Australian Government
Department of Health and Ageing
Office of the Gene Technology Regulator

The Biology of *Torenia* spp. (torenia)

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This document provides an overview of baseline biological information relevant to risk assessment of genetically modified forms of the species that may be released into the Australian environment.

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PREAMBLE

This document describes the biology of *Torenia* spp. with particular reference to the Australian environment, cultivation and use. Information included relates to the taxonomy and origins of cultivated *Torenia* spp., general descriptions of their morphology, reproductive biology, biochemistry, and biotic and abiotic interactions. This document also addresses the potential for gene transfer to occur to closely related species. The purpose of this document is to provide baseline information about the parent organism for use in risk assessments of genetically modified (GM) torenia that may be released into the Australian environment.

Torenia, wishbone flowers and blue wings are common garden names for various species of torenia (*T. fournieri, T. concolor. T.* x *hybrida*[1] *and T. asiatica*), which are popular throughout the world for growing in home gardens and landscaped areas, often in hanging baskets and patio planters. The common name 'wishbone' is derived from the chicken wishbone-shaped stamens.

T. fournieri was, for many years, the most important species in the genus for ornamental use but since the mid 1990s *T.* x *hybrida* cultivars (see Section 2.5) have also become popular. For this reason, the focus of this document will largely be confined to these two species.

SECTION 1 TAXONOMY

Plants of *Torenia* spp. are dicotyledons, belonging to the class *Magnoliopsida*, order *Scrophulariales* and family *Scrophulariaceae*, which is commonly known as the 'figwort family'. The *Scrophulariaceae* comprise 306 genera and approximately 5850 species. There are discrepancies in the number of torenia species mainly because the genus has been insufficiently studied in the Indochina region. Based on herbarium specimens, Yamazaki (1985) reported a total of 50 torenia species, 20 of which were from Cambodia, Laos and Vietnam, and 19 from Thailand. Other reports indicate that there are 40 species of torenia (Fischer 2004; Spencer 2006).

SECTION 2 ORIGIN AND CULTIVATION

2.1 Centres of diversity and domestication

The origin of *Torenia* spp. is not known (Fischer 2004), although they are reportedly native to South East Asia, Africa and Madagascar (Yamazaki 1985). Details on the distribution of a few well known species of torenia are given in Table 1. *Torenia* x *hybrida* describes those plants derived from controlled crosses between *T. fournieri* and *T. concolor* (see Section 2.5). The flowers of *T.* x *hybrida* are both male and female sterile (Tanaka 2008)[2]

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[1] The prefix 'x' in front of the epithet indicates the hybrid nature of the species

[2] This is a personal communication. As at January 2006, Yoshikazu Tanaka was a scientist in the genetic modification program at Suntory Ltd (Osaka, Japan).

Members of the family *Scrophulariaceae* are found mostly in tropical mountains, particularly in open rather than forested areas. Torenia plants are prevalent in disturbed habitats such as roads and paths, clearings, plantations and around settlements (Fischer 2004). There are reports of torenia growing in 'open swamps', wet rocks on open slopes or by streams, moist ground in evergreen forests and lowlands, and at altitudes of 300-1200m (Yamazaki 1985).

Table 1. Distribution of some *Torenia* spp. (Yamazaki 1985; Tanimoto & Harada 1990; Flora of China 1994; Harvard University 2006)

Botanical name	Common name	Occurrence / distribution
T. asiatica	wishbone	China, Japan, Vietnam
T. flava	unknown	Assam, Burma, Malaysia, Indonesia, Thailand, Laos, Vietnam, S. China, Taiwan, Cambodia, India
T. benthamiana	unknown	Vietnam, S. China and Taiwan
T. concolor	torenia wishbone	Laos, Vietnam, S. China, Taiwan, Ryukyu Islands (Japan)
T. fournieri	blue wings torenia wishbone	Thailand, Cambodia, Laos, Vietnam, Cochin China

T. polygonoides is listed as a native species of conservation significance (ie rare) in the tropical savannas region (Cape York Peninsula) of Queensland (Queensland Government 2006; North Australian Land Manager 2008).

2.2 Uses

Torenia spp. have an extended history of cultivation and safe use as an ornamental plant and are usually found in nurseries, home gardens and landscaping situations. Torenia have been grown as a front border plant especially in lightly shaded areas and are popularly grown in hanging baskets or as trailing specimens in patio planters (Starman 2005). Flowers of *T. fournieri* are reportedly edible and can be used as salad material (Shindu et al. 2008).

Apart from its ornamental value, *T. fournieri* is a useful experimental model plant for cytogeneticists to study fertilization, because of the ease of observing pollination and fertilisation due to the protruding nature of the embryo sac (see Sections 3.2 & 4.2). *T. fournieri* is also used to study the location and movement of chromosomes and their centromeres in the early stages of embryogenesis in interspecific hybrid plants (Kikuchi et al. 2005).

2.3 Cultivation in Australia

Two *T.* x *hybrida* cultivars[3] – ‘Sunrenilabu’ and ‘Sunrenirirepa’ are listed in the Plant Varieties Journal of the Department of Agriculture, Forestry and Fisheries (DAFF) and have been approved for breeding and cultivation in Australia (Anon. 1999; Anon. 2000). *T.* x *hybrida* and *T. fournieri* have been introduced into most States of Australia, including the tropics and subtropics. *T.* x *hybrida* cultivars are commercially available from nurseries and are being grown in Victoria, New South Wales (NSW), South Australia, and the Australian Capital Territory (ACT) with limited availability in Queensland.

2.4 Cultivation practices

T. fournieri is propagated mainly by seed and grown as an annual[4] either outdoors or as a houseplant; seeds remain uncovered as light apparently aids germination (Cornell University 2006). In central Australia, the recommended seed sowing time is from August to December (DPIFM Darwin 2004). *T. fournieri* can also be grown from cuttings under controlled conditions (such as bottom heat, aerated medium, misting and plant growth regulator application) that optimise rooting and growth (Ecke Ranch 2007). T*orenia* x *hybrida* does not produce viable seeds and is vegetatively propagated from mother plants or by tissue culture at specialist nurseries. Commercially-grown *T.* x *hybrida* may be propagated in pots in a soil-less medium (peat and perlite) with good drainage and nutrient-holding capacity (Starman 2005). The optimum soil pH is reportedly 5.5-6.5 (Starman 2005). Optimum growth within a greenhouse is at a day/night temperature of 24/18°C, with moderate to high light and good air circulation (Starman 2005). All torenia need to be kept well watered (Starman 2005).

T. fournieri is recommended for growing in part shade and in moist, well-drained soil (Gilman & Howe 1999); plants may be “pinched” back to promote growth (Cornell University 2006). Pinching is not considered necessary for *T.* x *hybrida* (Starman 2005) nor is the removal of old flowers (Jackson & Perkins Wholesale Inc 2007). *T.* x *hybrida* also grows well in partial shade to shady areas (Jackson & Perkins Wholesale Inc 2007). *Torenia* spp may also be grown in full sun (Black 2006) and in fact some newer varieties of *T.* x *hybrida* exhibit heat and sun tolerance (Starman 2005).

For the home gardener, monthly fertilisation is recommended, while constant feeding of nitrogen:phosphorus:potassium (200:65:125 ppm) is recommended for the commercial grower (Jackson & Perkins Wholesale Inc 2007). Over-fertilisation can

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[3] The word ‘cultivar’ is a contraction of ‘cultivated variety’ and describes a group of cultivated plants within a species that are significant in agriculture, forestry or horticulture and have clearly distinguished, heritable characteristics. ‘Cultivar’ is synonymous with the term ‘variety’. However it is not analogous with the category ‘botanical variety’ that is used to refer to naturally occurring variants within a species (Hartmann & Kester 1975). Cultivars/varieties mentioned in this document are indicated in quotation marks eg. ‘Summer Wave® Blue’

[4] The term ‘annual’ is used in the sense that plants will not produce flowers during prolonged low temperatures and gardeners will often dig up and destroy the plants or transfer them inside for winter. Plants will not survive frost but at slightly higher temperatures can survive through winter although growth is retarded and leaves develop a red colour.

result in the promotion of vegetative growth rather than flower production (Jackson & Perkins Wholesale Inc 2007).

2.5 Crop improvement

Torenia cultivars or varieties that produce violet flowers, were the only types commercially available for a long time until Pan American Seed (Illinois, USA) released the 'Crown' series in 1988, which included pink, white and reddish/purple flower colour lines. Since then, there has been the development of a number of series (eg 'Summer Wave', 'Moon', 'Catalina', 'Panda' and 'Lovely'), the release of which has increased the popularity of torenia in Japan, North America and Australia (Anon. 2000; Aida & Shibata 2001).

Since the mid 1990s a wide range of *T.* x *hybrida* cultivars have been released. Many of these cultivars make up the 'Summer Wave' series. Some of the cultivars are direct hybrids [e.g. 'Sunrenidibu' (Miyazaki 1999)], but there are also those that have arisen in a hybrid as a result of spontaneous mutation [e.g. 'Sunreniririepa' - commercially known as 'Summer Wave® Amethyst' (Miyazaki 2003); and 'Sunrenilabu' - commercially known as 'Summer Wave® Blue' (Tamura & Miyazaki 1999)] and those that have arisen as a result of artificial chromosome doubling in a hybrid [e.g. 'Sunrenilamu - commercially known as 'Summer Wave® Large Violet' (Kazunari 2005)].

As an indication of the features of the hybrids, Table 2 shows some of the key characteristics of *T.* x *hybrida* 'Sunrenidibu' in comparison with its female parent *T. fournieri* 'Crown Violet' and male parent *T. concolor* 'Con Color'.

Table 2. Horticultural characteristics of a *T.* x *hybrida* cultivar ('Sunrenidibu') with its *T. fournieri* (Crown Violet) and *T. concolor* (Con Color) parents (Miyazaki 1999).

Characteristic	**Crown Violet (♀)**	**Con Color (♂)**	**Sunrenidibu**
Growth habit	Erect	Decumbent	Semi-erect
Plant height	20 – 30 cm	10 – 15 cm	15 – 20 cm
Plant width	30 – 50 cm	50 – 70 cm	50 – 60 cm
Branching	Slight	Profuse	Medium
Leaf length	2 – 4 cm	1 – 2 cm	2 – 3 cm
Leaf width	2 – 3 cm	1 – 2 cm	1.5 – 2.5 cm
Colour of floral tube	Soft violet	Moderate purple	Brilliant purple
Petal colour	Purplish white all over	Deep purple all over	Brilliant purple (upper petal); deep purple (lower petal)

In addition to improving the morphological characteristics, the breeder of 'Sunrenidibu' (Miyazaki 1999) also specified a number of valuable horticultural characteristics that were incorporated into the hybrid including resistance to heat, a moderate resistance to diseases and the ability to grow and flower in the shade.

2.5.1 Conventional Breeding

Current torenia breeding programs aim to produce varieties with novel flower colours, increased flower production, early flowering and enhanced survival and disease resistance.

Torenia is generally a diploid plant. The basic chromosome number varies with species (see Section 9.1). As mentioned in the previous section, tetraploids can be induced by colchicine treatment of young seedlings and have relatively large flowers offering potential for developing better horticultural varieties. However these tetraploids exhibit significant reduction in pollen viability, seed setting and unequal distribution of chromosomes at anaphase when compared to their diploid progenitors (Tandon & Bhutani 1965).

The production of interspecific hybrids (*T.* x *hybrida*) requires that after crossing the ovules are removed from ovaries and cultured *in vitro* (Miyazaki 1999). As the hybrid plants are male and female sterile and do not produce viable pollen or set seed, it is not possible to expand the variation in desirable horticultural characteristics without selecting spontaneous mutants or using techniques such as chromosome doubling (as described above), artificial mutagenesis (Miyazaki et al. 2006b) or gene transformation techniques (Nakamura et al. 2006).

Recently *T.* x *hybrida* was identified as a versatile model system for use in transformation technology to study morphogenetic responses. Given that torenia can be grown easily under laboratory/field conditions and has high genetic transformation efficiency, scientists and horticulturist have now recognised its potential for the production of new and significant cultivars (Aida et al. 1998; Tao & Li 2006). *In vitro* tissue culture requirements for *T.* x *hybrida* have been well established (Tanimoto & Harada 1990) and plant tissue can readily differentiate into adventitious structures and regenerate plants (transformation frequency of 90%) (Aida & Shibata 2001).

2.5.2 Mutation Breeding

Irradiation with heavy ion beams such as nitrogen, carbon or neon has been used to generate flower colour mutants of *T.* x *hybrida* (Miyazaki et al. 2006a) and *T. fournieri* (Sasaki et al. 2008) and has been proposed as a method that can be used in conjunction with genetic modification for improving commercial traits within a short time (Sasaki et al. 2008).

2.5.3 Genetic modification

Aida & Shibata (Aida & Shibata 2001) suggested that genetic modification would be important in improving flower colour and the single-type flower of torenia.

Early experiments with torenia established plant tissue culture regeneration systems, a necessary precursor to successful transformation. The main pathway of regeneration is via shoot regeneration and young leaf tissue is the preferred explant (Bridgen 1999). Aida and Shibata (1995) were the first to genetically modify torenia and co-culturing of leaf discs with *Agrobacterium tumefaciens* has been the standard transformation method for torenia. Floral traits that have been introduced include wavy colour patterns, new colours and extended longevity (Aida et al. 1998; Aida et al. 2000a; Aida et al. 2000b; Aida et al. 2001; Ono et al. 2006). Initial experiments on genetic modification used *T. fournieri* but *T.* x *hybrida* has now also been a target species. One of the limitations in *T.* x *hybrida* (ie the 'Summer Wave®' Series) was the restriction of flower colour to amethyst, violet, blue and lavender. Attempts to extend this range have concentrated on suppressing endogenous genes responsible for flower colour (eg by downregulating anthocyanin synthesis through RNA interference) with or without expression of genes responsible for other flower colours (Suzuki et al.

2000; Fukusaki et al. 2004; Ono et al. 2006). Two GM flower colour lines of *Torenia* spp. (events 1165 & 1382) were approved for general release in Japan in 1998 [see information provided in Bruderer & Leitner (2003)]. Approval for a limited and controlled release of *T.* x *hybrida* genetically modified for flower colour has been approved in Australia (OGTR 2006).

Other traits have also been introduced through *A. tumefaciens*-mediated transformation:

- In light of recent concerns with using antibiotic selection markers for the selection of transgenic plants other alternatives have been tested. Success has been obtained in genetically modified *T.* x *hybrida* and *T. fourneri* using mannose as the selective agent (Seitz et al. 2007; Li et al. 2007).

- *Torenia fournieri* genetically modified to over express the *AtNHX5* gene from thale cress (*Arabidopsis thaliana*) showed enhanced salt tolerance, *in vitro* shoot regeneration frequency and increased survival compared to wild-type plants (Shi et al. 2008).

Other methods of transformation have been tested:

- *A. rhizogenes* has been used to successfully modify *T. fournieri* (Tao & Li 2006).

- Argon-fluoride (ArF) excimer laser-induced shock waves have been used to introduce gold particles coated with the green fluorescent protein gene into tissue samples from *T.* x *hybrida* for the purpose of developing a novel transformation system (Kajiyama et al. 2007).

SECTION 3 MORPHOLOGY

3.1 Plant morphology

Detailed morphological descriptions of the torenia plant can be found in numerous publications (Yamazaki 1985; Flora of China 1994; Flora of Taiwan 1998; Gilman & Howe 1999; Klingaman 2000; Starman 2005; Cornell University 2006). Here, the description is dealt with in more general terms.

Plants of *Torenia* spp. are herbs with quadrangular, branched, erect or prostrate stems. Leaves are simple and oppositely arranged on the stem and usually have a stalk (petiole). The leaf blades have a variety of shapes ranging from lanceolate (lance shaped 3-4 times long as broad and tapering at the apex) to ovoid or circular, with serrated margins.

As an example of the variation that occurs between species Table 2 provides information on three of the most widely used horticultural species.

3.2 Reproductive morphology

Flowers are borne on stalks (pedicels) and may be solitary or may be part of an inflorescence that is classed as a cyme or raceme (see Fig. 1)

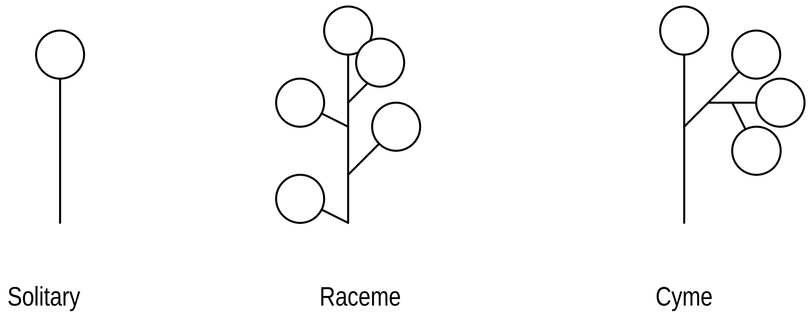

Figure 1. Inflorescence types

The calyx (sepals) of the flower is tubular, often bilabiate (2-lipped), usually 5-lobed and with wings or ribs. The corolla (petals) is a cylindrical tube, 2.5 – 3.5 cm long that, in wild species is yellow, purple, blue or white in colour. As a result of breeding and gene manipulation, the cultivars of *Torenia* spp. exhibit a wider range of flower colours, including pink, and violet and are often multicoloured due to differences in the concentrations, ratios and types of anthocyanins and flavonoids produced. Petals are bilabiate, with the lower lip having 3 lobes and the upper lip having 2 lobes. (Yamazaki 1985; Flora of China 1994; Flora of Taiwan 1998; Fischer 2004).

The flowers are hermaphrodite and have four stamens arranged in pairs. Each pair is fused at the anthers giving rise to two wishbone-shaped structures that are characteristic of the *Torenia* genus. One stamen pair is longer than the stigma and the other stamen pair is shorter than the stigma (Miyazaki 1999). As with all members of the Scrophulariacea, there is a single style with a 2-lobed stigma and a superior ovary divided into two compartments (locules) containing numerous ovules [eg 300 – 500 in *T. fournieri*; >500 in *T. concolor* (Higashiyama et al. 2006)].

The embryo sac within an ovule of *Torenia* spp. is of interest in studies on pollination and fertilization because it is exserted (protrudes through the micropyle) and is thus easily accessible for observation (Wallwork & Sedgley 2000; Higashiyama et al. 2001). The arrangement of the embryo sac can be seen diagrammatically in Fig 2.

Following fertilization, the ovary develops into a fruit classified as a capsule, containing tiny seeds (0.5mm long and 0.3mm wide). Flowers of *T.* x *hybrida* do not produce seeds (see Section 4.1.2).

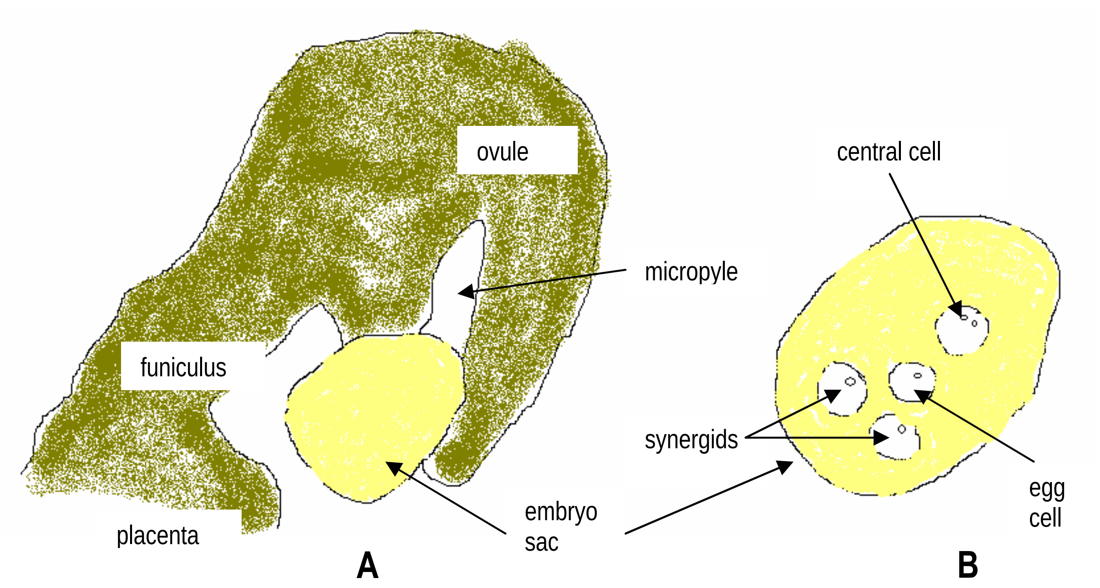

Figure 2. A). Representation of the arrangement of the torenia embryo sac inside an ovule (as seen in transverse section); B). Arrangement of the cells inside the embryo sac [based on interpretation of diagrams and photographs in Wallwork & Sedgley (2000) and Higashiyama et al. (2001)].

SECTION 4 DEVELOPMENT

4.1 Reproduction

4.1.1 Asexual reproduction

Torenia stem cuttings are used for commercial propagation (see Section 2.4) under optimal horticultural conditions to maximise success. Detached stem pieces or cuttings do not have the propensity for asexual reproduction under natural conditions (Tanimoto & Harada 1990). Under favourable *in vivo* conditions of adequate moisture and ambient temperature, it is possible for trailing stems of *Torenia* spp. to form adventitious roots if in contact with the soil.

4.1.2 Sexual reproduction

A number of horticultural conditions and environmental factors influence flower development in torenia. Light, temperature, pest control, timing of fertiliser application, soil type and pH, and horticultural practices are some of the important factors that determine profuse flowering. Torenia plants grow well in moist to wet soils and require warm days, with temperatures ranging between 21-25 ºC to stimulate profuse flower production.

T. fournieri can be pollinated artificially and set seed under *in vitro* tissue culture conditions, and this is one of its merits as an experimental plant (Aida & Shibata 2001).

Using *in vitro* techniques a comparative study on ovules and pollen viability between *T. fournieri*, *T. concolor* and *T.* x *hybrida* has been conducted[5]. Microscopic examination revealed that pollen grains were misshapen and non-viable in *T.* x *hybrida* (of the 1091 pollen grains examined, 97% derived from the other *Torenia* spp. germinated compared to 0% germination in *T.* x *hybrida*). Other experiments indicated that 99.5% of 637 ovules were non-aborted in *T. fournieri and T. concolor*) compared to all 864 ovules being aborted in *T.* x *hybrida.*

4.2 Pollination and pollen dispersal

The genera of *Scrophulariaceae* are mainly insect pollinated. *Torenia* spp. (excluding *T.* x *hybrida*) are apparently insect pollinated and produce numerous seeds. One of the parents of *T.* x *hybrida* (*T. fournieri*) is mainly bee pollinated (Yamazaki 1985; Fischer 2004). In Thailand, pollen from *T. fournieri* is a food source for several species of stingless bees (Jongjitvimol & Wattanachaiyingcharoen 2006). In North America, hummingbirds are reportedly attracted to the flowers of *T. fournieri* (Cornell University 2006) and may therefore act as potential pollinators.

Some flowering plants show the development of mimic stamens that help the flower to overcome the disadvantages of having conspicuous stamens (such as exposure of pollen to wind and rain, consumption of pollen) while still attracting pollinators. In some instances it is thought that the mimic stamens (which often manifest as yellow floral guides) have evolved to a stage where they have taken over other functions in the flower. It is suggested that the prominent yellow color patch on the lower lip of wild *T. fournieri* is one such example (Lunau 2006).

Torenia anthers produce numerous tiny pollen grains that are approximately 22±4 x 33±7 µm in size (Watanabe et al. 2006). The pollen is not shed during anthesis and requires that mechanical pressure be applied to the anthers before pollen is released by a propelling lever action (Armstrong 1992). A flange like outgrowth of the lateral pollen sac wall of the anther forms each of four levers which when pressed, cause an infolding of the thin pollen sac wall which then ruptures thereby pushing the pollen grains out. It was estimated that a force of 1.0 - 1.5g pressing against the four levers of an anther, could result in the forcible shedding of approximately 2,000-3,000 pollen grains in two parallel rows. The pollinator, in foraging in a flower, is covered with pollen grains that can be transferred to stigmas as the pollinator visits other flowers.

As in all angiosperms, following germination on the stigma, the pollen grows a pollen tube (which contains two sperm cells and a tube nucleus) down the style to an ovule (Raven et al. 1999). Inside the ovule (see Fig. 2) is an embryo sac and in *Torenia* spp. two of the cells within the embryo sac (the synergids) produce an, as yet unidentified, chemical which acts as a guidance cue for the pollen tube (Higashiyama et al. 2006). The chemical that is produced is species specific and may serve as a hindrance to interspecific pollination since pollen tube growth is delayed or fails when the pollen and embryo sac are from different species. In addition, once fertilized, an embryo sac

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[5] This information was provided to the Japanese regulatory authority for an application for general release approval of GM lines of *Torenia* spp. in Japan (see Section 2.5.3)

no longer attracts pollen tubes even if an intact synergid is present (Higashiyama et al. 2001). This is consistent with the idea that fertilization prevents additional pollen tubes from entering the embryo sac, thus avoiding the problem of polyspermy.

The pollen tube enters the embryo sac at the micropylar end, pushes between the two synergid cells, and explosively releases its contents (Higashiyama et al. 2000). There then follows a double fertilization where one of the sperm cells unites with the egg cell to form the embryo and one sperm cell unites with the central cell (containing two fused polar nuclei) that develops into the seed endosperm (Wallwork & Sedgley 2000).

4.3 Fruit development and seed dispersal

The most common fruit type in the family *Scrophulariaceae* is the capsule. In Torenia spp. (*T. fournieri, T. concolor and T. asiatica*), the capsule dehisces transversally by splitting at the septum and separating from the placenta that is positioned between two movable valves. A special case of poricidy occurs in *T. fournieri* where the fruiting body opens adjacent to the septum on each side producing longitudinal pores. This is followed by the septa separating from the placenta emptying the capsule while the septum splits (Kadereit 2008). At the time of poricidal dehiscence of the capsule the calyx splits longitudinally. In *Torenia* spp. (excluding *T.* x *hybrida*), seeds are numerous, minute and wind dispersed (Yamazaki 1985; Fischer 2004). *T. fournieri* may self seed each year (Gilman & Howe 1999).

4.4 Seed dormancy and germination

Seeds of *T. fournieri* germinate at temperatures of 21-24°C and take 7-17 days to emerge (Cornell University 2006). There is no information to suggest that torenia seeds exhibit dormancy.

SECTION 5 BIOCHEMISTRY

5.1 Toxins and allergens

Throughout the world *Torenia* spp. are widely grown ornamental plants and are not known to be poisonous to people or other organisms. A comprehensive search of the scientific literature[6] and an examination of a number of toxic plant databases[7] (Cornell University Poisonous Plant Information Database; Canadian Poisonous Plants Information System; FDA Poisonous Plant Database; Toxic Plant Database, Veterinary Library, University of Illinois, USA; Toxic Plant Database, University of Purdue, USA) revealed no evidence that torenia has any toxic or allergenic potential to people or is toxic to other organisms.

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[6] A variety of databases and search engines were utilised using "torenia" as the search term (PubMed; Toxnet; Scirus and Google Advanced). The search was then refined using the term "toxicity".

[7] http://www.ansci.cornell.edu/plants/ http://www.cbif.gc.ca/pls/pp/poison?p_x=px http://www.cfsan.fda.gov/~djw/plantox.html http://www.library.uiuc.edu/vex/toxic/intro.htm http://www.vet.purdue.edu/depts/addl/toxic/cover1.htm

5.2 Beneficial phytochemicals

The edible flower of *T. fournieri* exhibited antioxidant activity in an *in vitro* rat brain homogenate model, which was attributable to the presence of acteoside, luteolin-7-*O*-β-glucoside, apigenin-7-*O*-α-rhamnosyl-(1→6)-β-glucoside and apigetrin (0.19, 0.09, 0.16 and 0.27 mg/g dry weight, respectively) (Shindu et al. 2008).

5.3 Torenia flower colour

Aesthetically, flower colour is one of the most important characteristics for the floriculture industry. While the types, ratios and concentrations of floral pigments play a key role in determining flower colour, other important factors include vacuolar pH (the site of pigment accumulation), the structure of the pigments, the presence of co-pigments, light reflection and the size, shape and surface striations of petal epidermal cells (Aida et al. 2000b; Yamaguchi et al. 2001; Nakayama et al. 2003).

Besides carotenoids and betalins, the main pigments contributing to flower colour in *Torenia* spp. are the anthocyanins, which are a subclass of flavonoids. Anthocyanins are water-soluble pigments, responsible for the red, blue and purple colour of flowers (and other plant tissues such as fruit and leaves). Anthocyanins exist as glycosides or acyglycosides of their respective aglycone anthocyanidins. There are six major anthocyanidins: pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin (Suzuki et al. 2000).

SECTION 6 ABIOTIC INTERACTIONS

6.1 Abiotic stresses

T. x *hybrida* is cold and frost sensitive (Black 2006; Jackson & Perkins Wholesale Inc 2007). Foliage will bronze at temperatures below 15°C (Starman 2005). *T.* x *hybrida* does not tolerate dry conditions and requires moist soil to survive (Jackson & Perkins Wholesale Inc 2007).

Torenia exhibits poor salt tolerance (Black 2006).

6.2 Abiotic tolerances

T. x *hybrida* is heat tolerant when watered well (Starman 2005). It is reportedly tolerant to a variety of soil types including clay, sand, acidic and loam (Gilman & Howe 1999).

SECTION 7 BIOTIC INTERACTIONS

Torenia spp. are reported to be susceptible to common pests of herbaceous ornamental plants, such as thrips, aphids and mites. Viruses can be transmitted by insects, primarily aphids, leafhoppers and thrips.

Torenia spp. are reportedly susceptible to certain fungal pathogens like *Botrytis* and powdery mildew (*Oidium* spp.) (Holocomb 1999). Symptoms include leaf distortion and yellowing. Conditions like high relative humidity, crowded conditions and poor air circulation favour spread of powdery mildew. Powdery mildew can be prevented by spraying with a broad spectrum fungicide and watering the plant early in the day (Starman 2005). Other powdery mildew pathogens reported on *T. fournieri* are *Sphaerotheca fuliginea* (Schlechtend: Fr.) Pollacci in Finland and Japan and an

Erysiphe spp. in Japan (Holocomb 1999). In the US, *T. fournieri* is reportedly resistant to root knot nematodes (Hagan 2005) and has shown very light infestation with one or a few scattered galls (Black 2006).

In recent years Tobacco Mosaic Virus and Impatiens Necrotic Spot Tospovirus have been reported on torenia spp., (*T. fournieri*) showing necrotic spots on stems and apices (Roggero et al. 1999; University of Connecticut 2006).

T. fournieri is reportedly susceptible to many viruses including abelia latent tymovirus, apple mosaic ilarvirus, arabis mosaic nepovirus, carnation mottle carmovirus, carnation ringspot dianthovirus, cherry leaf roll nepovirus, cloverwound tumor phytoreovirus, clover yellow mosaic potexvirus, clover yellow vein potyvirus, cymbidium ringspot tombusvirus, dogwood mosaic nepovirus, foxtail mosaic potexvirus, groundnut eyespot potyvirus, maize eyespot virus, peach enation nepovirus, pelargonium line pattern carmovirus, peanut clump furovirus, peanut stunt cucumovirus, strawberry latent ringspot nepovirus, sweet potato mild mottle ipomovirus, tobacco etch potyvirus, tobacco ringspot nepovirus, tobacco streak ilarvirus, tomato black ring nepovirus and watermelon mosaic 2 potyvirus (VIDE 1997).

SECTION 8 WEEDINESS

Weeds are plants that spread and persist outside their natural geographic range or intended growing areas such as farms or gardens. Weediness in Australia is often correlated with weediness of the plant, or a close relative, elsewhere in the world (Panetta 1993; Pheloung et al. 1999). The likelihood of weediness is increased by repeated intentional introductions of plants outside their natural geographic range that increase the opportunity for plants to establish and spread into new environments, e.g. escapes of commonly used garden plants (Groves et al. 2005).

Characteristics in plants that are generally associated with weediness include prolonged seed dormancy, long persistence of seeds in the soil, germination under a broad range of environmental conditions, rapid vegetative growth, short lifecycle, very high seed output, high seed dispersal and long-distance seed dispersal (Keeler 1989; Keeler et al. 1996).

8.1 Weediness on a global scale

A number of *Torenia* spp. are classified as “naturalised” or “weeds” in various parts of the world including *T. asiatica* L. (US), *T. bicolour* Dalz. (global), *T. concolor* Lindl. (global), *T. flava* Bth. (Java), *T. fournieri* Linden ex Fourn. [Guyana, Surinam French Guiana and the US, (Florida)], *T. polygonoides* Benth. (Thailand), *T. spicata* Engl. (global), *T. thouarsii* (Cham. & Schltdl.) Kuntze. (global; Guyana, Surinam French Guiana) and *T. violacea* (Azaola ex Blanco) Pennell (Java, Global, Indonesia, The Tropics and China) (Randall 2002). *Torenia asciatica* and *Torenia glabra* are classified as potentially invasive in Hawaii (Staples et al. 2000). *Torenia.* x *hybrida* does not possess any characteristics typical of weeds since it does not produce any viable seed, cannot spread by vegetative means under natural conditions and does not produce any persistent vegetative structures.

8.2 Weediness status in Australia

The CRC for Weed Management, in its online *Introduced Flora of Australia and its Weed Status*, lists *T. fournieri* as being a weed of agriculture (see http://weeds.cbit.uq.edu.au/). On the other hand, other authorities do not list any *Torenia* spp. as being weeds in Australia (Groves et al. 2003; Weeds Australia 2006). As *T.* x *hybrida* was only introduced relatively recently (about 5 years ago) to Australian horticulture, and has no means of sexual reproduction and limited asexual reproduction, it would not be expected to have developed weed status. In addition to *T. fournieri* and *T.* x *hybrida*, the species *T. baillonii* is also permitted as an import (nursery stock) to Australia (AQIS 2008).

Related genera in the family *Scrophulariaceae* found in NSW and listed as noxious weeds are *Veronica arvensis* and *V. persica* (Persian speedwell), *Linaria dalmatica* syn *Antirrhinum dalmaticum* L., *Linaria grandiflora* Desf. (commonly known as Dalmatian toadflax) and *Verbascum thapsus* (commonly known as great mullein) (Holm et al. 1997; Parsons & Cuthbertson 2001).

SECTION 9 POTENTIAL FOR GENE TRANSFER

An extensive search of the scientific literature revealed no evidence of gene transfer occurring naturally either within the *Torenia* genus nor between *Torenia* spp. and related plant species (den Nijs et al. 2004; Fischer 2004; AGBIOS 2005). Since *T.* x *hybrida* is male sterile it is not able to transfer genes.

9.1 Crossing under experimental conditions

These crosses, while possible under controlled conditions, do not occur naturally and therefore are of significance only in the context of broadening an understanding of unaided gene transfer (OECD 2006).

In a large study looking at pollen tube growth, Kikuchi et al. (2001) used four species of torenia [*T. fournieri* (2n = 18), *T. baillonii* (2n = 16), *T. concolor* and *T.* x *hybrida* (2n = 26, or 52 if an amphidiploid)] and twelve species of other genera belonging to the Scrophulariacea (*Antirrhinum majus, Asarina barclaiana, Bacopa diffusus, Cymbalaria muralis, Diascia barberae, Linaria japonica, Mimulus hybridas, Mimulus nepalensis, Nemesia denticulata, Tetranema roseum, Veronica arvensis, Veronica persica*) in interspecific or intergeneric crosses (using only *T. fournieri* as the female parent). Freshly dehisced anthers were collected and hand-pollinated on stigmas of castrated flowers of *in vivo* grown plants.

The results can be summarised as follows

- While pollen tube growth, fertilisation and early embryogenesis occurred in all hybrid crosses between *T. fournieri, T. baillonii* and *T. concolor*, fertile seeds were obtained only in a *T. fournieri* (♀) x *T. baillonii* (♂) cross (n = 17). [It should be noted that while plants can be obtained from the cross between *T. fourneri* (♀) x *T. concolor* (♂) there is a requirement that after crossing the ovules are removed from ovaries and cultured *in vitro* (Miyazaki 1999). In the resulting *T.* x *hybrida* plants there is no natural mechanism for gene transfer between sexually compatible or incompatible species since this hybrid does not produce viable pollen or seeds]. The *T. fournieri* x *T. baillonii* hybrid has been used to study the behaviour of the centromeres of each genome during mitosis and meiosis as a model in interspecific hybridization (Kikuchi et al. 2007).

- Five classifications were made for the intergeneric crosses between *T. fournieri* and the 12 related species (none of the crosses resulted in fertilization):
    1. No pollen grain germination: *Asarina barclaiana; Linaria japonica; tetranema roseum; Veronica arvensis; Veronica persica*
    2. Pollen grain germination + 10% of pollen tubes grew a short distance down through the stigma: *Antirrhinum majus; Cymbalaria muralis*
    3. Pollen grain germination + 29% of pollen tubes grew a longer distance down through the stigma: *Diascia barberae; Namesia denticulata*
    4. Pollen grain germination + 61% of pollen tubes grew a short distance down style: *Bacopa diffusus; Mimulus nepalensis*
    5. Pollen grain germination + 65% of pollen tubes grew to the ovary: *Mimulus hybridas*

These results show the inherent difficulty of even interspecific crosses within *Torenia* spp. and the unlikelihood of successful intergeneric crosses involving *Torenia* spp.

SECTION 10 REFERENCES

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