

Breeding of an Indigo *Phalaenopsis* by Intergeneric Hybridization: *Rhynchosopsis* Tariflor Blue Kid ‘1030-4’

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Breeding for new phalaenopsis varieties has been conducted for many decades. With the efforts of breeders, a lot of varieties have been bred and sold in the market, including many colorful varieties with various flower sizes. However, new varieties are constantly being bred and selected every year and are expected to create new colors or new types that are different from those on the market. Breeding for indigo flowers has been a common goal for many breeders in the world. Currently, indigo *Phalaenopsis* is rarely seen on the commercial market. Most of them are crossed or backcrossed from certain species or commercial varieties such as *Phal. equestris*, *Phal. pulcherrima*, *Phal. violacea*, *Phal. Kenneth Schubert*, and *Phal. Purple Martin*, and only a few native blue species have been applied to blue *Phalaenopsis* hybridization (Tsao et al., 2020). Blue flowers of most plants are the result of delphinidin accumulation or expression (Qingyu and Silan, 2004), and in only a few cases (such as *Phalaenopsis*) is the blue color a result of cyaniding in flowers (Tatsuzawa et al., 2004). Blue flowers of most *Phalaenopsis* varieties faded easily without delphinidin expression as a result of the lack of the flavonoid 3',5'-hydroxylase gene. It is necessary to introduce other genes to the

Phalaenopsis genus by crossing with other genera or by transferring a gene or genes from other genera to create unique flower colors or characteristics. In our study, we introduced a stable blue color to *Phalaenopsis* from the genus *Rhynchostylis* to breed *Rhynchosopsis* Tariflor Blue Kid ‘1030-4’ successfully with a deep and nonfading blue color.

Origin

Rhynchosopsis Tariflor Blue Kid ‘1030-4’. All the breeding, including crossing, selection, and performance studies, was carried out under natural greenhouse conditions at the Floriculture Research Center. Flowering plants of *Phal. Fire Cracker* and *Rhynchostylis coelestis* were grown in a pad and fan greenhouse. *Phal. Fire Cracker* was used as the seed parent that was crossed with *Rhy. coelestis* in Aug. 2007. Mature capsules were surface-sterilized with 70% ethanol, followed by 1.0% sodium hypochlorite solution for 20 min and rinsed three times with sterile distilled water. Capsules were cut open and the seeds were scooped out with forceps onto Murashige and Skoog medium that had half-strength macroelements and full-strength microelements (Murashige and Skoog, 1962) supplemented with 100 mg·L⁻¹ myo-inositol, 0.5 mg·L⁻¹ niacin, 0.5 mg·L⁻¹ pyridoxine hydrochloride, 0.1 mg·L⁻¹ thiamine hydrochloride, 2.0 mg·L⁻¹ glycine, 20,000 mg·L⁻¹ sucrose, 7000 mg·L⁻¹ Difco Bacto agar, and 1000 mg·L⁻¹ activated charcoal. The pH of the medium was adjusted to 5.7 before autoclaving at 121 °C, 1.05 kg·cm⁻² for 20 min. The seeds were incubated at 25 ± 1 °C under a 14-h photoperiod at an irradiance of 40 μmol·m⁻²·s⁻¹ (daylight cool-white fluorescent light). Two hundred ninety-seven

seedlings from flasks were grown in sphagnum moss under natural greenhouse conditions (20 to 30 °C, 12–13.5-h daylength). Irrigation was performed once every 1 to 2 weeks during the cultivation period. Peters 20N–20P₂O₅–20K₂O at 1.0 g·L⁻¹ (Scotts Company, Marysville, OH) was added to tap water as the fertilizer solution. Seedling plants flowered in July 2010, and this hybrid was registered as *Rhmps. Tariflor Blue Kid*. The cultivar ‘1030-4’ was selected from more than 100 seedlings because of its darker blue flowers in July 2010. After that, the stalk was used to be propagated by tissue culture.

Description and Performance

Rhmps. Tariflor Blue Kid ‘1030-4’ was bred and selected by crossing *Phal. Fire Cracker* with *Rhy. coelestis*. The characteristics of new cultivar are similar to those of *Phalaenopsis. Phal. Freed’s Beautiful Girl* was used as the control variety and is compared to the new line. *Rhmps. Tariflor Blue Kid ‘1030-4’* had 24 traits that distinguish it from *Phal. Freed’s Beautiful Girl* (Table 1) based on the distinctness, uniformity, and stability (DUS) test guideline for *Phalaenopsis* (International Union for the Protection of New Varieties of Plants, 2013). DUS testing is a way of judging whether a new variety differs from existing varieties within the same species (the distinctness part), whether the characteristics used to establish distinctness are expressed uniformly (the uniformity part), and whether these characteristics change (or not) through subsequent generations (the stability part) (An et al., 2014). The following color description was based on The Royal Horticultural Society’s (RHS) color chart (Royal Horticultural Society, 2015). Plants of *Rhmps. Tariflor Blue Kid ‘1030-4’* are of medium size, with oval leaves, spots on the upper side of leaves, and darker green leaves. Plants of *Phal. Freed’s Beautiful Girl* are large, with oval leaves, no spots on the upper side of leaves, and lighter green leaves. *Rhmps. Tariflor Blue Kid ‘1030-4’* has a greater average flower count (n = 15) than that of *Phal. Freed’s Beautiful Girl* (n = 8). The flower diameter of *Rhmps. Tariflor Blue Kid ‘1030-4’* and *Phal. Freed’s Beautiful Girl* is 3.5 cm and 5 cm, respectively (Fig. 1). The dorsal sepal of *Rhmps. Tariflor Blue Kid ‘1030-4’* and *Phal. Freed’s Beautiful Girl* is moderately compressed and moderately elongated, respectively. The cross section of the dorsal sepal for *Rhmps. Tariflor Blue Kid ‘1030-4’* is concave, and the ground color of the upper side is moderate violet (RHS 86A). The cross section of the dorsal sepal for *Phal. Freed’s Beautiful Girl* is straight, and the ground color of the upper side is strong purple (RHS N82A). The ground color of the upper side for the lateral sepal of *Rhmps. Tariflor Blue Kid ‘1030-4’* and *Phal. Freed’s Beautiful Girl* is strong purple (RHS N87A) and strong purple (RHS N82A), respectively. The cross section of the petal for *Rhmps. Tariflor Blue Kid ‘1030-4’* is concave, and the ground color of the upper side is strong purple (RHS

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Table 1. Comparisons of *Rhmps.* Tariflor Blue Kid ‘1030-4’ and *Phal.* Freed’s Beautiful Girl plant, leaf, and flower based on the distinctness, uniformity, and stability (DUS) test guideline of *Phalaenopsis*.

Trait	<i>Rhyn.</i> Tariflor Blue Kid ‘1030-4’ Result	<i>Phal.</i> Freed’s Beautiful Girl
Plant length (cm)	Medium: $25 \leq x < 40$	Long: $40 \leq x \leq 65$
Leaf spots on upper side	Present	Absent
Leaf color of upper side	Medium green	Light green
Inflorescence, no. of flowers	Medium	Few
Flower width in front view	Very narrow	Narrow
Dorsal sepal shape	Moderately compressed	Moderately elongated
Dorsal sepal shape in cross section	Concave	Straight
Dorsal sepal ground color of upper side	Moderate violet (RHS 86A)	Strong purple (RHS N82A)
Lateral sepal ground color of upper side	Strong purple (RHS N87A)	Strong purple (RHS N82A)
Lateral sepal overcolor (if present)	Strong purple (RHS N87B)	Light purple (RHS N82C)
Petal shape of cross section	Concave	Straight
Petal ground color of upper side	Strong purple (RHS N82B)	Strong purple (RHS N87A)
Petal overcolor (if present)	Light purple (RHS N82D)	Strong purple (RHS N87B)
Lip, size of lateral lobe relative to apical lobe	Equal	Larger
Apical lobe ground color	Very pale violet (RHS 91D)	Very pale purple (RHS 85D)
Apical lobe tip color	Moderate violet (RHS 86A)	Strong purple (RHS N87A)
Apical lobe overcolor	Very pale violet (RHS 91D)	Very light purple (RHS 85C)
Apical lobe tip overcolor	Strong violet (RHS 86B)	Strong purple (RHS N87B)
Apical lobe color of spots	Moderate violet (RHS 86A)	Deep purple (RHS 83B)
Lateral lobe ground color	Strong violet (RHS 86B)	Strong purple (RHS N87B)
Lateral lobe overcolor	Strong violet (RHS 86C)	Brilliant purple (RHS N87C)
Callus color	Very pale violet (RHS 91D)	Brilliant yellow (RHS 12B)
Callus shape of pattern	Spots	None
Column apex color	Strong purple (RHS N82A)	Strong purple (RHS N87B)

RHS = Royal Horticultural Society.



Fig. 1. Flower of *Rhmps.* Tariflor Blue Kid ‘1030-4’ (left) and *Phal.* Freed’s Beautiful Girl (right).

N82B). The cross section of the petal for *Phal.* Freed’s Beautiful Girl is straight, and the ground color of the upper side is strong purple (RHS N87A). The size of the lateral lobe relative to the apical lobe for *Rhmps.* Tariflor Blue Kid ‘1030-4’ is equal and large for *Phal.* Freed’s Beautiful Girl, respectively. Lip traits of *Rhmps.* Tariflor Blue Kid ‘1030-4’ are as follows: ground color, tip color, and

spot color of the lip apical lobe are very pale violet (RHS 91D), moderate violet (RHS 86A), and moderate violet (RHS 86A) respectively. Lip traits of *Phal.* Freed’s Beautiful Girl are as follows: ground color, tip color, and spot color of the lip apical lobe are very pale purple (RHS 85D), strong purple (RHS 87A), and deep purple (RHS 83B), respectively. The callus color of *Rhmps.* Tariflor Blue Kid ‘1030-4’ is very pale violet (RHS 91D) with spots, whereas the callus color of *Phal.* Freed’s Beautiful Girl is brilliant yellow (RHS 12B) without spots. The apex color of the column of *Rhmps.* Tariflor Blue Kid ‘1030-4’ and *Phal.* Freed’s Beautiful Girl is strong purple (RHS N82A) and strong purple (RHS N87B), respectively.

Availability

Questions concerning *Rhynchonopsis* Tariflor Blue Kid ‘1030-4’ and the research should be addressed to Dr. Jung-Yi Wu (E-mail: iris12@tari.gov.tw) at the Floriculture Research Center, Taiwan Agriculture Research Institute, Council of Agriculture, Taiwan.

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