Morphology and Inheritance of Double Floweredness in *Catharanthus roseus*

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Abstract. A double-flowered periwinkle [*Catharanthus roseus* (L.) G. Don.] mutant TYV1 was identified and the morphology and inheritance of the double-flowered phenotype was studied. TYV1 has an outer salverform whorl of petals and an additional inner funnel-shaped whorl of petals originating from the apex of the corolla. The apex of corolla tube forms a narrow opening. There are hairs under the opening at the apex. The stigma in this mutant is set below the anthers. The overlap between the top end of the pistil and bottom ends of anthers in TYV1 flowers at 1 to 2 days after anthesis is 0.56 ± 0.01 mm. TYV1 could be used as either the male or female parent in crossing. Self-pollinated TYV1 produced all double-flowered progeny compared with self-pollinated single-flowered cultivars Little Pinkie and Titan Burgundy, which produced all single-flowered progeny. F₁ plants between TYV1 and 'Little Pinkie' or 'Titan Burgundy' were all single. Three F₂ populations segregated into 3 single: 1 double ratio. Backcrossing F₁ to seed parents also indicated that a double-flowered form was controlled by a recessive allele. A single dominant gene expressed in the homozygous or heterozygous state resulted in the single-flowered phenotype. All the young seedlings of self-pollinated TYV1 and double-flowered progeny had distorted leaves before the sixth pair of leaves emerged.

Periwinkle [*Catharanthus roseus* (L.) G. Don.], a member of the Apocynaceae family, is endemic to Madagascar. This plant species is known for its production of terpenoid indole alkaloids that may be used to treat cardiac diseases and certain tumors in mammals (Zhou et al., 2009). Periwinkle has become pantropical by escaping from cultivation and has become naturalized in many tropical/subtropical regions (Levy, 1981). Periwinkle has been among the top ranked bedding/garden plants in the United States as a result of its tolerance to heat and drought, and air pollution (Howe and Waters, 1994; USDA, 2010).

The flower of periwinkle is morphologically close to cleistogamous (Miyajima, 2004). The stigmatic head, with sticky secretion, is normally below the anthers and takes up the shed pollen. Periwinkle is a self-compatible plant species. However, the receptive portion is mainly on the base of the stigmatic head and thus automatic intraflower self-pollination does not normally occur (Kulkarni et al., 2005; Sreevalli et al., 2000). Nectar-seeking pollinators with probosces such as butterflies and hawkmoths are required for effective pollination by pressing pollen from dehiscent anthers

to the basal stigma (Miyajima, 2004). Automatic intraflower self-pollination, however, occurred in cultivars/strains with the continued growth of the gynoecium beyond the base of the anthers, i.e., the overlap between stigma and anthers (Kulkarni et al., 2001, 2005; Miyajima, 2004).

Flower doubleness commonly increases the number of petals at the expense of anthers or carpels and thus affects the pollination mechanism (Comba et al., 1999). A related double-flowered variety of lesser periwinkle (*Vinca minor* fl. pl.) was found in the wild with partial or complete transformation of stamens in the third whorl into petaloid organs (Wang et al., 2011). Flower doubling has been reported to be controlled by genes, either recessive in *Nicotiana* (Zainol and Stimart, 2001) or dominant in *Petunia* (Sink, 1973). We found a double-flowered mutant of periwinkle and released a new double-flowered cultivar through crossing between the mutant and a line derived from a commercial cultivar (Chen and Yeh, 2012). We report the morphology and inheritance of flower doubleness in *Catharanthus roseus*.

Materials and Methods

Seeds of *Catharanthus roseus* 'Pacifica Polka Dot' were purchased from PanAmerican Seed and 900 seeds were sown at Taoyuan District Agricultural Research and Extension Station (TDARES). A double-flowered mutant was identified from the 'Pacifica Polka Dot' population. Because of showy double petals

and compact characteristics, the plant was selected, mass-propagated with tip cuttings, and labeled as TYV1 (Fig. 1A). A corolla tube of a flower was cut longitudinally 1 to 2 d after anthesis with a scalpel (Fig. 1B). Dissected flowers were observed under a binocular microscope (Model SMZ-U; Nikon Co., Tokyo, Japan) with a CCD digital camera (Optronics MicroFire® True Color Firewire Digital CCD Camera; Meyer Instruments Inc., Houston, TX). The overlap between the top end of the pistil and bottom ends of anthers was measured under the binocular microscope and calculated using image analysis software (Picture Frame 2.3; Optronics, CA).

TYV1 was self-pollinated and outcrossed with single-flowered, cutting-propagated *Catharanthus roseus* 'Titan Burgundy' and 'Little Pinkie' (seeds purchased from TAIHORT Inc., Taipei, Taiwan, and we propagated the seedlings by tip-cutting). Emasculatation and pollination followed the method described by Miyajima (2004). F₁ and F₂ progeny were produced. Additional crosses were made to facilitate the determination of double-flower inheritance (Tables 1 and 2).

Seeds were sown and young plants were planted in 12-cm diameter plastic pots each containing 3 sphagnum peat (Fafard No. 1; Conrad Fafard, Agawam, MA): 1 perlite (by volume). Plants were fertilized weekly with water-soluble 20N–8.6P–16.6K (Scotts, Marysville, OH) at 200 mg·L⁻¹ nitrogen. Plants were arranged in a completely randomized design and scored phenotypically for single or double flowers. Single-flowered plants were categorized as normal and double-flowered as possessing an additional whorl of petals. Pollination, plant raising, and evaluation were conducted between 2008 and 2011 under natural greenhouse conditions (20 to 30 °C, 12- to 13.5-h daylengths) at TDARES. Data were subjected to χ^2 test for goodness of fit to compare actual single to double flower ratios to expected ratios.

Results

The outer whorl of petals in *Catharanthus roseus* TYV1 is salverform, whereas the additional inner whorl of petals is funnel-shaped, originating from the apex of the corolla (Fig. 1). The apex of the corolla tube forms a narrow 1-mm opening (Fig. 2). There are hairs under the opening at the apex. A cylindrical corolla tube encloses five stamens and one pistil. The stigma is set below the anthers. The overlap between the top end of pistil and bottom ends of anthers in TYV1 flowers at 1 to 2 d after anthesis was 0.56 ± 0.01 mm.

Self-pollinated TYV1 produced all double-flowered progeny with the first five pairs of leaves showing distortion (Fig. 3A; Table 1). Plants had normal leaves after the sixth leaf pair. Self-pollinated single-flowered 'Little Pinkie' produced all single-flowered progeny (Table 1). All F₁ plants of TYV1 × 'Little Pinkie' were single-flowered and the F₂ generation, derived from TYV1 × 'Little Pinkie', fit a 3 single:1 double segregation

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Fig. 1. Intact flower (A) and longitudinal section of a flower (B) of TYV1, a double-flowered mutant of *Catharanthus roseus*.

Table 1. Segregation for flower form in progeny of self-pollinated and crossed TYV1, a double-flowered mutant of *Catharanthus roseus* and a single-flowered wild-type 'Little Pinkie'.

Parents/generation	Test ratio	Flower form ^z			
	– : +	–	+	χ^2	P
<i>Parents self-pollinated</i>					
TYV1	0: 1	0	61		All double
Little Pinkie	1: 0	362	0		All single
<i>F₁, F₂, and backcross</i>					
TYV1 × Little Pinkie					
F ₁	1: 0	141	0		All single
F ₂	3: 1	147	42	0.78	0.37
F ₁ × TYV1	1: 1	101	94	0.25	0.62
Little Pinkie × TYV1					
F ₁	1: 0	189	0		All single
F ₂	3: 1	126	33	1.53	0.22
F ₁ × Little Pinkie	1: 0	203	0		All single

^aSingle (–) or double (+) flower form.

Table 2. Segregation for flower form in progeny of self-pollinated and crossed TYV1, a double-flowered mutant of *Catharanthus roseus* and a single-flowered wild type 'Titan Burgundy'.

Parents/Generation	Test ratio	Flower form ^a			
	– : +	–	+	χ^2	P
<i>Parents self-pollinated</i>					
TYV1	0: 1	0	61		All double
Titan Burgundy	1: 0	182	0		All single
<i>F₁, F₂, and backcross</i>					
TYV1 × Titan Burgundy					
F ₁	1: 0	118	0		All single
F ₂	3: 1	138	27	6.56	0.01
F ₁ × TYV1	1: 1	63	54	0.69	0.41
Titan Burgundy × TYV1					
F ₁	1: 0	189	0		All single
F ₂	3: 1	112	31	0.84	0.36
F ₁ × Titan Burgundy	1: 0	162	0		All single

^aSingle (–) or double (+) flower form.

ratio ($\chi^2 = 0.78$, $P = 0.37$). Progeny from backcrosses of the F₁ generation to TYV1 segregated 1 single:1 double ($\chi^2 = 0.25$, $P = 0.62$). All F₁ plants of 'Little Pinkie' × TYV1 produced single-flowered progeny and the F₂ generation, derived from 'Little Pinkie' × TYV1, fit a 3:1 segregation ratio ($\chi^2 = 1.53$, $P = 0.22$). Backcrosses of the F₁ generation to 'Little Pinkie' produced all single-flowered progeny (Table 1).

Self-pollinated single-flowered 'Titan Burgundy' produced all single-flowered progeny (Table 2). All F₁ plants of TYV1 × 'Titan Burgundy' were single-flowered. The

F₂ generation, derived from TYV1 × 'Titan Burgundy', failed to fit the expected 3:1 segregation ratio. Progeny from backcrosses of the F₁ generation to TYV1 segregated 1 single:1 double ($\chi^2 = 0.69$, $P = 0.41$). All F₁ plants of 'Titan Burgundy' × TYV1 produced single-flowered progeny. The F₂ generation, derived from 'Titan Burgundy' × TYV1, fit a 3:1 segregation ratio ($\chi^2 = 0.84$, $P = 0.36$). Backcrosses of the F₁ generation to 'Titan Burgundy' produced all single-flowered progeny (Table 2).

Segregation of leaf growth and flower form in all the crossed progeny were identical

in that plants with distorted leaves during the one to five leaf-pair stage produced double flowers later on, whereas those with normal leaves were single-flowered (see example in Fig. 3B).

Discussion

The additional petals of *Catharanthus roseus* TYV1 originated from the apex of the corolla and did not develop at the expense of stamens or carpels (Fig. 1). In contrast, flower doubleness resulted from stamens in *Vinca minor* fl. pl. (Wang et al., 2011). The observation that the distal part of the upper corolla tube is thickened by an adaxial meristem in *Vinca rosea* L. (Boke, 1948) suggests that the additional whorl of petals in TYV1 might have originated from this meristem.

TYV1 could be used as either a male or female parent in crossing (Tables 1 and 2). The narrow opening of the corolla tube and hairs below the opening (Figs. 1 and 2) could block entry of alien pollen and protect the stamens and pistil as reported by Miyajima (2004). We have rarely seen automatic intra-flower self-pollinated capsules in TYV1, and even so, each capsule contains only two to three seeds as compared with 25 to 34 seeds in other commercial cultivars (Miyajima, 2004). Possible reasons for the limited self-pollination are as follows. The additional whorl of erect petals in TYV1 (Figs. 1 and 2) could serve as a barrier to pollinators to touching stigma and anthers through the narrow openings of the corolla tubes. The overlap between anthers and stigma after anthesis in TYV1 was ≈ 0.56 mm, close to the lower limit required for automatic intra-flower self-pollination in two periwinkle cultivars (Miyajima, 2004). Hence, automatic intraflower self-pollination does not occur because of spatial separation of the stigma and anthers. Three alleles at two loci were shown to determine the occurrence of automatic intraflower self-pollination in periwinkle through either ovary or style elongation (Kulkarni et al., 2005).

We propose that a nuclear recessive gene controls the double-flowering phenotype of *Catharanthus roseus* and that the dominant allele conditions single flowers in either the homozygous or heterozygous state. Segregation data obtained from F₁, F₂, and backcross families largely support the genetic model proposed (Table 1). Similar examples for a monogenic recessive gene conditioning flower doubling have been reported in *Nicotiana glauca* Link & Otto (Zainol and Stimart, 2001) and *Papaver somniferum* L. (Dhawan et al., 2007).

The only progeny that failed to fit the proposed genetic model was F₂ progeny of TYV1 × 'Titan Burgundy' (Table 2). The reason was unclear and environmental variables were unlikely to have caused the deviation because the plants were grown under similar greenhouse conditions. All the young seedlings of self-pollinated TYV1 and double-flowered progeny (Fig. 3) had distorted leaves

Fig. 2. Structure of the apex of a corolla tube of TYV1, a double-flowered mutant of *Catharanthus roseus*. Outer petal (op), inner petal (ip), hairs (h), anthers (a), and stigma (s). Bar = 1 mm.

Fig. 3. A young seedling derived from self-pollination of a double-flowered mutant (TYV1) (A) and a backcross generation between the double-flowered mutant, TYV1, and a single-flowered wild-type 'Titan Burgundy' segregating for the distorted/non-distorted leaf trait (B).

during vegetative growth. Ecker et al. (1994) have reported that the allele for distinct leaf morphology is tightly linked to the recessive allele for double flowering in stock [*Matthiola*

incana (L.) R. Br.]. It was possible that the double-floweredness allele was linked to the leaf distortion allele in *Catharanthus* as in the case of *Matthiola* or the allele itself was

responsible both for the petal and leaf morphogenesis. Growers or breeders might discard the periwinkle plants with distorted leaves before flowering and also their inability to set seeds by autogamy. This could partially explain why the double-flowered periwinkle cultivars are not common on the markets. We suggest tip-cutting propagation for the double-flowered periwinkles for commercial production because these plants produce few seeds.

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