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RESEARCH PAPER

Phylogenetic Relationship of Ramie and Its Wild Relatives Based on Cytogenetic and DNA Sequence Analyses

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Abstract: To study the relationship between cultivated ramie (*Boehmeria nivea*) and its wild relatives and the origin of *B. nivea*, the karyomorphology of interphase nuclei and chromosome numbers were investigated in 19 species and 5 varieties of ramie and sequences of the internal transcribed spacers (ITS) of nuclear ribosomal DNA and the chloroplast *trn*L-F were analyzed in 18 species and 9 varieties of ramie. On the basis of cytological observation of interphase karyomorphology, the interphase nuclei of sections Tilocnide and Boehmeria were prochromosome type and those of sections Zollingeriana, Phyllostachys, and Duretia were prochromosome or diffuse type. The cytogenetic examination of *B. nivea* and its 23 wild relatives showed that 19, 3, 1, and 1 taxa were diploid ($2n = 28$), triploid ($2n = 42$), tetraploid ($2n = 56$), and pentaploid ($2n = 70$), respectively, of which triploid taxa were all from section Duretia. From the phylogenetic trees based on ITS and *trn*L-F sequences, *Boehmeria* taxa were grouped into 3 clades and several subclades. Different individuals or clones of *B. clidemioides* var. *diffusa* fell into different clades, indicating the possible hybridization and reticulate evolution among species in *Boehmeria*, and the introgression between *B. nivea* and *B. clidemioides* var. *diffusa*. The core species of wild ramie resources should not only involve *B. nivea* and its 3 varieties but also *B. malabarica* var. *leioclada* and *B. clidemioides* var. *diffusa*. It was inferred that 2 evolution routes existed in *Boehmeria*: prochromosome evolution line and diffuse evolution line. In the former route, section Tilocnide originated from section Boehmeria. In the latter route, the evolution direction was section Boehmeria → Zollingeriana → Phyllostachys → Duretia. Both routes probably originated from the same ancestor. Based on the morphological traits of interphase nucleus and molecular experiment, close relationships were found among sections Zollongerie, Phyllostachys, and Duretia. However, their systematic relationships based on morphological traits were not supported by the cytological evidence and phylogenetic trees of ITS and *trn*L-F sequences.

Keywords: *Boehmeria*; phylogenetic relationship; interphase nucleus; chromosome number; internal transcribed spacers (ITS); *trn*L-F

Ramie (*Boehmeria nivea*) has been cultivated as an important fiber crop for about 4700 years in China and East Asia [1]. *Boehmeria nivea* var. *tenacissima* has been extensively cultivated in South Asia. Some taxa of *Boehmeria* such as *B. tricuspic* are medicinal plant. There are about 120 species in the genus *Boehmeria* Jacq., which are mainly distributed in the tropical and the subtropical zones and rare in the temperate zone. In the genus *Boehmeria*, about 75 species are distributed in Asia, 30 species in America, and few in Australia and Africa; 31 species and 12 varieties are distributed in China [2–5].

Genus *Boehmeria* was first constructed by Jacquin in 1760 with the species of *B. ramiflora* Jacq. Blume et al. have recorded 74 species and classified these species into 6 groups based on the characteristics of leaf, inflorescence, and ovary; Weddell et al. grouped 47 *Boehmeria* species according to the characteristics of inflorescence (axillary glomerule or cone-shaped) and leaf (alternate or opposite) [2, 3]. Satake [6] reported 39 *Boehmeria* species in Japan and its neighbors, and classified these taxa into 2 subgenus and 8 sections. Wang [2–4]

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recorded 31 species and 12 varieties in *Boehmeria* in China, and classified them into 5 groups, i.e., sections Boehmeria, Tilocnide, Zollingeriana, Phyllostachys, and Duretia. Chen and Friis [5] classified *Boehmeria* species from China into 25 species and 9 varieties. To date, there is no identical taxonomy of genus *Boehmeria*, and the phylogeny and relationship of *Boehmeria* spieces have been rarely studied. On the basis of morphological characteristics of phyllotaxy, inflorescence, male flower, and achene, Wang [2–4] suggested 5 sections of *Boehmeria*, of which section Boehmeria is an original group and radially evoluted to the other 4 sections. Tilocnide originated directly from section Boehmeria. Comparing morphology of 13 *Boehmeria* species in 5 sections and palynology of 10 *Boehmeria* species in 4 sections in China, the origin of section Duretia was suggested to be section Boehmeria, and then derived into 2 clades: sections Tilocnide and Phyllostachys; i.e., section Tilocnide originated from section Duretia [7–9].

The length of chromosome of *Boehmeria* is about 1–4 μm, which is small and difficult for karyotype analysis [10]. Thus, there are few reliable karyotype pictures published. Currently, the chromosome numbers of *Boehmeria* have been counted for only 29 species [11]. It is still far away from comprehensive understanding of *Boehmeria* chromosomes. Because of the short chromosomes, little information is available for phylogenetic analysis of *Boehmeria*.

In most plants, the phylogenetic significance is often ignored due to the slight morphological variation of interphase nucleus. However, the morphology of interphase nucleus sometimes showed diversity in plants with small chromosomes. For instance, the interphase nucleus of Orchidaceae is classified into 7 types, i.e., densely diffuse, simple chromocenter, complex chromocenter, gradient, rod-shaped prochromosome, round prochromosome, and simple diffuse type [12, 13]. The morphology of interphase nucleus is often used in phylogenetic analysis among families or genus [14]. In this study, distinct variation of interphase nuclei was found among *Boehmeria* species.

Guo [15] reconstructed random amplified polymorphic DNA (RAPD) fingerprinting on the genomic DNA of *Boehmeria* using 19 samples from 15 species, including 1 species and 3 varieties in section Tilocnide, 2 species in section Boehmeria, 2 species in section Phyllostachys, 1 species in section Zollingeriana, and 6 species in section Duretia. These 15 taxa were classified into 5 sections, which is in accordance with Wang's taxonomy [4]. Guo [15] also outlined the relationships among the 5 sections of *Boehmeria* (Fig. 1), and deduced the evolution direction in section Tilocnide as *B. nivea* var. *viridula* → *B. nivea* var. *tenacissima* → *B. nivea* var. *nipononivea* → *B. nivea*.

Which relative taxa can hybridize with *B. nivea* and its 3 varieties? Except for *B. nivea* and its 3 varieties, which relative taxa constitute the core wild germplasm resources? Which taxa are the external germplasm resources? How about the relationship between section Tilocnide and other taxa? These unsolved questions are restrictive to the utilization and protection of *B. nivea* and its wild resources. In this paper, the systematic relationship between *B. nivea* and its wild relatives was studied based on morphological performance of interphase nucleus, chromosome numbers, sequences of the internal transcribed spacers (ITS) of nuclear ribosomal DNA and *trn*L-F.

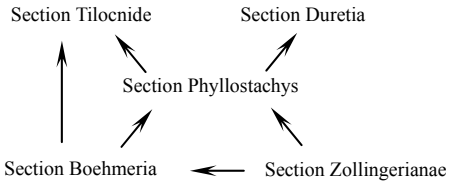

Fig. 1 Relationship among sections in *Boehmeria* (According to Guo [15])

1 Materials and methods

1.1 Plant materials

Materials and voucher specimens are listed in the Table. The voucher specimens were deposited in the Herbarium of Jiujiang University, Jiujiang, Jiangxi, China, and the other materials were collected from the germplasm nursery of Institute of Bast Fiber Crops of Jiangxi Province, Yichun, China.

1.2 Cytologenetic analysis

Young ramie shoots were collected at 15–20°C from 8:00–12:00 am, pretreated with 0.1% colchicine solution for 2–3 h, and then fixed in a fixative (ethanol: glacial acetic acid = 3:1 *V*/*V*) for over 6 h. The fixed shoot tips were macerated with 1 mol L^{-1} HCl at 60°C for 9 min, rinsed with distilled water for 4–5 h, and stained with carbol fuchsin for more than 12 h. The chromosome spreads were prepared by conventional squash technique. Thirty cells were used for chromosome count and interphase nucleus observation. The cells at the interphase were photographed using Nikon 80i microscope (Shanghai, China).

1.3 Genomic DNA extraction

Genomic DNA was extracted using improved cetyltrimethylammonium bromide (CTAB) method [16] from silica gel dried young shoot.

1.4 Amplification and sequencing

Full length of ITS was amplified with primers ITS1 (5′-AGA AGTCGTAACAAGGTTTC-3′) and ITS4 (5′-TCCTCCGCT TATTTATATGC-3′) [17]. *Trn*L-F region was amplified with