

Nymphaea ‘Silk-road Red’: A New Intersubgeneric Cultivar

Meihua Yang, Zhixian Jiang, Xinkai Zheng, Zhuojun Liu, and Nueraihemaiti Maihemuti

Agricultural College of Shihezi University, Shihezi 832003, China

Yuanzhi Wang

School of Medicine, Shihezi University, Shihezi 832002, China

Yingchun Xu

College of Horticulture, Nanjing Agricultural University, Nanjing 210095, China

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Waterlily, belonging to the Nymphaeaceae family and the genus *Nymphaea*, is divided into six subgenera, namely *Brachyceras*, *Anecphyra*, *Nymphaea*, *Hydrocallis*, *Confluentes*, and *Lotos* (Huang et al. 2008; Sun et al. 2018). *Nymphaea candida* is a cold-tolerant native species found in the Xinjiang Uyghur Autonomous Region of China, Siberia, Central Asia, and Europe. Its population has declined due to habitat degradation and overcollection. This species is classified as a national second-class protected wild plant in China. *N. candida* holds ornamental value. Both its flowers and leaves are aesthetically pleasing, making it a precious plant material for wetland ecological construction (Liu et al. 2023). Additionally, *N. candida* is a traditional medicinal herb. It contains flavonoids, saponins, and phenolic compounds, which possess pharmacological effects such as antibacterial, anti-inflammatory, and antioxidant properties (Yang et al. 2022). *Nymphaea hybrid* is a tropical waterlily. It was introduced from the American fragrant waterlily as a breeding material during the 1970s. *N. hybrid* has been developed into nine varieties with different colors, including blue, purple, and yellow. Additionally, these cultivars possess a delicate and pleasant fragrance (Xu et al. 2008; Zhang et al. 2021).

To create new traits, breeders have developed new cultivars through intersubgeneric hybridization, such as *Nymphaea* ‘William Phillips’ (Doran et al. 2004; Les et al. 2004) and *Nymphaea* ‘Zi Yan’ (Ou et al. 2021), among others. This article introduces a new intersubgeneric cultivar of the waterlily named *Nymphaea* ‘Silk-road Red’, developed through

hybridization between *N. candida* (subgenus *Nymphaea*) as the seed parent and a purple-petaled cultivar of *N. hybrid* (subgenus *Brachyceras*) as the pollen parent. Its identity was confirmed by morphological and genetic sequence similarity analyses. The new cultivar, optimized through genetic traits, combines the advantages of tropical and cold-tolerant waterlilies, not only enhancing the ornamental value of waterlilies but also improving their adaptability and market competitiveness.

Origin

N. candida is characterized by its purely white flower colors and extended blooming period. Its petals are white (RHS 155D), the stamens are yellow (RHS 3A), and the

leaves have smooth margins and are spotless. *N. candida* possesses advantages in cold resistance, as well as alkalinity and salt tolerance. In hybrid breeding, its cold tolerance traits offer natural hybrid vigor (Huang et al. 2008, 2022).

N. hybrid was used as the pollen parent in this study. It produces a large number of flowers with a long blooming period and excellent ornamental value. The petals are slender, and their colors are diverse. The leaf margins of *N. hybrid* have irregular serrations, and the leaf surfaces have spots. This study focused on the hybrid breeding and selection using the purple-flowered series of this variety.

In the hybridization experiment, stamens were removed with tweezers from donor plants during morning anthesis for pollen collection. After collection, the stamens were placed in parchment bags and refrigerated at 4°C. Prebloom seed parent flowers were opened with tweezers to access bud stamens, which were excised at the base to prevent self-pollination. The flowers were then covered with gauze to block insect pollination during blooming. On the first day of blooming, the prepared anthers are placed into the sticky fluid at the center of the seed parent’s flower to complete pollination. Postpollination, the flowers were rebagged and marked. Seeds obtained that year were sown in Summer 2020, cultivating a novel flower color lineage. The new cultivar, designated ‘Silk-road Red’ (Fig. 1), was officially registered with the International Waterlily and Water Gardening Society in Oct 2022.



Fig. 1. New waterlily cultivar *Nymphaea* ‘Silk-road Red’.

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M.Y. and Z.J. contributed equally to this work.

M.Y. is the corresponding author. E-mail: ymh_agr@shzu.edu.cn.

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Table 1. Morphological features of the new cultivar *Nymphaea* ‘Silk-road Red’.

Traits	<i>Nymphaea</i> ‘Silk-road Red’
General overall shape of the flowers	Star shaped
Flower petal color(s)	Purple (RHS N78)
Filament color	Yellow (RHS 4A)
Anther color	Purple (RHS 75B)
Sepal color abaxial (underside)	Green (RHS 138B)
Sepal color adaxial (top side)	Purple (RHS N79A)
Sepal number	4
Pedicel (flower stalk) color	Yellow-green (RHS 145A)
Diameter of flower (cm)	2.5 to 5
Petal number	15 to 18
Emergence height above water (cm)	8 to 10
General overall shape of the leaves	Ovate
General margins of the leaves	Irregularly dentate
Leaves average length × width (cm)	11 × 10
Diameter of plant (cm)	120 × 150
Leaves abaxial (underside) color	Gray-purple (RHS 186C)
Leaves adaxial (top side) color	Green (RHS N137A)
Petiole color	Leaves with mottling brown (RHS 200B)
Is the plant viviparous?	Gray-orange (RHS 165A)
Lobes of the leaves	No
Pedicel glabrous or pubescent (with fine hairs)?	Lobes open
Is the cultivar fragrant?	No
	Yes

RHS = Royal Horticultural Society color chart number.

Description

The morphological characteristics of *Nymphaea* ‘Silk-road Red’ were investigated and recorded according to the files applicable for registering a Nymphaeaceae cultivar name as appointed by the International Society for Horticultural Science (Table 1). The colors of the plant organs were described according to the 2015 Royal Horticultural Society color chart.

Plant habit. *Nymphaea* ‘Silk-road Red’ is a medium-sized, cold-tolerant, tuberous, perennial, aquatic herb that belongs to an intersubgeneric hybrid of waterlilies. The plant diameter is 120 × 150 cm. The emergence height above water is 8 to 10 cm.

Flower. The flowers are star-shaped with a diameter ranging from 2.5 to 5 cm and are fragrant. The four sepals are obovate in shape. The color of the sepals’ upper surfaces is purple (RHS N79A), and the undersides of the sepals are green (RHS 138B). There are 15 to 18 petals. The new cultivar inherits the fragrance characteristic of *N. candida* and also carries the purple petal flower color from a cultivar of *N. hybrid*. The filament color is yellow (RHS 4A), and the anther color is purple (RHS 75B).

Leaves. The leaf blades are ovate, about 11 cm long, and 10 cm wide. The adaxial leaf surface is green (RHS N137A) with irregular brown (RHS 200B) mottlings. The abaxial leaf surface is gray-purple (RHS 186C). Leaf margins are irregularly dentate. The lobes of the leaves are open. The petiole color is gray-orange (RHS 165A), and the pedicel color is yellow-green (RHS 145A).

Compared with *N. candida* and *N. hybrid*, the new cultivar has fewer petal numbers and stamen petaloids. ‘Silk-road Red’ resembles *N. hybrid*, with the difference that the plant is smaller and has stamens, stigma, and outer

petals that differ in color from *N. hybrid*. Furthermore, this new cultivar is a rare variety among hardy waterlilies with purple petals, exhibiting good cold resistance.

In addition to morphological identification, this study performed species characterization by analyzing double-stranded DNA from the chloroplast *trnK* locus (including the 5' intron flanking a portion of the *matK* coding region) and the nuclear ribosomal internally transcribed spacer (nrITS) region (including ITS-1, 5.8 S recombinant DNA, ITS-2 loci) (Moody and Les 2002) extracted from parental plants (both paternal and maternal) and their hybrid offspring. Each sequence was submitted to the GenBank database (<http://www.ncbi.nlm.nih.gov/genbank>) and assigned five GenBank accession numbers (PV423498, PV485213, PV485214, PV551125, and PV551126). To further analyze homology among the paternal and maternal lines and their hybrid, BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used based on ITS region and the chloroplast DNA (cpDNA) (*trnK* 5' intron). The alignment results indicated that (i) the pollen parent was identified as *N. hybrid*, and the seed parent was identified as *N. candida*; (ii) the hybrid offspring, designated as a new cultivar named *Nymphaea* ‘Silk-road Red’, shared 96% genetic similarity with *N. hybrid*; and (iii) the cpDNA (*trnK* 5' intron) sequences of *N. candida* and *Nymphaea* ‘Silk-road Red’ were highly similar, with 99.40% sequence similarity. The match of the cpDNA sequences confirmed *N. candida* as the maternal parent of *Nymphaea* ‘Silk-road Red’.

Cultivation

‘Silk-road Red’ is a cold-tolerant waterlily. The suitable temperature range for planting is 8 to 15 °C. It can be grown in ponds or

in pots. The optimal planting time is from late April to mid-May in Xinjiang Uyghur Autonomous Region, northwestern China. When planting, choose a clay loam with high viscosity and rich in organic matter, with a pH level of 6 to 8, such as swamp peat soil. As autumn and winter approach, move the waterlily indoors to a 5 to 10 °C environment. Return it outside when temperatures exceed 15 °C the following year.

Availability

The cultivar *Nymphaea* Silk-road Red is maintained by the Agricultural College of Shihezi University (Shihezi, China). Requests for the cultivar should be addressed to author Meihua Yang (E-mail: ymh_agr@shzu.edu.cn).

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