

Ning Qing 3: A New Holly Cultivar with Peculiar Leaf Morphology

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Keywords. hybridization, leaf character, ornamental plant

The *Ilex* L. (holly) is the largest woody dioecious angiosperm genus in Aquifoliaceae, composed of ~700 deciduous and evergreen trees and shrubs (Chong et al. 2022; Manen et al. 2010; Yao et al. 2022). The genus is distributed worldwide from tropical to temperate regions, with centers of species diversity located in East Asia and South America (Su et al. 2020; Yao et al. 2016). For tens of decades, *Ilex* species have been used as ornamentals, beverages, pharmaceutical plants, and industrial materials (Li et al. 2022; Yao et al. 2022; Zhou et al. 2022). The flowers and fruits of holly are fairly uniform, but leaf characters vary greatly among species in this genus, including size, shape, margins, and texture. Many *Ilex* species are widely cultivated as ornamental plants because of their red drupes and often distinctive leaves. Therefore, many breeders and consumers pay much attention to the leaf traits of *Ilex* plants.

China has abundant germplasm resources of *Ilex* both in nature and cultivation; more than 200 species have been documented in southwestern China (Chong et al. 2023; Yao et al. 2020). Traditional cross breeding is the

main way to create new holly cultivars. ‘Ning Qing 3’ was selected and released from the holly breeding program of Institute of Botany, Jiangsu Province and Chinese Academy of Sciences (Nanjing Botanical Garden Memorial Sun Yat-Sen). Its moderate-olivaceous (RHS 146A) (Royal Horticultural Society 2015), shiny, leathery, and oblong leaves have sparked a lot of interest, enriching the *Ilex* germplasm resources, and significantly improving their ornamental values. ‘Ning Qing 3’ has excellent ecological characteristics, including cold resistance, wide adaptability, pruning resistance, and fewer diseases and pests. Therefore, the cultivar is the ideal tree species for afforestation and landscaping.

Origin

In Spring 2014, *Ilex dabieshanensis* (♀) was crossbred with *Ilex latifolia* (♂) at the Holly Resource Collection Garden of Nanjing Botanical Garden Memorial Sun Yat-Sen, Jiangsu, China (118°49′55″E, 32°3′32″N). More than 300 cross-pollinated seeds were collected in winter for sand storage to break seed dormancy. In Spring 2015, seeds were sown in a seedbed with a perlite and peat mixture as substrate. After germination, seedlings were transferred into the field with a 30 × 30-cm spacing. In Jun 2017, an individual plant with olive green (RHS 146A), oblong, and leathery leaves was observed and selected for further phenotypic identification, which was named ‘Ning Qing 3’. After 3 years of softwood/hardwood cutting and branch grafting (2018–20), and 5 years of

field observation (2018–22), the cutting and grafted seedlings exhibited the same morphological characteristics as the mother (donor) plant, which confirmed their phenotypic stability. Thus, ‘Ning Qing 3’ has stable genetic traits and was cultivated successfully. The seedlings grew vigorously, and have wide adaptability. The plants exhibited good tolerance to high (37 to 40 °C) and low temperatures (–4 to 0 °C) in Jiangsu (118°49′E, 32°03′N, US Department of Agriculture plant hardiness zones ≈9b/10a). Few incidents of leaf spot, branch blight, root rot, and aphids were observed.

Description

Compared with its parents, *I. dabieshanensis* and *I. latifolia*, the distinguishing characteristic of ‘Ning Qing 3’ is its leathery, oblong leaves with shiny moderate-olivaceous color (RHS 146A) (Table 1, Fig. 1). The morphological characteristics are described as follows.

Tree. ‘Ning Qing 3’ is an evergreen tree with an upright and semiopen growth habit, forming an elegant ovoid crown (Fig. 2A). It can grow to 2 m tall with a 1.2-m spread at 5 years of age.

Twigs and foliage. The twigs are yellow green (RHS 146C) without lenticels. The leaves are simple and leathery, and connected with short petioles (0.8–1.1 cm). Leaf blades are oblong (7.2–7.8 cm in length × 2.5–3.0 cm in width) with narrowly cuneate leaf bases and acuminate leaf apices. Leaf apices have three obtuse spines, and leaf margins contain three to four pair spines. The color of leaves is moderate olive green (RHS 146A) (Fig. 2B). Both leaf surfaces are glabrous with midvein impressed adaxially.

Flower. Greenish yellow flowers (RHS 1A) are small, inconspicuous, and not showy. Umbellate inflorescences with 9 to 16 flowers are axillary on the current year’s branchlets (Fig. 2C). Pedicels are glabrous (3–5 mm). Each flower contains four petals and four stamens, but the pistil is degenerated. Petals are obovate-oblong (4.0–4.2 mm in length × 2.4–2.6 mm in width) and basally slightly connate (Fig. 2D and E). In Jiangsu Province of China, the blossoming time of the cultivar starts in early April and usually lasts for 14 d. This cultivar is a male plant and does not bear fruit.

Propagation

‘Ning Qing 3’ is regenerated mainly by softwood cutting (June to July) or hardwood cutting (late November to late March before budbreak). For softwood cutting, the current year’s semilignified branches were selected and cut into short cuttings 6 to 10 cm long

Table 1. Comparison of leaf phenotypic attributes of ‘Ning Qing 3’, *Ilex dabieshanensis*, and *Ilex latifolia*.

Attribute	‘Ning Qing 3’	<i>I. dabieshanensis</i>	<i>I. latifolia</i>
Leaf texture	Leathery	Thickly leathery	Thickly leathery
Leaf color	Moderate olive green (RHS 146A)	Grayish olive green (RHS NN137A)	Dark yellowish green (RHS 139A)
Leaf shape	Oblong	Ovate-oblong, ovate, or elliptic	Ovate-oblong

Received for publication 11 Apr 2023. Accepted for publication 15 May 2023.

Published online 12 Jul 2023.

X.C. and X.W. contributed equally to this work. This work was supported by the Natural Science Foundation of Jiangsu Province (BK20210163, BK20201244), Opening Project of Zhejiang Provincial Key Laboratory of Forest Aromatic Plants-based Healthcare Functions (SLFX2205), National Natural Science Foundation of China (32202535), Jiangsu Agricultural Science and Technology Innovation Fund (CX(21)3020), and Open Fund of Jiangsu Key Laboratory for the Research and Utilization of Plant Resources (JSPKLB202209).

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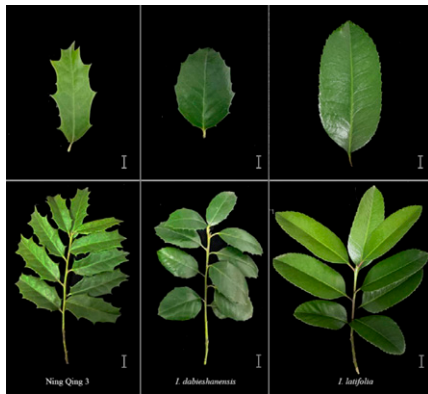


Fig. 1. Comparison of leaf attributes of 'Ning Qing 3', *Ilex dabieshanensis*, and *Ilex latifolia*. Bars: 1 cm.

with two to three leaves kept at the top, whereas for hardwood cutting, thick and healthy annual branches were selected and cut into cuttings 10 to 15 cm in length, with a cutting depth of ~8 to 10 cm. The cuttings were treated with 2000 ppm indole-3-butyric acid for 8 to 10 s, inserted into the substrate, and kept in moderate humidity, and placed under sprinkler irrigation. Generally, the cuttings can take root after 30 d, with a rooting percentage of 90%.

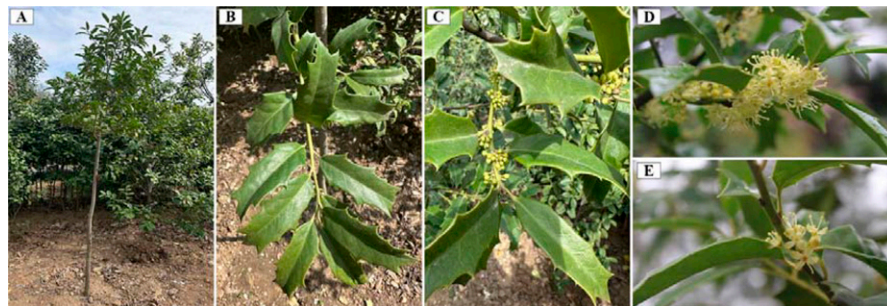


Fig. 2. Phenotypic characteristics of 'Ning Qing 3'. (A) Upright and semiopen growth habit. (B) Leaves and twigs. (C) Axillary umbellate inflorescence. (D, E) Greenish yellow flowers.

The cultivar can also be propagated by grafting in early spring using *I. dabieshanensis* seedlings as the rootstock and the current year's branches with healthy and plump axillary buds of the cultivar as the scion. The grafted union is wrapped in plastic film to prevent desiccation, which should be removed after scion and stock plants are firmly connected.

Cultivation

This cultivar likes sufficient light and can tolerate semishaded conditions. It prefers to grow in well-drained acidic soil. It is suggested that plants should be transplanted with soil slightly in early spring. After that, the plants should be watered thoroughly, and appropriate pruning and reshaping can be carried out as required. During the rainy season, timely drainage is of great importance. In winter and spring, plants can be treated with some well-rotted organic fertilizer. Leaf spot, stick blight, root rot, and aphids rarely cause damage to young tips and leaves.

Availability

'Ning Qing 3' relevant plant material and research information can be obtained from Dr. Hong (chenhong@cnbg.net), Institute of Botany, Jiangsu Province, and the Chinese Academy of Sciences (Nanjing Botanical Garden Memorial Sun Yat-Sen).

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