HortScience 60(9):1441–1446. 2025. https://doi.org/10.21273/HORTSCI18737-25

Crossability and Inheritance of Leaf Traits in *Primulina*

Sheng-Wen Chen and Der-Ming Yeh
Department of Horticulture and Landscape Architecture, National Taiwan University, Taipei, 106319, Taiwan

Keywords. leaf margin, leaf vein, pollen fertility, pollen germination, reciprocal cross

Abstract. ***Primulina*** **plants exhibit diverse leaf shapes and venation patterns, demonstrate tolerance to low light conditions, and are commonly used as flowering foliage plants. However, scientific studies on their hybridization and the inheritance of leaf traits remain limited. In this study,** ***Primulina*** **species, cultivars, and hybrid progeny were investigated to evaluate pollen germination, crossability, and the inheritance of leaf characteristics. The results showed that self-pollination of the tested four cultivars Aiko, Nakako, New York, and Rachel or using them as pollen parents in crosses with the wild species resulted in no fruit set. However, using the four tested cultivars as seed parents in crosses with the wild species did result in fruit set. In BK medium culture, the pollen of the cultivars did not germinate, whereas the pollen of** ***P. liujianensis***, ***P. longgangensis***, ***P. ningmingensis***, **and** ***P. sinovietnamica*** **showed germination. Pollen from hybrids of same-clade species showed 9% to 37% germination, whereas no germination occurred in hybrids from different clades. The results of trait segregation ratio in the hybrid progeny showed that white leaf veins are dominant over green leaf vein. Netted white veins are dominant over veined white veins. Cleft leaves display incomplete dominance over the entire leaves, and crenate margins are dominant over the entire margins.**

Primulina (Gesneriaceae) comprises perennial herbaceous plants native to tropical and subtropical karst limestone regions particularly in southern China and northern Vietnam and is known for its high species diversity (Christenhusz and Byng 2016; Yang et al. 2023). Adapted to warm, low-light environments, these plants are widely used as ornamental potted plants due to their attractive foliage and flowers (Baptiste and Fang 2023; Shalit 2000).

Phylogenetic relationships significantly influence hybridization success and progeny fertility in Gesneriaceae ornamentals. Xu et al. (2019) classified key *Primulina* species into clades I, II-1, II-2, and II-3. Crosses between closely related, sympatric species *P. depressa* and *P. danxiaensis* resulted in F_1 and F_2 hybrids with reduced pollen fertility (Feng et al. 2020). *Sinningia speciosa* (Sinningia clade) crosses poorly with *S. eumorpha* (Dircaea clade), producing hybrids with low pollen fertility (Clayberg 1996; Perret et al. 2001). *Sinningia tubiflora* (Corytholoma clade), which normally produces ~20,000 seeds per capsule, yielded only one hybrid when crossed with *S. eumorpha* or *S. cardinalis* (Dircaea clade) (Clayberg 1996). Hybrid fertility correlates with parental phylogeny: intraclade hybrids (e.g., *S. eumorpha* × *S. cardinalis*) exhibited up to 70% pollen fertility, whereas interclade hybrids (e.g., *S. speciosa* × *S. aggregata*) showed less than 0.2% (Clayberg 1996; Perret et al. 2001).

Some *Primulina* species such as *P. pungentisepala*, display white-veined leaves caused by intercellular air spaces (Chen et al. 2022). In *Caladium* (Deng et al. 2008), *Dieffenbachia* (Henny 1983), and *Sinningia speciosa* (Kan et al. 2021), white veins are dominant over green. In *Saintpaulia*, serrated or ruffled leaf margins are dominant over smooth edges (Smith 2000). Traits such as white veins, lobed, and serrated leaves contribute to the ornamental appeal of *Primulina* (Weber et al. 2011), yet their genetic inheritance remains unclear. Understanding hybridization potential and the inheritance of leaf traits is essential for effective parent selection and breeding in *Primulina*. This study investigates these aspects using ten species and four cultivars, aiming to provide practical insights for ornamental plant breeders.

Materials and Methods

Expt. 1. Crosses between primulina species and cultivars. The species and cultivars tested included: *P. guizhongensis* (clade II-1), *P. heterochroa* (clade II-3), *P. hochiensis* (clade I), *P. huangii* (clade II-2), *P. liujiangensis* (clade II-1), *P. longgangensis* (clade II-3), *P. longzhouensis* (clade II-3), *P. ningmingensis* (clade II-3), *P. sinovietnamica* (clade II-3), *P. zhoui* (clade I), *P.* 'Aiko' [*P. lutea* (clade II-3) × *P. fimbrisepala*

Table 1. Fruit set percentage of crosses between tested *Primulina* species/cultivars.

Cross (cross no.)[i]	No. of pollination	Fruit set (%)
'Aiko' ⊗ (1)	2	0
'New York' ⊗ (2)	3	0
'Rachel' ⊗ (3)	2	0
'Aiko' × *P. longgangensis* (4)	1	100
P. longgangensis × 'Aiko' (5)	1	0
'Aiko' × *P. longzhouensis* (6)	1	100
P. longzhouensis × 'Aiko' (7)	1	0
'Nakako' × *P. liujiangensis* (8)	12	50
P. liujiangensis × 'Nakako' (9)	3	0
'Nakako' × *P. longzhouensis* (10)	1	0
P. longzhouensis × 'Nakako' (11)	1	0
'New York' × *P. liujiangensis* (12)	1	100
P. liujiangensis × 'New York' (13)	1	0
'New York' × *P. longgangensis* (14)	1	100
P. longgangensis × 'New York' (15)	1	0
'Rachel' × *P. longgangensis* (16)	4	100
P. longgangensis × 'Rachel' (17)	1	0

[i] Numbers in parentheses serve as cross-references within the text.

Table 2. Pollen germination percentage of interspecific *Primulina* hybrid progeny.

Cross (cross no.)[i]	Pollen germination (%) in BK medium at 25 °C
Intraclade[ii]	
P. longgangensis × *P. ningmingensis* (18)	30.3 b[iii]
P. longzhouensis × *P. ningmingensis* (19)	37.0 a
P. ningmingensis × *P. sinovietnamica* (20)	9.3 d
P. sinovietnamica × *P. longgangensis* (21)	20.0 c
Interclade	
P. hochiensis × *P. liujiangensis* (22)	0.0 e
P. hochiensis × *P. ningmingensis* (23)	0.0 e
P. liujiangensis × *P. ningmingensis* (24)	0.0 e
P. longgangensis × *P. guizhongensis* (25)	0.0 e

[i] Numbers in parentheses serve as cross-references within the text.
[ii] Clade I: *P. hochiensis*; clade II-1: (1) *P. guizhongensis* (2) *P. liujiangensis*; clade II-3: (1) *P. longgangensis* (2) *P. longzhouensis* (3) *P. ningmingensis* (4) *P. sinovietnamica.* Clade classification was based on Xu et al. (2019).
[iii] Mean separation within column by least significant difference test at $P < 0.05$.

Received for publication 20 May 2025. Accepted for publication 16 Jun 2025.
Published online 31 Jul 2025.
This paper is a portion of an MS thesis submitted by S.-W. Chen.
D.-M.Y. is the corresponding author. E-mail: dmyeh@ntu.edu.tw.
This is an open access article distributed under the CC BY-NC license (https://creativecommons.org/licenses/by-nc/4.0/).

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-09-04 via Open Access. This is an open access article distributed under the CC BY-NC license (https://creativecommons.org/licenses/by-nc/4.0/). https://creativecommons.org/licenses/by-nc/4.0/

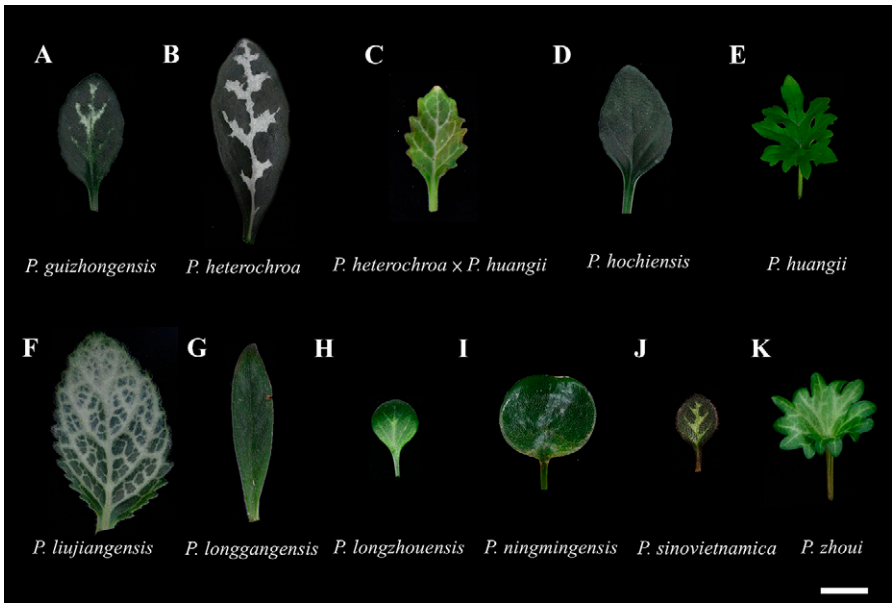

Fig. 1. Leaf traits observed in *Primulina* plants in this study. Leaf vein color: green vein (**D, E, G, I**), veined white vein (**A, H, J**), and netted white vein (**B, C, F, K**). Leaf lobing: deeply cleft (**E, K**) and shallow cleft (**C**). Crenate leaf margin (**F**). Bar = 2 cm.

(clade I)], *P.* 'Nakako' [*P. lutea* (clade II-3) × *P. dryas* (clade I)], *P.* 'New York' [*P. heterotricha* (clade II-3) hybrid], and *P.* 'Rachel' [*P. sclerophylla* (clade II-1) × *P. linearifolia* (clade II-3)]. One potted plant of each species was used to perform self- or cross-pollination (see Table 1 for crosses). Clade classification was based on the system proposed by Xu et al. (2019). The parentage of the four cultivars tested was obtained from The Gesneriad Society (2025).

Plants were grown in a controlled environment room maintained at an average temperature of 23.6 °C, under a light intensity of 26 μmol·m^{-2}·s^{-1} photosynthetic photon flux density (PPFD) at the canopy level, provided by fluorescent lamps (Master TL5 HE 28 W/865, Royal Philips Co., Amsterdam, The Netherlands) for 12 h per day. A 20N–8.6P–16.6K soluble fertilizer (Peters 20–20–20; The Scotts Co., Marysville, OH, USA) was applied weekly at a concentration of 1 g·L^{-1}, and plants were watered as needed. Emasculation was performed at or 1 d before anthesis by manually removing the corolla tube and attached stamens from the base of the flower, leaving the pistil intact. Pollen from flowers within 2 d of anthesis was applied to the stigma after style elongation and once the stigma had opened or secreted fluid. Pollination was conducted daily between 1300 and 1500 HR. The number of pollinated flowers varied due to inconsistencies in flower number on single plant and asynchronous flowering among different species/cultivars. In general, multiple flowers per plant were pollinated when possible. Fruit set was recorded based on ovary enlargement.

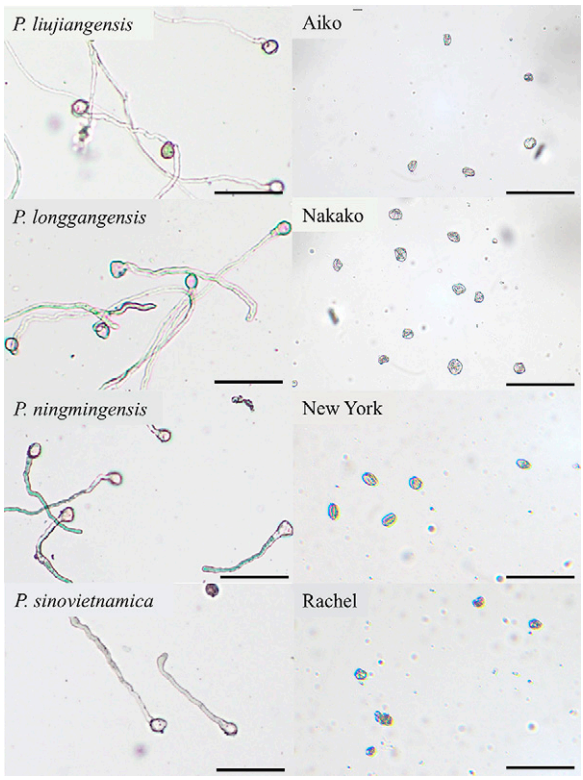

Fig. 2. Pollen germination of *Primulina* species and cultivars in BK medium cultured at 25 °C for 2 h. Bar = 100 μm. Note that pollen grains from the four cultivars were comparably shriveled or smaller than the four tested species.

Expt. 2. Pollen germination of primulina cultivars, species, and hybrid progeny. Preliminary observations from Expt. 1 showed that some crosses failed to set fruit, suggesting possible issues with pollen fertility. To investigate this, pollen germination was evaluated in four *Primulina* species, four cultivars, four intraclade hybrid progeny, and four interclade hybrid progeny (see Table 2). Fresh pollen was collected from flowers within 2 d of anthesis and cultured in modified BK (Brewbaker and Kwack 1963) medium, containing 300 mg·L^{-1} boric acid (H_3BO_3), 200 mg·L^{-1} magnesium sulfate ($MgSO_4{\cdot}7H_2O$), 300 mg·L^{-1} calcium nitrate [$Ca(NO_3)_2{\cdot}4H_2O$], and 100 mg·L^{-1} potassium nitrate (KNO_3), and 10% sucrose dissolved in deionized water. Medium pH was adjusted to 5.6–5.7 measured with a pH meter (Jenco Model 6171; Jenco Instruments Inc., San Diego, CA, USA). For each sample, pollen from one stamen was placed into a microtube, and 0.5 mL of the culture medium was added. The mixture was vortexed (G-650; Scientific Industries, Bohemia, NY, USA) and incubated in the dark at 25 °C for 2 h. Pollen germination was observed under a microscope (Nikon E600; Nikon Co., Tokyo, Japan), and images were captured using a digital microscope camera (MicroFire, Optronics, Fremont, CA, USA) with PictureFrame 2.3 software (Optronics). Pollen was considered germinated if the pollen tube length exceeded the diameter of the pollen grain. The experiment followed a completely randomized design with three replicates for each species, cultivar, and cross combination. Each microtube was treated as one replicate, and 100 pollen grains were assessed per replicate. The percentage of pollen germination was calculated as follows: pollen germination (%) = (number of germinated pollen grains/total number of pollen grains) × 100%. The difference in pollen germination among cross combinations was analyzed by the least significant difference at $P < 0.05$ using CoStat 6.4 (CoHort Software, Monterey, CA, USA). Percentage data were transformed using Bliss transformation tables before statistical analysis.

Expt. 3. Leaf traits investigation. The seeds obtained from interspecific crosses in

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-09-04 via Open Access. This is an open access article distributed under the CC BY-NC license (https://creativecommons.org/licenses/by-nc/4.0/). https://creativecommons.org/licenses/by-nc/4.0/

Table 3. Segregation of leaf vein color in progeny of *Primulina* crosses.

Cross (cross no.)[i]	Leaf vein: Netted ($V^r\_$)[ii]	Leaf vein: Veined (V^pV^p, V^pv)	Leaf vein: Green (vv)	Expected ratio	χ^2	P
White netted × White netted						
P. liujiangensis ⊗ (26)	34	0	10	3:0:1	0.121	0.728
P. liujiangensis × *P. heterochroa* (27)	19	0	0	1:0:0	0	1
White netted × White veined						
P. heterochroa × *P. sinovietnamica* (28)	70	0	0	1:0:0	0	1
P. zhoui × *P. longzhouensis* (29)	49	0	0	1:0:0	0	1
P. zhoui × *P. sinovietnamica* (30)	93	0	0	1:0:0	0	1
White veined × White netted						
P. guizhongensis × *P. liujiangensis* (31)	34	41	0	1:1:0	0.653	0.419
P. longzhouensis × *P. heterochroa* (32)	40	0	0	1:0:0	0	1
P. longzhouensis × *P. zhoui* (33)	8	0	0	1:0:0	0	1
White veined × White veined						
P. longzhouensis × *P. guizhongensis* (34)	0	21	0	0:1:0	0	1
P. longzhouensis × *P. sinovietnamica* (35)	0	74	0	0:1:0	0	1
P. sinovietnamica × *P. longzhouensis* (36)	0	40	0	0:1:0	0	1
White netted × Green						
P. liujiangensis × *P. longgangensis* (37)	12	0	11	1:0:1	0.043	0.835
P. liujiangensis × *P. ningmingensis* (38)	26	0	35	1:0:1	1.328	0.249
Green × White netted						
P. hochiensis × *P. liujiangensis* (39)	38	0	36	1:0:1	0.054	0.816
P. longgangensis × *P. heterochroa* (40)	53	0	0	1:0:0	0	1
P. ningmingensis × *P. zhoui* (41)	73	0	0	1:0:0	0	1
White veined × Green						
P. heterochroa × *P. huangii* (42)	0	8	0	0:1:0	0	1
P. longzhouensis × *P. ningmingensis* (43)	0	16	0	0:1:0	0	1
P. sinovietnamica × *P. longgangensis* (44)	0	23	0	0:1:0	0	1
P. sinovietnamica × *P. ningmingensis* (45)	0	104	0	0:1:0	0	1
Green × White veined						
P. longgangensis × *P. guizhongensis* (46)	0	127	0	0:1:0	0	1
P. longgangensis × *P. longzhouensis* (47)	0	70	0	0:1:0	0	1
P. longgangensis × *P. sinovietnamica* (48)	0	114	0	0:1:0	0	1
P. ningmingensis × *P. sinovietnamica* (49)	0	53	0	0:1:0	0	1
Green × Green						
P. hochiensis ⊗ (50)	0	0	35	0:0:1	0	1
P. longgangensis × *P. ningmingensis* (51)	0	0	90	0:0:1	0	1
P. ningmingensis ⊗ (52)	0	0	55	0:0:1	0	1
P. ningmingensis × *P. hochiensis* (53)	0	0	89	0:0:1	0	1

[i] Numbers in parentheses serve as cross-references within the text.
[ii] Listed in the parenthesis is the inferred genotype for leaf vein color.

Expt. 1 were sown in 6-cm plastic petri dishes filled with fine-grain akadama soil (Sanbonsen Shosei Akadama; Plantation Iwamoto Co., Ibaraki, Japan). Once the seedlings developed one pair of leaves, they were transplanted into 288-cell tray filled with a 1:1 mixture of peat (Potgrond; Klasmann-Deilmann, Geeste, Germany) and fine-grain akadama soil.

Table 4. Segregation of leaf lobing in progeny of *Primulina* crosses.

Cross (cross no.)[i]	Leaf lobing: Deeply cleft (CC)[ii]	Leaf lobing: Shallow cleft (Cc)	Leaf lobing: Entire (cc)	Expected ratio	χ^2	P
Deeply cleft × Deeply cleft						
P. huangii ⊗ (54)	5	0	0	1:0:0	0	1
Deeply cleft × Entire						
P. zhoui × *P. longgangensis* (55)	0	3	0	0:1:0	0	1
P. zhoui × *P. longzhouensis* (56)	0	49	0	0:1:0	0	1
P. zhoui × *P. sinovietnamica* (57)	0	93	0	0:1:0	0	1
Entire × Deeply cleft						
P. heterochroa × *P. huangii* (58)	0	8	0	0:1:0	0	1
P. longzhouensis × *P. zhoui* (59)	0	8	0	0:1:0	0	1
P. ningmingensis × *P. zhoui* (60)	0	73	0	0:1:0	0	1
Entire × Entire						
P. heterochroa × *P. sinovietnamica* (61)	0	0	70	0:0:1	0	1
P. longgangensis × *P. guizhongensis* (62)	0	0	127	0:0:1	0	1
P. longgangensis × *P. longzhouensis* (63)	0	0	70	0:0:1	0	1
P. longgangensis × *P. ningmingensis* (64)	0	0	90	0:0:1	0	1
P. longgangensis × *P. sinovietnamica* (65)	0	0	114	0:0:1	0	1
P. ningmingensis × *P. hochiensis* (66)	0	0	80	0:0:1	0	1
P. sinovietnamica × *P. ningmingensis* (67)	0	0	104	0:0:1	0	1

[i] Numbers in parentheses serve as cross-references within the text.
[ii] Listed in the parenthesis is the inferred genotype for leaf lobing.

Plants were fertilized weekly with 1 $g \cdot L^{-1}$ of a 20N–8.6P–16.6K soluble fertilizer (Peters 20–20–20, The Scotts Co.). When seedlings produced three to four pairs of leaves, they were transplanted into 9-cm pots containing a 1 peat:1 perlite (No. 2; Nanhai Vermiculite Industrial Co., New Taipei City, Taiwan) mixture and grown in a greenhouse. The average temperature was 27.1 °C, the average daily light intensity was 127.7 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD, and the daylength averaged 12.2 h during the experiment.

When progeny developed seven to nine pairs of leaves, traits including leaf vein color, leaf lobing, and leaf margin crenation (Fig. 1) were recorded. The distribution of white leaf veins was categorized as either netted or veined. Netted white veins extended from the midrib through the primary, secondary, and tertiary veins, whereas veined patterns were limited to the midrib and lateral veins. Leaf lobing was classified into three categories: deeply cleft, shallow cleft, and entire. Deeply cleft leaves had lobes separated by pronounced indentations, whereas shallow cleft leaves exhibited only slight separation between lobes. Entire leaves lacked lobes or indentations. Chi-square analysis was used to

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-09-04 via Open Access. This is an open access article distributed under the CC BY-NC license (https://creativecommons.org/licenses/by-nc/4.0/). https://creativecommons.org/licenses/by-nc/4.0/

Table 5. Segregation of leaf margin in progeny of *Primulina* crosses.

Cross (cross no.)[i]	Leaf margin		Expected ratio	χ^2	*P*
	Crenate (*Cr_*)[ii]	Entire (*crcr*)			
Crenate × Crenate					
P. liujiangensis ⊗ (68)	44	0	1:0	0	1
Crenate × Entire					
P. liujiangensis × *P. heterochroa* (69)	7	0	1:0	0	1
P. liujiangensis × *P. ningmingensis* (70)	61	0	1:0	0	1
Entire × Crenate					
P. guizhongensis × *P. liujiangensis* (71)	74	0	1:0	0	1
P. hochiensis × *P. liujiangensis* (72)	74	0	1:0	0	1
P. ningmingensis × *P. liujiangensis* (73)	30	0	1:0	0	1
Entire × Entire					
P. heterochroa × *P. sinovietnamica* (74)	0	70	0:1	0	1
P. longgangensis × *P. guizhongensis* (75)	0	127	0:1	0	1
P. longgangensis × *P. heterochroa* (76)	0	53	0:1	0	1
P. longgangensis × *P. longzhouensis* (77)	0	70	0:1	0	1
P. longgangensis × *P. ningmingensis* (78)	0	90	0:1	0	1
P. longzhouensis × *P. heterochroa* (79)	0	40	0:1	0	1
P. ningmingensis × *P. hochiensis* (80)	0	89	0:1	0	1

[i] Numbers in parentheses serve as cross-references within the text.
[ii] Listed in the parenthesis is the inferred genotype for leaf margin.

test the goodness-of-fit between observed and expected segregation ratios for each trait. The chi-square value and corresponding probability (*P* value) were calculated to determine statistical significance.

Results

Fruit set from various cross combinations. No fruit set was observed following self-pollination of the three cultivars [crosses 1–3 (Table 1)]. Reciprocal crosses showed that viable fruit set occurred only when the cultivar was used as the seed parent, with the exception of the cross combination between 'Nakako' and *P. longzhouensis*, which did not produce any fruit set (crosses 10). In contrast, when the cultivar served as the pollen parent in crosses with species, no fruit set occurred (crosses 4–17).

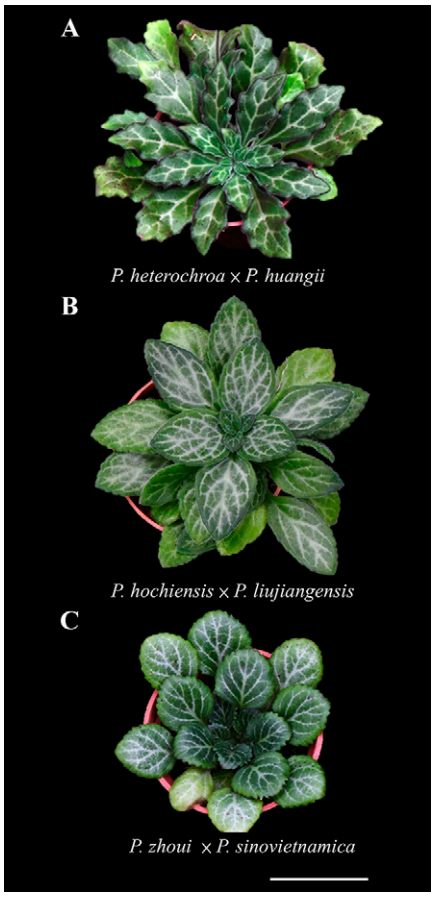

Fig. 3. Hybrid progeny from interspecific crosses. *P. heterochroa* × *P. huangii* (**A**); *P. hochiensis* × *P. liujiangensis* (**B**), and *P. zhoui* × *P. sinovietnamica* (**C**). Bar = 5 cm.

Pollen germination of primulina cultivars, species, and hybrid progeny. Fresh pollen cultured in BK medium germinated successfully in all four tested species but failed to germinate in the four tested cultivars (Fig. 2). Pollen grains from the cultivars appeared smaller or shriveled compared with those from the species.

The progeny from intraclade crosses [crosses 18–21 (Table 2)] exhibited pollen germination percentages ranging from 9.3% to 37.0%, whereas no germination was observed in progeny from interclade crosses (crosses 22–25).

Inheritance of leaf traits in F_1 progeny. Progeny from selfing white-netted *P. liujiangensis* segregated into a 3:1 ratio of white-netted veins: green veins [cross 26 (Table 3)]. Progeny from crosses between white-netted and white-veined species predominantly exhibited white-netted veins (crosses 27–30, 32–33), except for *P. guizhongensis* × *P. liujiangensis* (cross 31), which segregated into a 1:1 ratio of white-netted veins: white-veined veins. Crosses between white-veined species yielded progeny with only white-veined veins (crosses 34–36). Crosses between *P. liujiangensis* and green-veined species showed a 1:1 segregation ratio of white-netted: green veins (crosses 37–39). With the exception of the cross involving *P. liujiangensis*, progeny from crosses between white-netted and green-veined species all exhibited white-netted veins (crosses 40–41). Progeny from crosses between white-veined and green-veined species were all white-veined (crosses 42–49). Progeny from selfing or crosses between green-veined species all exhibited only green veins (crosses 50–53). These results suggest that white veins, whether netted or veined, are dominant over green veins in *Primulina,* with the white-netted type exhibiting dominance over the white-veined type. We propose that V^f and V^p represent the netted and veined types, respectively, as alleles at the *V* locus, with *v* representing green veins.

Progeny from selfing *P. huangii* all produced deeply cleft leaves, as observed in the parent [(cross 54) Table 4]. Progeny from crosses between *P. zhoui* (deeply cleft leaf) and three species with non-cleft (entire) leaves exhibited shallow cleft leaves (crosses 55–60). Progeny from crosses between noncleft species all produced entire leaves (crosses 61–67).

Progeny resulting from selfing *P. liujiangensis*, which has crenate leaf margins, as well as from interspecific crosses involving *P. liujiangensis*, all exhibited crenate margins [crosses 68–73 (Table 5)]. Progeny from crosses between species with entire leaf margins uniformly displayed entire margins (crosses 74–80). These results suggest that crenate leaf margins (*Cr_*) are dominant over entire margins (*crcr*). Both homogeneous (*CrCr*) or heterogeneous (*Crcr*) genotypes exhibit similar crenate leaf margins.

Discussion

Reciprocal crosses between *Primulina* cultivars and species resulted in various fruit set percentages (Table 1). Similar variations in fruit set percentages in reciprocal crosses have been reported in other ornamental plants, such as *Neoregelia* bromeliad (Liang et al. 2020) and *Angelonia* (Tsai et al. 2024). Pollen from the tested cultivars failed to germinate and no fruit set was observed following self-pollination of *Primulina* cultivars or the cultivars served as the pollen parent in crosses with species (Table 1; Fig. 2). The difference in fruit set percentages between reciprocal crosses in *Primulina* is likely due to pollen sterility, a mechanism distinct from that in *Neoregelia*, where it is caused by pollen tube and the style interaction (Liang et al.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-09-04 via Open Access. This is an open access article distributed under the CC BY-NC license (https://creativecommons.org/licenses/by-nc/4.0/). https://creativecommons.org/licenses/by-nc/4.0/

Table 6. Phenotype and proposed genotypes of *Primulina* species based on selfing or F_1 crosses.

	Leaf vein		Leaf lobing		Leaf margin	
Species	Phenotype	Genotype	Phenotype	Genotype	Phenotype	Genotype
Clade I						
P. hochiensis	Green	*vv*	Entire	*cc*	Entire	*crcr*
P. zhoui	Netted white	V^fV^f	Deeply cleft	*CC*	Entire	*crcr*
Clade II-1						
P. guizhongensis	Veined white	V^pV^p	Entire	*cc*	Entire	*crcr*
P. liujiangensis	Netted white	V^fv	Entire	*cc*	Crenate	*CrCr*
Clade II-2						
P. huangii	Green	*vv*	Deeply cleft	*CC*	Entire	*crcr*
Clade II-3						
P. heterochroa	Netted white	V^fV^f	Entire	*cc*	Entire	*crcr*
P. longgangensis	Green	*vv*	Entire	*cc*	Entire	*crcr*
P. longzhouensis	Veined white	V^pV^p	Entire	*cc*	Entire	*crcr*
P. ningmingensis	Green	*vv*	Entire	*cc*	Entire	*crcr*
P. sinovietnamica	Veined white	V^pV^p	Entire	*cc*	Entire	*crcr*

2020), or in *Angelonia*, where it results from various ploidy levels (Tsai et al. 2024).

The progeny from intraclade crosses exhibited pollen germination percentage ranging from 9.3% to 37.0%, whereas no germination was observed in progeny from interclade crosses (Table 2). These results suggest that the tested *Primulina* cultivars, derived from interclade hybridization (The Gesneriad Society 2025), exhibit male sterility and can function only as maternal parents in breeding programs. A similar phenomenon has been reported in *Sinningia*, where significantly higher pollen fertility was observed in progeny derived from crosses between the same clade, but very low pollen fertility in progeny from interclade crosses (Clayberg 1996; Perret et al. 2001).

Crossability can also be affected by differences in chromosome numbers. Crossing between parents with different chromosome numbers or ploidy levels can lead to male sterility (Rieseberg and Carney 1998). However, Kang et al. (2014) reported that all 61 *Primulina* species examined share the same chromosome number ($2n = 36$). Furthermore, Feng et al. (2020) demonstrated that progeny sterility can occur even between closely related *Primulina* species, suggesting that chromosome number imbalance is not the primary factor affecting crossability.

In *Primulina*, white leaf veins are complete dominant over green leaf veins and are likely governed by a single locus with multiple alleles (Table 3). A similar genetic model has been reported in *Caladium* (Deng et al. 2008), *Dieffenbachia* (Henny 1983), and *Sinningia speciosa* (Kan et al. 2021). *Primulina* species with white-veined leaves are theoretically expected to be homozygous, but interestingly, when *P. liuliangensis* was crossed with green-veined wild types, progeny with green veins did appear (Table 3). In China, *P. liuliangensis* individuals with green veins have also been documented (Plant Photo Bank of China 2025), suggesting that the white-vein trait in *P. liuliangensis* is heterozygous. In *Primulina pungentisepala*, the formation of white leaf veins was found to result from the development of air spaces between water storage tissue, spherical palisade tissue cells, and reduced chlorophyll content (Chen et al. 2022). Further research is required to determine whether these mechanisms or other factors contribute to the variation in white vein distribution observed in this study.

Cleft leaf shape exhibits incomplete dominance over the entire leaf shape in *Primulina* (Table 4). In zucchini (*Cucurbita pepo*), crosses between lobed-leaf and entire-leaf plants also produced intermediate leaf types in the F_1 generation, and the F_2 generation segregated into a 1 lobed:2 intermediate: 1 entire leaf ratio, indicating that lobed leaves are controlled by a single gene with incomplete dominance (Bo et al. 2022).

Among the tested accessions, only *P. liujiangensis* exhibited crenated leaf margins. When crossed with species possessing entire leaf margins, all progeny displayed a similar crenated margin phenotype (Table 5). This is similar to another Gesneriaceae ornamental, the African violet (*Saintpaulia*), where different cultivars may exhibit serrated or ruffled leaf margins, traits that are dominant over entire margins (Smith 2000). In basil (*Ocimum basilicum*), leaf margins can also be categorized as either entire or serrated. Crosses between entire- and serrated-margined plants produced F_1 progeny with all entire margins, whereas F_2 progeny segregated in a 3 entire: 1 serrated ratio, indicating that serrated margins are recessive in basil (Phippen and Simon 2000).

Distinct hybrid progeny were obtained from various interspecific crosses, each exhibiting dominant traits inherited from their respective parents. For example, the progeny resulting from the cross between *P. heterochroa* and *P. huangii* (Fig. 3A) displayed netted white veins, derived from *P. heterochroa* (Fig. 1B), and a shallower leaf cleft, inherited from *P. huangii* (Fig. 1E). Similarly, the hybrid progeny (Fig. 3B) from *P. hochiensis* (Fig. 1D), which has green veins, and *P. liujiangensis* (Fig. 1F), characterized by netted white veins and crenate leaf margin, exhibited netted white veins and crenate margin. In another cross between *P. zhoui* (Fig. 1K) and *P. sinovietnamica* (Fig. 1J), a species with veined white veins and entire leaves, the resulting progeny (Fig. 3C) also displayed pronounced netted white veins and a shallow leaf cleft—traits distinct from either parent. This report provides valuable information for effective parent selection and breeding in *Primulina*. The inferred genotypes of leaf traits for the ten *Primulina* species tested are summarized in Table 6. Understanding the inheritance of leaf traits can help improve breeding efficiency and facilitate the development of new cultivars with desirable characteristics.

References Cited

Baptiste FJ, Fang JY. 2023. Study on pollen viability and stigma receptivity throughout the flowering period in the selected taxa of the Gesneriaceae family. Folia Hortic. 35(1):123–133. https://doi.org/10.2478/fhort-2023-0009.

Bo K, Duan Y, Qiu X, Zhang M, Shu Q, Sun Y, He Y, Shi Y, Weng Y, Wang C. 2022. Promoter variation in a homeobox gene, *CpDll*, is associated with deeply lobed leaf in *Cucurbita pepo* L. Theor Appl Genet. 135(4):1223–1234. https://doi.org/10.1007/s00122-021-04026-3.

Brewbaker JL, Kwack BH. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. Am J Bot. 50(9):859–865. https://doi.org/10.1002/j.1537-2197.1963.tb06564.x.

Chen J, Li Y, He D, Bai M, Li B, Zhang Q, Luo L. 2022. Cytological, physiological and transcriptomic analysis of variegated leaves in *Primulina pungentisepala* offspring. BMC Plant Biol. 22(1):419. https://doi.org/10.1186/s12870-022-03808-1.

Christenhusz MJ, Byng JW. 2016. The number of known plants species in the world and its annual increase. Phytotaxa. 261(3):201. https://doi.org/10.11646/phytotaxa.261.3.1.

Clayberg CD. 1996. Interspecific hybridization in *Sinningia* (Gesneriaceae). Baileya. 23:184–194.

Deng Z, Goktepe F, Harbaugh BK. 2008. Inheritance of leaf spots and their genetic relationships with leaf shape and vein color in caladium. J Am Soc Hortic Sci. 133(1):78–83. https://doi.org/10.21273/JASHS.133.1.78.

Feng C, Yi H, Yang L, Kang M. 2020. The genetic basis of hybrid male sterility in sympatric *Primulina* species. BMC Evol Biol. 20(1):1–12. https://doi.org/10.1186/s12862-020-01617-4.

Henny RJ. 1983. Inheritance of the white foliar midrib in *Dieffenbachia* and its linkage with the gene for foliar variegation. J Hered. 74(6):483–484. https://doi.org/10.1093/oxfordjournals.jhered.a109848.

Kan PW, Cheng YC, Yeh DM. 2021. Mechanism of leaf vein coloration and inheritance of leaf vein color, flower form, and floral symmetry in

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-09-04 via Open Access. This is an open access article distributed under the CC BY-NC license (https://creativecommons.org/licenses/by-nc/4.0/). https://creativecommons.org/licenses/by-nc/4.0/

gloxinia. J Am Soc Hortic Sci. 146(3):178–183. https://doi.org/10.21273/JASHS05034-20.
Kang M, Tao J, Wang J, Ren C, Qi Q, Xiang QY, Huang H. 2014. Adaptive and nonadaptive genome size evolution in Karst endemic flora of China. New Phytol. 202(4):1371–1381. https://doi.org/10.1111/nph.12726.
Liang CC, Wei TY, Yeh DM. 2020. Pollination timing and hybrid seed production of *Neoregelia*. HortScience. 55(12):1970–1973. https://doi.org/10.21273/HORTSCI15385-20.
Perret M, Chautems A, Spichiger R, Peixoto M, Savolainen V. 2001. Nectar sugar composition in relation to pollination syndromes in *Sinningieae* (Gesneriaceae). Ann Bot. 87(2):267–273. https://doi.org/10.1006/anbo.2000.1331.
Phippen WB, Simon JE. 2000. Anthocyanin inheritance and instability in purple basil (*Ocimum basilicum* L.). J Hered. 91(4):289–296. https://doi.org/10.1093/jhered/91.4.289.
Plant Photo Bank of China 2025. *Primulina liujiangensis*. https://ppbc.iplant.cn/sp/291915 [accessed 18 May 2025].
Rieseberg LH, Carney SE. 1998. Plant hybridization. New Phytol. 140(4):599–624. https://doi.org/10.1046/j.1469-8137.1998.00315.x.
Shalit P. 2000. Breeding gesneriads, p 155–173. In: Callaway DJ, Callaway MB (eds). Breeding Ornamental Plants. Timber Press, Portland, OR, USA.
Smith JL. 2000. Breeding African violets, p 133–154. In: Callaway DJ, Callaway MB (eds). Breeding Ornamental Plants. Timber Press, Portland, OR, USA.
The Gesneriad Society 2025. *Primulina* (*Chirita*) 'Nakako'. https://gesneriadsociety.org/registry/primulina-chirita-nakako/. [accessed 18 May 2025].
Tsai TS, Liao YC, Wei TY, Yeh DM. 2024. Triploid formation and heat tolerance of *Angelonia angustifolia* with various ploidy levels. HortScience. 59(11):1656–1660. https://doi.org/10.21273/HORTSCI18154-24.
Weber A, Middleton DJ, Forrest A, Kiew R, Lim CL, Rafidah AR, Sontag S, Triboun P, Wei YG, Yao TL, Möller M. 2011. Molecular systematics and remodelling of *Chirita* and associated genera (Gesneriaceae). Taxon. 60(3):767–790. https://doi.org/10.1002/tax.603012.
Xu WB, Chang H, Huang J, Chung KF. 2019. Molecular systematics of Chiritopsis-like *Primulina* (Gesneriaceae): One new species, one new name, two new combinations, and new synonyms. Bot Stud. 60(1):18–21. https://doi.org/10.1186/s40529-019-0266-x.
Yang ZM, Chou WC, Maciejewski S, Wei YG. 2023. *Primulina nymphaeoides* (Gesneriaceae), a new species from Guangxi, China. Taiwania. 68:44–50. https://doi.org/10.6165/tai.2023.68.44.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-09-04 via Open Access. This is an open access article distributed under the CC BY-NC license (https://creativecommons.org/licenses/by-nc/4.0/). https://creativecommons.org/licenses/by-nc/4.0/