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Yuan Chun: A New *Paphiopedilum* Cultivar

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Paphiopedilum, also known as slipper orchids, are superior and unique ornamental flowers admired around the world. They have a unique saccular lip, bright and beautiful colors, and are long lived (Cribb, 1998; Liu et al., 2009). Several species, including Paphiopedilum armeniacum, Paphiopedilum micranthum, and Paphiopedilum malipoense, have frequently won awards at world horticultural fairs. However, the genus Paphiopedilum is also one of the most endangered plant groups in the world and is acknowledged as the "Flagship" genus of orchid conservation (Zeng et al., 2016). All wild species of Paphiopedilum are listed in Appendix I of the CITES (Convention on International Trade in Endangered Species) and their trade is prohibited. Breeding of superior cultivars can be used in commercial production to reduce the overmining of wild species and provide an alternative strategy for conservation. At the South China Botanical Garden (SCBG), researchers have successively bred and released 10 new cultivars of Paphiopedilum, including SCBG Yingchun (Deng et al., 2017), SCBG Lüfeicui (He et al., 2017), SCBG Ziguang (Wen et al., 2019),

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SCBG Zijun (Mao et al., 2019), SCBG Chunyun (Xu et al., 2020), SCBG Wenfei (Wu et al., 2020), Zhongke Huanghou (Xie et al., 2021), and Zhongke Ziban (Fu et al., 2021). Particularly, the new cultivars SCBG Purple Spots and SCBG Star, also bred at the SCBG, have won the special award at the World Horticultural Expo 2019, Beijing, China.

Here, we report the release of the new *Paphiopedilum* cultivar Yuan Chun (Yue Ping Hua 20210023), which blooms from January to April, precisely during the Spring Festival of China and until Qingming Festival. This newly bred cultivar was developed from an interspecific cross between *Paphiopedilum villosum* and *Paphiopedilum spicerianum*, both of which flower from December through February.

Origin

Parents. In Dec 2008, 10 individuals of P. villosum and 20 individuals of P. spice-rianum were introduced from Yunnan Baoshan Lvboyuan Flower Company and planted in a greenhouse at the SCBG of the Chinese Academy of Sciences. An outstanding, healthy P. villosum 'V-1' individual with vigorous growth and bright purple stripes on

sepals and petals was selected as the female parent, and a healthy and excellent *P. spiceria-num* 'Z-1' was selected as the male parent. More than 95% of the inbred offspring of both *P. villosum* 'V-1' and *P. spicerianum* 'Z-1' were consistent and stable from breeding through selfing.

Breeding process. P. villosum 'V-1' (Fig. 1A), was used as female parent for crossing to P. spicerianum 'Z-1' (Fig. 1B) in Dec 2013. Immature fruits were collected in Aug 2014. The method of Zeng et al. (2012) was used for asymbiotic seed germination and seedling cultivation. Then, in Dec 2016, the first 1000 F₁ in vitro seedlings were obtained and planted for phenotypic observation. In Dec 2017, anther 1000 F₁ in vitro seedlings from the second hybridization in 2014 were obtained and planted for phenotypic observation. The first and second batches of hybrid offspring bloomed for the first time in Jan 2019 and Jan 2020, respectively. From 2019 to 2020, multiple-site experiments were conducted in a greenhouse at SCBG and at Foshan planting base of Guangdong Huada Agricultural Development Co., Ltd. The data obtained from 2019 to 2020 showed that the F₁ hybrid plants were consistent and stable, and showed vigorous growth, high resistance, beautiful flower shape, bright color, and a longer flowering period. Furthermore, the flowering season coincided with the Spring Festival. Therefore, this new cultivar was officially authorized by the Guangdong Crop Variety Approval Committee (Yue Ping Hua 20210023) and named 'Yuan Chun' (Fig. 1C), on 31 Aug 2021.

Description

In 2020, 100 plants of the *P*. Yuan Chun cultivar and each of its parents, *P. villosum* and *P. spicerianum*, were planted in the greenhouse at SCBG. Eighteen morphological characters (Table 1) were recorded for 30 randomly selected plants (i.e., three replicates with 10 plants/replicate). Flower color was rated according to the Royal Horticultural Society (RHS) Color Chart. One-way analysis of variance was conducted on the data collected using SPSS Statistics 24.0 (IBM Corp, Armonk, NY, USA).

The morphological traits of cultivar Yuan Chun, including plant width, number of leaves per plant, leaf length, leaf width, and leaf thickness, did not significantly vary with respect to the female parent, *P. villosum* (Table 1). But cultivar Yuan Chun shows better flowering; each inflorescence in the parent plants *P. villosum*









Fig. 1. New cultivar Yuan Chun and its parents. Single flower of female parent *Paphiopedilum villosum* (A), male parent *Paphiopedilum spicerianum* (B), and new cultivar Yuan Chun (C). The potted plants of new cultivar Yuan Chun and its parents (D).

Table 1. Morphological traits of its parents *Paphiopedilum villosum* and *Paphiopedilum spicerianum* and new cultivar Yuan Chun.

Trait ⁱ	P. villosum	P. spicerianum	Yuan Chun
Plant height (cm)	$20.51 \pm 3.48 \text{ a}$	$6.79 \pm 0.98 \text{ c}$	12.96 ± 1.09 b
Plant width (cm)	$35.21 \pm 4.31 \text{ a}$	$30.12 \pm 3.31 \text{ b}$	$41.43 \pm 8.11 \text{ a}$
Scape length (cm)	$34.85 \pm 5.14 a$	$27.90 \pm 4.61 \text{ a}$	$30.52 \pm 2.89 \text{ a}$
Scape thick (cm)	4.45 ± 0.23 a	$2.64 \pm 0.47 \text{ b}$	3.24 ± 0.60 ab
Number of flowers	$1.00 \pm 0.00 \text{ a}$	$1.00 \pm 0.00 \text{ a}$	$1.33 \pm 0.58 \text{ a}$
per plant (cm)			
Flower transverse	$8.86 \pm 0.60 \text{ a}$	$7.03 \pm 1.12 \text{ b}$	8.68 ± 0.76 ab
diameter (cm)			
Flower longitudinal	$9.17 \pm 0.30 \text{ b}$	$7.08 \pm 1.07 \text{ a}$	$11.07 \pm 1.21 \text{ c}$
diameter (cm)			
Middle sepal length (cm)	$5.69 \pm 0.87 \text{ a}$	$3.39 \pm 0.73 \text{ b}$	$6.21 \pm 0.30 \text{ a}$
Middle sepal width (cm)	$4.27 \pm 0.56 \text{ b}$	$4.99 \pm 0.62 \text{ b}$	$6.09 \pm 0.15 \text{ a}$
Petal length (cm)	$6.88 \pm 0.29 \text{ a}$	$4.15 \pm 0.60 \text{ b}$	6.64 ± 0.24 a
Petal width (cm)	$3.14 \pm 0.22 \text{ a}$	$1.11 \pm 0.19 \text{ b}$	$2.86 \pm 0.03 \text{ a}$
Petal thickness (mm)	$0.57 \pm 0.02 \text{ a}$	$0.41 \pm 0.03 \ c$	$0.52 \pm 0.02 \text{ b}$
Lip length (cm)	$53.77 \pm 2.23 \text{ a}$	$42.09 \pm 3.44 \text{ b}$	$52.76 \pm 1.62 \text{ a}$
Lip width (cm)	$27.28 \pm 3.21 \text{ ab}$	$23.74 \pm 2.63 \text{ b}$	$29.94 \pm 2.11 a$
Number of leaves per plant	$3.67 \pm 0.58 \text{ a}$	$4.33 \pm 0.58 \text{ a}$	$4.33 \pm 0.58 \text{ a}$
Leaf length (cm)	$32.24 \pm 4.37 \text{ a}$	$20.03 \pm 2.14 \text{ b}$	$35.68 \pm 2.45 \text{ a}$
Leaf width (cm)	$3.63 \pm 0.68 \text{ ab}$	$2.94 \pm 0.31 \text{ b}$	4.26 ± 0.17 a
Leaf thickness (mm)	$2.25 \pm 0.47 \text{ a}$	$1.33 \pm 0.35 \text{ b}$	$2.42 \pm 0.23 \text{ a}$
Flowering period (day)	$40.67 \pm 1.15 \text{ b}$	$34.5 \pm 3.95 \text{ a}$	$54 \pm 5.21 \text{ c}$

¹ Data were collected from 30 randomly selected plants (10 plants/replication in three replications) at the flowering stages of each genotype in South China Botanical Garden, Chinese Academy of Sciences, from 2019 to 2020.

Different letters in the same line indicate significant differences for the same traits between 'Yuan Chun' and its parents (P < 0.05).

and P. spicerianum had a single flower, but a few individuals of cultivar Yuan Chun showed two flowers. Especially, the flower shape of cultivar Yuan Chun differed significantly from that of both parents (Figs. 1-3). The middle sepal is the most different floral part: that of 'Yuan Chun' is longer (6.21 \pm 0.30 cm) and wider $(6.09 \pm 0.15 \text{ cm})$ than both of its parents. The position of the petals was also different, with the petals of cultivar Yuan Chung tilting down 45° at full bloom, whereas petals of the female parent are horizontal and those of the male parent are slightly tilted down. Flower longitudinal diameter and lip width in plants of cultivar Yuan Chun were larger than those of both parents as well (Figs. 1-3). As to the length of flowering period, that of cultivar Yuan Chun (54 \pm 5.21 d) was significantly longer than those of P. villosum (40.67 \pm 1.15 d) and P. spicerianum $(34.5 \pm 3.95 \text{ d})$ (Table 1). Also, the flowering time of cultivar Yuan Chun is from January to April, precisely at the time of the Chinese Spring Festival, whereas both parents flower from December to February.

The colors of 'Yuan Chun' are markedly different from those of both parents (Fig. 1).

The dorsal sepal of *P. villosum* is moderately brown (RHS 200D) in the middle, surrounded by a yellow-green (RHS 150 A) circle, with a white (RHS 155 C) edge; the midrib of the dorsal sepal is dark grayreddish-brown (RHS 200A). The petals are yellow (RHS 11A) with vivid red (RHS 44A) stripes and moderately red (RHS N34A) midveins. The lip is brilliant yellow (RHS 11A) with grayed-purple (RHS 185A) grouped spots. The synsepal is yellow-green (RHS N144C), with dense, moderately brown (RHS 200D) linear stripes at the base. The dorsal sepal of P. spicerianum is white (RHS NN155 C), with purple (RHS 60A) midvein and yellow-green (RHS N144C) at the base. The petals are vellow-green (RHS N144C). The lip is slightly reddish-brown (RHS 177B). Last, the synsepal is yellow-green (RHS N144C). The dorsal sepal of P. 'Yuan Chun' is white (RHS NN155C) with a moderately purplish-red stripe and midrib (RHS 64A). Petals are divided by a moderately purplish- red (RHS 64A) midrib into a vellowgreen (RHS N144C) upper part and a vividly red-striped (RHS 44A) lower part. The lip of 'Yuan Chun' is moderately reddish-orange (RHS N172B). The synsepal is yellow-green (RHS N144C) with strongly greenish-yellow (RHS 153C) sparse linear stripes.

In summary, there are many significant differences between cultivar Yuan Chun and its parents, *P. villosum* and *P. spicerianum*. The most obvious features are plant width, shape, and color of the dorsal sepal and petal, and an earlier and longer flowering period. Overall, cultivar Yuan Chun shows outstanding growth and flowering characteristics.

Asymbiotic Seed Germination and Seedling Development

- 1) Aseptic seeding: Ten-month-old immature pods were disinfected with 70% alcohol for 30 s, then with 0.1% mercuric solution for 15 min, and then washed with sterile water five times. After that, they were placed on sterile filter paper to absorb water, and cut open with a scalpel, and the seeds were scattered on the seed germination culture medium [1/2 Murashige & Skoog + 1.0 g/L activated carbon + 100 mL/L coconut milk + 1-Naphthaleneacetic acid (NAA) 1.0 mg/L].
- 2) Seedling development: The protocorms from seeds at 65 d after germination were transferred to the same culture medium for seedling differentiation. Then, seedlings were cultured on rooting and seedling growth medium (Hyponex NO.1 1.0 g/L + Hyponex NO.2 1.0 g/L + peptone 2.0 g/L + activated carbon 1.5 g/L + NAA 0.5 mg/L + 6-BA 0.2 mg/L + 50 g/L banana juice). The rooting rate observed was 100%
- 3) Seedlings in vitro transplanting: Culture bottles were placed in the greenhouse to train seedlings for I to 2 weeks; then, trained seedlings were washed clean of the attached culture medium, the roots were wrapped with watersoaked moss for more than 24 h and squeezed dry. Subsequently, seedlings were individually planted in 1.7-inch pots. The appropriate humidity and ventilation were maintained during cultivation, and after 2 weeks, seedlings were transferred to the greenhouse for cultivation and handled following standard watering and fertilizer management practices.

The culture medium used in this experiment was added with 1.5% sucrose and 0.6% agar, pH was maintained at 5.2 \sim 5.4, culture temperature was 25 \pm 2 $^{\circ}$ C, light intensity was 30 \sim 40 μ mol·m⁻²·s⁻¹, and photoperiod duration was 12 h/d.

Cultivation Methods

The mixture of Zhijing stone for orchids (Northridge Enterprise Co. Ltd., Taibei, Taiwan, China) and soaked pine bark (v/v = 1:1) is a suitable cultivation medium. Water should be supplied according to seasonal evaporative demand, cultivation medium, and plant growth and development. For young seedlings, spraying 3000 to 4000 times of solution of water-soluble fertilizer containing nitrogen, phosphorus, and potassium (20–20–20) (Peters Professional 20–20–20; Scotts Co., Marysville, OH, USA) was performed every week. For intermediate

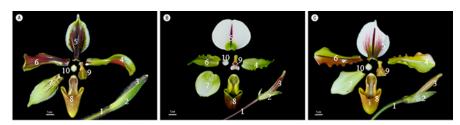


Fig. 2. The flower anatomic structure of female parent *Paphiopedilum villosum* (**A**), male parent *Paphiopedilum spicerianum* (**B**), and new cultivar Yuan Chun (**C**). Their peduncle (1), bract (2), ovary (3), petal reverse (4), dorsal sepal (5), petal obverse (6), synsepal (7), lip (8), staminode (9), and stigma (10) are shown.



Fig. 3. The flower anatomic structure comparison of new cultivar Yuan Chun and its parents. The dorsal sepal obverse (A) and reverse (B); petal reverse and obverse (C); lip (D), staminode (E); stigma (F); peduncle, bract, and ovary (G); and synsepal obverse (H) and reverse (I) (from left to right are *Paphiopedilum villosum*, *Paphiopedilum spicerianum*, and 'Yuan Chun').

and large seedlings, in addition to spraying or root irrigating 2000 to 3000 times of solution of fertilizer containing nitrogen, phosphorus, and potassium (20-20-20) every half month, we applied 10 to 15 grains of Nutricote slow-release fertilizer containing nitrogen, phosphorus, and potassium (14-12-14) (Asahi Kasei Corporation, Tokyo, Japan) for 180 d to each pot. In addition, 3 to 4 months before flowering, 2000 to 3000 times of solution of 20N-20P-20K fertilizer (Peters Professional) was sprayed at 15-d intervals. The most suitable temperature for the growth of large seedlings is 25 to 30 °C during the day and 18 to 20 °C at night, although seedlings can withstand high temperatures (>35 °C) and temperatures as low as 0 °C. However, temperatures should not exceed 32 °C during the day nor fall below 5 °C at night, for small seedlings to grow successfully. Paphiopedilum cultivar Yuan Chun is suitable for planting in greenhouses and other facilities equipped with water-curtain cooling in Guangdong Province.

Applications

In summary, our observations led us to conclude that 'Yuan Chun' is a superior cultivar. We found that it shows great vigor and high environmental adaptability. Particularly, 'Yuan Chun' blooms easily and has a significantly long flowering period. It blooms precisely during the Chinese Spring Festival, and the flowering period of some individuals can last until the Qingming Festival. Because of all these advantages, 'Yuan Chun' is an outstanding New Year flower and suitable for family planting.

Availability

Inquiries about research or request for plant materials can be made to Prof. Songjun Zeng (e-mail: zengsongjun@scib.ac.cn) at the SCBG of the Chinese Academy of Sciences.

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