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Cytoplasmic DNA disclose high nucleotide diversity and different phylogenetic pattern in *Taihangia rupestris* Yu et Li

Yueqin Cheng [a, 1], Jingmian Duan [b, 1], Zhenbin Jiao [b], G. Geoff Wang [c], Fengming Yan [a], Hongwei Wang [a, c, *]

[a] College of Plant Protection, Henan Agricultural University, No. 95, Wenhua Road, Zhengzhou 450002, China
[b] College of Life Science, Henan Agricultural University, No. 95, Wenhua Road, Zhengzhou 450002, China
[c] School of Agricultural, Forest, and Environmental Science, Clemson University, South Carolina 29634, United States

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ABSTRACT

Taihangia rupestris Yu et Li is an ancient species endemic to the Taihang Mountains, where it originated and evolved. Six chloroplast DNA (cpDNA) (*trnT*^{UGU}-*trnL*^{UAA} 5′exon, *trnL*^{UAA} 5′exon-3′exon, *trnL*^{UAA} 3′exon-*trnF*^{GAA}, *trnH*^{GUG}-*trnK*^{UUU}, *psbC-trnS* and *rps12-rpl20*) and five mitochondrial DNA regions (*rrn5*/*rrn18*-2, *nad1*/*2-3*, *nad4*/*3-4*, *nad7*/*1-2* and *nad7*/*2-3*) were employed to investigate the nucleotide diversity and subspecies differentiation. The results showed that *T. rupestris* had high nucleotide diversity at the species level, and *T. rupestris* var. *ciliate* possessed higher levels of haplotype and nucleotide diversity than did *T. rupestris* var. *rupestris*. Cytoplasmic DNA revealed a phylogenetic pattern of individuals that was in disagreement with the patterns obtained from nuclear DNA. These results primarily reflect the past habitat fragmentation of the species and different modes of inheritance and spread of these genomes. Together, our results advance our understanding of plant speciation in this region.

1. Introduction

The Taihang Mountains in central China occupy a special geographic position. First, they are in a region where the North China Plain converges with the Loess Plateau. Second, this place has been affected by climate change, especially a reduction of precipitation, in the recent geologic period (Wang et al., 2011). Finally, it was a corridor through which plants migrated back and forth during ancient climatic fluctuations (Wang, 1992). Therefore, the study of species differentiation and population history could help elucidate ancient climate oscillations and their effects on biodiversity in this region.

Taihangia rupestris Yu et Li is an ancient species that is endemic to the Taihang Mountains, where it originated and evolved (Yü and Li, 1980). Thus, *T. rupestris* is a suitable species for investigating speciation in the region. *T. rupestris* is a perennial herb, and it is the most primitive diploid species in the tribe Dryadeae (Rosaceae) according to its morphology (Yü and Li, 1983). At present, it has a disjointed distribution and occurs sporadically across the limestone region of the eastern edge of the Taihang Mountains (Wang et al., 2011).

* Corresponding author. College of Plant Protection, Henan Agricultural University, No. 95, Wenhua Road, Zhengzhou 450002, China.
E-mail address: whwcas@163.com (H. Wang).
[1] These authors contributed equally to this work.

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Based on leaf features such as shape, the type of sawtooth edge, and the number of hairs on the edge and petiole, Yü and Li (1980) divided *T. rupestris* into two subspecies, *T. rupestris* var. *ciliate* and *T. rupestris* var. *rupestris*. Such discrimination of subspecies was supported by genetic evidence from nuclear markers (Tang et al., 2010; Wang et al., 2011). Those studies used RAPD and ISSR markers and showed the same population structure, namely, that all of sampled populations were grouped into two clades that corresponded to two subspecies. In addition, these markers disclosed high genetic diversity at the species level of *T. rupestris* (Tang et al., 2010; Wang et al., 2011).

In addition to nuclear DNA, chloroplast and mitochondrial genomes (the cytoplasmic genome) exist in plant cells. For most angiosperm species, the nuclear genome is biparentally inherited and can be spread by pollens and seeds, whereas the cytoplasmic genome is maternally inherited and spread only by seeds (Greiner et al., 2015). Moreover, the rate of evolution in the cytoplasm genome is different from that of the nuclear genome, and the former is generally lower than the latter. In this study, we sought to investigate the following questions: Is there a high genetic diversity of cytoplasmic DNA in *T. rupestris*? Is the subspecies differentiation of *T. rupestris* supported by the genetic information from the cytoplasmic genome? The answers to these questions will advance our understanding of plant speciation in this region.

2. Materials and methods

2.1. Material

Young leaf samples from 40 individuals were collected from 10 populations across the species range, with four samples per population (Fig. S1). To avoid getting the same clonal individual, leaves were collected from different crevices for each population. These sampled leaves were dried instantly in zip bags with silica gel and then stored in a laboratory environment.

2.2. Total DNA extraction, fragment amplification and sequencing

Total DNA was extracted from the dried leaves using a modified CTAB method (Fang et al., 2009) and purified with the Universal DNA Purification Kit (Tiangen Biotech, Beijing, China). The concentration and quality of the isolated DNA were checked by electrophoresis on 0.8% (w/v) agarose gels using the DL2000 marker, and the samples were then diluted to a concentration of 10–50 ng/μL for use in polymerase chain reaction (PCR).

Forty-five pairs of universal primers that have been used in other species were tested on five samples from five populations of *T. rupestris* (Taberlet et al., 1991; Demeasure et al., 1995; Dumolin-Lapegue et al., 1997; Hamilton, 1999; Shaw and Small, 2005). Of these primer pairs, 23 are for the chloroplast genome and 22 for the mitochondrial genome. Finally, we selected 11 primer pairs that amplified the expected PCR products and produced sequences of quality high enough to amplify all 40 *T. rupestris* DNA samples. Of these primer pairs, six (*trnT*^{UGU}-*trnL*^{UAA} 5′exon, *trnL*^{UAA} 5′exon-3′exon, *trnL*^{UAA} 3′exon-*trnF*^{GAA}, *trnH*^{GUG}-*trnK*^{UUU}, *psbC-trnS* and *rps12-rpl20*) are for chloroplast DNA (cpDNA), and five (*rrn5*/*rrn18-2*, *nad1*/*2-3*, *nad4*/*3-4*, *nad7*/*1-2* and *nad7*/*2-3*) for the mitochondrial genome (Table 1). Amplification reactions were performed in a volume of 15 μL containing 1× PCR Mix (Tiangen Biotech, Beijing, China), 1.25 μM of each primer, and 10–50 ng genomic DNA. Amplification was carried out under the following conditions: 5 min initial denaturation at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 47.1–61.8 °C, 60–150 s at 72 °C and a final extension at 72 °C for 10 min.

The PCR products were directly sequenced using the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) on a MegaBACE 1000 Automatic DNA Sequencer (Amershan Pharmacia Biotech, Piscataway, NJ, USA). Singleton polymorphism was verified by re-amplifying. All haplotype sequences have been deposited in the GenBank database (accession numbers KC573070–KC573080).

Table 1
Length, polymorphic sites, substitution types and AT content of the amplified gene loci from *Taihangia rupestris*

Loci	Total length (bp)	No indels	No SNPs	A + T (%)	Transitions	Transversions
trnT^{UGU}-*trnL*^{UAA} 5′exon	710	2	14	75	0	14
trnL^{UAA} 5′exon-3′exon	567	1	2	67	2	0
trnL^{UAA} 3′exon-*trnF*^{GAA}	415	1	1	68	0	1
trnH^{GUG}-*trnK*^{UUU}	1564	3	13	66	9	4
psbC-trnS	1535	2	3	61	2	1
rps12-rpl20	763	2	4	65	2	2
Total	5554	11	37	67	15	22
rrn5/rrn18-2	1673	0	1	47	0	1
nad1/2-3	1533	1	2	47	1	1
nad4/3-4	2136	1	1	56	0	1
nad7/1-2	904	0	1	42	1	0
nad7/2-3	1069	0	1	50	0	1
Total	7315	2	6	48.2	2	4