* × *T. bicolor* and *T. fournieri* × *T. baillonii* were male sterile. Then the pollen of *T. bicolor* × *T. baillonii* was crossed with four species (*T. fournieri, T. hirsutissima, T. concolor* and *T. siamensis*). Five hybrids were obtained from cultured 250 ovules of *T. fournieri* × (*T. bicolor* × *T. baillonii*). But ovule did not germination when *T. hirsutissima*, *T. concolor* and *T. siamensis* were used as a mother plant. When *T. fournieri* was used as an ovary parent, progenies were obtained in the crossing with *T. baillonii* and *T. bicolor* by normal crossing, and with *T. hirsutissima* and *T. concolor* though ovule culture. Kikuchi et al., (2007) reported that only *T. fournieri* × *T. baillonii* showed seed setting in the reciprocal crosses among *T. fournieri*, *T. concolor*, and *T. baillonii*. These facts indicate that *T. fournieri* has higher cross compatibility as an ovary parent compared with other species. Three hybrids mentioned above were used as female parents and crossed with five *Torenia* species. Only two cross combinations produced hybrids. Three hybrids were obtained from cultured 250 ovules of (*T. fournieri* × *T. baillonii*) × *T. siamensis* and two hybrids were obtained from cultured 250 ovules of (*T. fournieri* × *T. baillonii*) × *T. concolor*. Although total 5 progenies were obtained from ovule of (*T. fournieri* × *T. baillonii*), no ovule germination were observed when *T. fournieri* × *T. bicolor* and *T. bicolor* × *T. baillonii* were used as mother plants. This means both male and female gamete of *T. fournieri* × *T. bicolor* might be sterile. In contrast, only male gamete of *T. bicolor* × *T. baillonii* was functional. The progenies obtained by tri-parental crossings 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. Higher shoot regeneration was observed at higher temperature conditions in both summer and winter seasons. Meristematic structure appeared in sub-epidermal tissue of the swollen part near vascular buddle at distal end of explants and then developed to adventitious shoot. Vegetative propagation by leaf cutting could be adopted most of tested genotype.

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.

Present study has demonstrated that interspecific hybridization is a promising way to expand of variation in both flower color and plant form. Establishment of both young plant production system and preservation of genetic resource with development of breeding method will ensure the development of *Torenia* in floriculture.

References

Aida, R. and Shibata M.1995. Agrobacterium-mediated transformation of torenia (*Torenia fournieri*). Breed Sci, 45: 71-74

Aida, R., Kishimoto S., Tanaka Y., Shibata M. 2000. Modification of flower color in torenia (*Torenia fournieri* Lind.) by genetic transformation. Plant Sci, 153 (1): 33-42

De Jeu, M.J. and Jacobsen, E. 1995. Early postfertilization ovule culture in *Alstroemeral* L. and barriers to interspecific hybridization. Euphytica, 86:15–23.

Fukusaki, E., Kawasaki K., Kajiyama S, An C., Suzuki I., Tanaka K ., Kobayashi A. 2004. Flower color modulation of torenia hybrid by down regulation of chalcone synthase genes with RNA interference. J biotechnol,111 : 229-240

Haruki, K., Hosoki, T., Nako, Y. and Ohta, K. 1997. Possibility of classification in some species of *Lilium* by PCR-PFLP of ribulose-1,5-biphosphate carboxylase large subunit (rbcL) gene and ribosomal RNA gene. J Japan Soc Hort Sci, 66(1):189–192.

Iwashina, T. and Kokubugta, G. 2014. Flavonoids from the Flowers and Aerial parts of *T. concolor* var *formosana*. Bull. Natl. Mus. Nat. Sci., Ser. B, 40 : 55-59

Kikuchi, S., Tanaka H., Shiba T., Mii M., Tsujimoto H. 2006. Genome size, karyotype, meiosis and a novel extra chromosome in *Torenia fournieri*, *T. baillonii* and their hybris. Chromosome Res, 14 (6): 665-672

Kikuchi, S., Kino H., Tanaka H., Tsujimoto H. 2007. Pollen tube growth in cross combinations between *Torenia fournieri* and fourteen related species. Breed Sci, 57:117-122

Miyazaki, K., Suzuki, K. I., Iwaki, K, Kusumi, T., Abe, T., Yoshida S., and Fukui, H. 2006. Flower pigment mutations induced by heavy ion beam irradiation in an interspecific hybrid of *Toreni*a. Plant Biotechnology, 23(2): 163-167.

Nakamura, N., Fukuchi-Mizutani, M., Fukui, Y., Ishiguro, K., Suzuki, K., Suzuki, H., Okazaki, K., Shibata, D., and Tanaka, Y. 2010. Generation of pink flower varieties from blue *Torenia hybrida* by redirecting the flavonoid biosynthetic pathway from delphinidin to pelargonidin. Plant Biotechnology, 27(5): 375–383.

Pathirana, R., Wiedow, C., Pathirana, S., Hedderley, D., Morgan, E., Scalzo, J., Frew, T. and Timmerman-Vaughan, G. 2015. Ovule culture and embryo rescue facilitate in interspecific hybridization in blueberry (*Vaccinium* spp.). Acta Hort, 1083.

Suzuki, K., Hui, M.X., Tanaka, Y., Fukui, Y., Mizutani, F.M., Murakami, Y., Katsumoyo, Y., Tsuda, S. and Kusumi, T. 2000. Flower color modification of torenia hybrid by cosuppression of anthocyanin biosynthesis genes. Mol breeding, 6 : 239-246

Tanaka, Y., Sasaki, N. and Ohmiya, A. 2008. Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. Plant J, 54, 733-749.

Tomasi, P., Dierig, D., Dahlquist, G. 2002. An ovule culture technique for producing interspecific *Lesquerella* hybrids. Amer Soc Hort Sci 208–212.

Tsung-Hsin, H. and Kuoh-Chieng, Y. 2002. Revision of *Torenia* L (Scrophulariaceae) in Taiwan. Taiwania, 47 (4): 281-289

Van Tuyl, J.M. and De Jeu, M.J. 1997. Methods for overcoming interspecific crossing barriers. Chapter 13. p.273-293. In: Sawhney and Shivanna (eds.), Pollen Biotechnology for Crop Production and Improvement, Cambridge University Press.

Van Tuyl, J.M., Van Dien, M.P., Van Creij, M.G.M., Van Kleinwee, T.C.M., Franken, J. and Bino, R.J. 1991. Application of in vitro pollination, ovary culture, ovule culture and embryo rescue for overcoming incongruity barriers in interspecific *Lilium* crosses. Plant Sci, 74:115–126.

Yamazaki, T. 1985. A Revision of the Genera Limnophila and Torenia from Indochina. J Fac Sci Univ Tokyo III, 13 : 575-642