Analysis of Genetic Relationships in Germplasms of Mugua in China Revealed by Internal Transcribed Spacer and its Taxonomic Significance

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Genetic relationships were studied among eight species of three taxa in the genus *Chaenomeles* by nuclear ribosomal internal transcribed spacer (ITS) analysis. A genetic distance matrix based on ITS sequences was estimated according to the formula of Kimura-2 parameter and a neighbour-joining phenogram, which were obtained with Clustalx4.1 software. The results showed that the germplasms of Mugua originate from *Ch. speciosa* (Sweet) Nakai, not including *Ch. sinensis* (Thouin) Kochne and *Ch. cathayensis* (Hemsl.) Schneid. The results also showed that 'Yao Mugua' and 'Ornamental Mugua' are the most distantly related species in germplasms.

Key words: Genetic Relationships, ITS, Mugua

Introduction

Mugua, the fruit of *Chaenomeles*, is a commonly used traditional Chinese medicinal herb. According to the Pharmacopoeia of the People's Republic of China (Pharmacopoeia Comittee of People's Republic of China, 2010), it is specified with the efficacy of removing dampness of the stomach to restore the normal function, calming the liver, and relaxing tendons. Chinese quince is widely distributed and cultivated throughout China, such as in Xuancheng City of Anhui Province, Langping County of Hubei Province, and Qijiang County of Chongqing City, where it is known as 'Xuan Mugua', 'Ziqiu Mugua', and 'Chuan Mugua', respectively.

According to the investigations by Peng *et al.* (2009), 'Xuan Mugua' and 'Ziqiu Mugua' are derived from the dried fruit of *Ch. speciosa* (Sweet) Nakai and developed into 5 common native breeds, while 'Chuan Mugua' is derived from the dried fruit of *Ch. speciosa* (Sweet) Nakai and *Ch. cathayensis* (Hemsl.) Schneid. The fruit of *Ch. sinensis* (Thouin) Kochne, commonly known as 'Smooth-surfaced Mugua', was misused as Yao Mugua in ancient China. Even now, in some areas in China, it is still mistaken as medical herb. *Ch. speciosa* (Sweet) Nakai is bred for ornamental tree species in many areas in China. Furthermore, 'Xuan Mugua' has been differentiated into three native varieties, namely 'Yao Mugua', 'Apple-shaped Mugua', and 'Luohanqi Mugua'. 'Ziqiu Mugua' also has been separated into two farmer varieties. Therefore, it is essential to prove the relationships among different species of Mugua in China, such as 'Xuan Mugua', 'Ziqiu Mugua' and 'Chuan Mugua', 'Smooth-surfaced Mugua' and 'Yao Mugua', and their respective germplasms.

In spite of the fact that some documents focused on relationships and differentiation among and within populations of *Chaenomeles* Lindl (Rosaceae) of *Chaenomeles* which were estimated by RAPD, cpDNA and isozymes (Bartish *et al.*, 2000; Kaneko *et al.*, 2000; Garkava *et al.*, 2000; Rumpunen and Bartish, 2002), there were no papers about molecular studies dealing with the relationships among different species of Mugua and their germplasms. Obviously, efficient studies to clarify the taxonomic status of germplasms of Mugua species in China are needed.

[§] These authors had the same contribution to the article.

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Table I. Sources and origins of the eight collected Mugua species.

Germplasm	Denomination	Species	Origin
1	Ziqiu Mugua	*Ch. speciosa* (Sweet) Nakai	Langping, Hubei, China
2	Ziqiu Mugua	*Ch. speciosa* (Sweet) Nakai	Langping, Hubei, China
3	Luohanqi Mugua	*Ch. speciosa* (Sweet) Nakai	Xuancheng, Anhui, China
4	Apple-shaped Mugua	*Ch. speciosa* (Sweet) Nakai	Xuancheng, Anhui, China
5	Yao Mugua	*Ch. speciosa* (Sweet) Nakai	Xuancheng, Anhui, China
6	Chuan Mugua	*Ch. cathayensis* (Hemsl.) Schneid.	Qijiang, Chongqing, China
7	Smooth-surfaced Mugua	*Ch. sinensis* (Thouin) Kochne	Qijiang, Chongqing, China
8	Ornamental Mugua	*Ch. speciosa* (Sweet) Nakai	Hefei, Anhui, China

In recent years, the authentication and identification of herbs has played an important role in ribosomal RNA and cDNA sequence analysis of plant species in the original appraisal (Shaw and Cao, 2004; Zhang *et al.*, 2008; Yuan *et al.*, 2009). ITS (internal transcribed spacer) is a range of 18S and 26S in the non-coding transcribed spacer, including a 5.8S coding region. Compared to the 18S and 26S, ITS undertakes rapid concerted evolution (Hillis *et al.*, 1991). Its detection, amplification, and sequencing are made easily with regard to the presence of highly conserved sequences flanking each of the two spacers (Baraket *et al.*, 2008). Therefore, analysis of ITS has been widely used for studying genetic relationships among and within groups of closely related plant taxa (Wen and Zimmer, 1996). In this paper, in order to make a research on Mugua's germplasms and the relevance between ITS genotypes, the PCR (polymerase chain reaction) techonology was used to analyse ITS1 and ITS4 gene sequencs of several Muguas derived from different origins.

Material and Methods

Plant materials

All of the accessions were collected in local areas in June 2008 by Huasheng Peng. Twenty tender fresh leaves from each randomly selected family per population were sampled for ITS analyses. All of the leaves were rapidly dried with silica gel and identified by Dequn Wang (Table I).

PCR amplification

Total cellular DNA was extracted from 50 mg of desiccated leaves by using the kit according to Dellaporta *et al.* (1983). The ITS region was amplified using PCR. For this purpose, the following primers were used after screening: ITS1 (5'TCCGTAGGTGAACCTGCGG3') and ITS4 (5'TCCTCCGCTTATTGATATGC3'). Amplifications were carried out according to the method of the kit (Kaneko *et al.*, 2000). PCRs were performed in a DNA thermocycler (Crocodile III; QBIOgene, Illkirch, France) as follows: reaction mixtures were heated at 96 °C for 5 min as an initial denaturation step before entering 12 cycles consisting of 30 s at 94 °C for denaturation, 30 s at 63 °C, 45 s at 72 °C, and then entering 35 cycles consisting of 94 °C for 30 s, 59 °C for 30 s, 72 °C for 45 s. A final extension step of 10 min at 72 °C was performed in the last cycle. Amplification products from DNA samples of families from different populations were run on the same gel (Fig. 1). Amplification products were purified by using the kit according to the manufacturer's instructions (Generay Biotech Co., Ltd., Shanghai, China).

Sequencing

Purification products were heated at 96 °C for 5 min as an initial denaturation step; magnifications were performed by 35 cycles of 10 s at 96 °C, 5 s at 50 °C and 4 min at 60 °C. The primers ITS1 and ITS4 were used for the first and second strand sequencing. The labeled reactions were

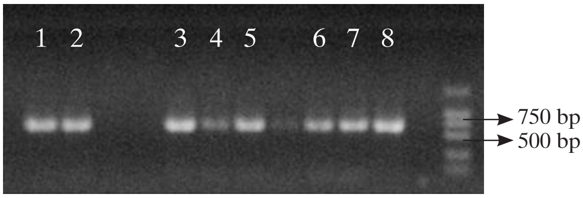

Fig. 1. Amplification products from DNA samples of different germplasms of Mugua run on the same gel. Lanes 1–8, see Table I.

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58624317
  1 TTCGGGCTCGGCGTCCCCTTATCCCGGGAGCCCGCTCCCGGGCGTACCAAACGAAAACCG
  1 TTCGGGCTCGGCGTCCCCTTATCCCGGGAGCCCGCTCCCGGGCGTACCAAACGAAAACCG
  1 TTCGGGCTCGGCGTCCCCTTAT----------------------TACCAAACGAAAACCG
  1 TTCGGGCTCGGCGTCCCCTTATCCCGGGAGCCCGCTCCCGGGCGTACCAAACGAAAACCG
  1 TTCGGGCTCGGCGTCCCCTTATCCCGGGAGCCCGCTCCCGGGCGTACCAAACGAAAACCG
  1 TTCGGGCTCGGCGTCCCCTTATCCCGGGAGCCCGCTCCCGGGCGTACCAAACGAAAACCG
  1 TTCGGGCTCGGCGTCCCCTTATCCCGGGAGCCCGCTCCCGGGCGTACCAAACGAAAACCG
  1 TTCGGGCCCGACGTCCCCTTATCCCGGGAGCCCGCTCCCGGGCGTAC-AAACGAACACCG

 61 GCGCGTGTCGCGCCAAGGAACCTGAACGAAAGAGCGCGCTCCCGCCGCCCCGGAAACGGT
 61 GCGCGTGTCGCGCCAAGGAACCTGAACGAAAGAGCGCGCTCCCGCCGCCCCGGAAACGGT
 39 GCGCGTGCTGCGCCAAGGAACCTCAACGAAAGAGCGCGCTCCCGTCGCCCCGGAAACGGC
 61 GCGCGTGCTGCGCCAAGGAACCTCAACGAAAGATCGCGCTCCCGCCGCCCCGGAAACGGT
 61 GCGCGTGCTGCGCCAAGGAACCTGAACGAAAGAGCGCGCTCCCGCCGCCCCGGAAACGGT
 61 GCGCGTGCTGCGCCAAGGAACCTCAACGAAAGATCGCGCTCCCGCCGCCCCGGAAACGGT
 61 GCGCGTGCTGCGCCAAGGAACCTCAACGAAAGATCGCGCTCCCGCCGCCCCGGAAACGGT
 60 GCGCGTGTTGCGCCAAGGAACCTGAACGAAAGATCGCGCTCCCGTCGCCCCGGAAACGGT

121 GCGCGCGCGGGTGCGTCGTCGTCTTCATTAAGTCAAAACGACTCTCGGCAACGGATATCT
121 GCGCGCGCGGGTGCGTCGTCGTCTTCATTAAGTCAAAACGACTCTCGGCAACGGATATCT
 99 GCGCGCGCGGGTGCGTCGCCCTCTTCATTATGTCAAAACGACTCTCGGCAACGGATATCT
121 GCGCGCGCGGGTGCGTCGTCGTCTTCATTACGTCAAAACGACTCTCGGCAACGGATATCT
121 GCGCGCGCGGGTGCGTCGTCGTCTTCATTACGTCAAAACGACTCTCGGCAACGGATATCT
121 GCGCGCGCGGGTGCGTCGTCGTCTTCATTACGTCAAAACGACTCTCGGCAACGGATATCT
121 GCGCGCGCGGGTGCGTCGTCGTCTTCATTACGTCAAAACGACTCTCGGCAACGGATATCT
120 GCGCCGGCGGAAGCGTCGTCGTCTTCGATACGTCAAAACGACTCTCGGCAACGGATATCT

181 CGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATC
181 CGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATC
159 CGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATC
181 CGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATC
181 CGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATC
181 CGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATC
181 CGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATC
180 CGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATC

241 CCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCTTCGGGCCGAGGGCACG
241 CCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCTTCGGGCCGAGGGCACG
219 CCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCTTCGGGCCGAGGGCACG
241 CCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCTTCGGGCCGAGGGCACG
241 CCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCTTCGGGCCGAGGGCACG
241 CCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCTTCGGGCCGAGGGCACG
241 CCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCTTCGGGCCGAGGGCACG
240 CCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCGTCAGGCCGAGGGCACG

301 CCTGCCTGGGCGTCACACGCCGTTGCCCCCCCC--AGCACCTCCCTCGGGAGGGTCGGGA
301 CCTGCCTGGGCGTCACACGCCGTTGCCCCCCCC--AGCACCTCCCTCGGGAGGGTCGGGA
279 CCTGCCTGGGCGTCACACGCCGTTGCCCCCCC---AGCATCTCCCTCGGGAGGGTCGGGA
301 CCTGCCTGGGCGTCACACGCCGTTGCCCCCCCC-AAGCACCTCCCTCGGGAGGGTCGGGA
301 CCTGCCTGGGCGTCACACGCCGTTGCCCCCCCC-AAGCACCTCCCTCGGGAGGGTCGGGA
301 CCTGCCTGGGCGTCACACGCCGTTGCCCCCCCCGAAGCACCTCCCTCGGGAGGGTCGGGA
301 CCTGCCTGGGCGTCACACGCCGTTGCCCCCCCCCAAGCACCTCCCTCGGGAGGGTCGGGA
300 CCTGCCTGGGCGTCACACGCCGTTGCCCCCCCC--CAGACCTCCTTCGGGAGCGTCGGGG

359 --GGGGCGGAGTATGGCCTCCCGTGCGTCACCCCGCGCGGTTGGCACAAATGCCGGGTCC
359 --GGGGCGGAGTATGGCCTCCCGTGCGTCACCCCGCGCGGTTGGCACAAATGCCGGGTCC
336 --GGGGCGGACGATGGCCTCCCGTGCGTCACCCCGCGCGGTTGGCGTAAATGTCGGGTCC
360 --GGGGCGGAGTATGGCCTCCCGTGCGTCACCCCGCGCGGTTGGCACAAATGCCGGGTCC
360 --GGGGCGGAGTATGGCCTCCCGTGCGTCACCCCGCGCGGTTGGCACAAATGCCGGGTCC
361 --GGGGCGGACTATGGCCTCCCGTGCGTCACCCCGCGCGGTTGGCACAAATGTCGGGTCC
361 --GGGGCGGACTATGGCCTCCCGTGCGTCACCCCGCGCGGTTGGCACAAATGCCGGGTCC
358 AGGGGGCGGAATATGGCCTCCCGTGAGTCACCCCTCGCGGTTGGCACAAATGCCGGGTCC

417 TCGGCGACGAACGCCACGACAATCGGTGGTCGTCGTACCTCGGTTGCCTGTTGTGCGCTT
417 TCGGCGACGAACGCCACGACAATCGGTGGTCGTCGTACCTCGGTTGCCTGTTGTGCGCTT
394 TCGGCGACGAACGCCACGACAATCGGTGGTCGTCGTACCTCGGTTGCCTGTTGTGCGCTT
418 TCGGCGACGAACGCCACGACAATCGGTGGTCGTCGTACCTCGGTTGCCTGTTGTGCGCTT
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419 TCGGCGACGAACGCCACGACAATCGGTGGTCGTCGTACCTCGGTTGCCTGTTGTGCGCTT
419 TCGGCGACGAACGCCACGACAATCGGTGGTCGTCGTACCTCGGTTGCATGTTGTGCGCTT
418 TCGGCGACGAACGCCACGACAATCGGTGGTCGTCAAGCCTCGGTTGCCTGTTGTGCGCCT

477 TCGTCGCGCCACGAGGGTCCCGCGACGCACCAC-GCTTCGCTTCGGCGGAGCTTTCAACG
477 TCGTCGCGCCACGAGGGGCCCGCGACGCACCAC-GCTTCGCTTCGGCGGAGCTTTCAACG
454 TCGTCGCGCCACGAGCGGCCCGCGACGCACCAC-GATTCGCTTCGGCGGAGCTTTCAACG
478 TCGTCTCGCCGCGAGCGGCCCGCGACGCACCAC-GCTTCGCTTCGACGGAGCTT-CAACG
478 TCGTCGCGCCACGAGCGTCCCGCGACGCACCAC-GCTTCGCTTCGGCGGAGCTTTCAACG
479 TCGTCTCGCCGCGAGCGGCCCGCGACGCACCAC-GCTTCGCTTCGACGGAGCTTTCAACG
479 TCGTCGCGCCGCGAGCGGCCCGCGACGCACCAC-GCTTCGCTTCGACGGAGCTTTCAACG
478 TCGTCGCGCCGCGAGCGGCCCGCGACCCCTCGCTGCTCTGTTTCGGCGGAGCCTTC----

536 -----------
536 -----------TGCGTCGTCGTCTTCATTAAGTCAAAACGACTCTCGGCAACGGATATCT
513 -----------TGCGTCGTCGTCTTCATTAAGTCAAAACGACTCTCGGCAACGGATATCT
536 CGACCCCAGTCTGCGTCGCCCTCTTCATTATGTCAAAACGACTCTCGGCAACGGATATCT
537 -----------TGCGTCGTCGTCTTCATTACGTCAAAACGACTCTCGGCAACGGATATCT
538 -----------TGCGTCGTCGTCTTCATTACGTCAAAACGACTCTCGGCAACGGATATCT
538 -----------TGCGTCGTCGTCTTCATTACGTCAAAACGACTCTCGGCAACGGATATCT
    -----------TGCGTCGTCGTCTTCATTACGTCAAAACGACTCTCGGCAACGGATATCT
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Fig. 2. nrDNA ITS sequences of eight germplasms of Mugua.

resolved and the DNA sequence was analysed by an ABI 3730XL automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). The sequences were spliced using the PHRAP software.

Alignment and sequence analysis

Nucleotide sequences were aligned with the use of appropriate programs of the Clustalx (V2.0) software. Pairwise sequence divergences between germplasms of Mugua were determined according to the Kimura-2 method using MEGA4.1 software. The genetic distance matrix was then calculated based on Kimura-2 parameter using MEGA4.1 software. The neighbour-joining method was practiced to generate phylogenetic trees, whose ramificate confidence level had an operated bootstrap of 1000 times.

Results

ITS sequence analysis

Starting from the total DNAs used as templates, the entire ITS region (ITS1 – 5.8S – ITS4) was successfully amplified and displayed a single DNA band of about 590 bp (Fig. 1). The resultant sequences are showed in Fig. 2.

The GC content of the amplified sequences varied from 64.56% to 65.70%, except for 'Ornamental Mugua' (*Ch. speciosa*, bred for ornamental tree species) with 73.03% in the ITS region (ITS1 + 5.8S + ITS4) (Table II).

After nucleotide sequences were aligned, they had 598 sites. Within them, 98 sites were changing. However, there were only 56 changing sites except the deletion sites.

ITS polymorphisms

A genetic distances matrix based on ITS sequences was estimated according to the formula of Kimura-2 parameter (Table III). Genetic distances varied from 0.0018 to 0.0918 with a mean of 0.0389. Thus, the germplasms of Mugua studied were characterized by significant divergence at the nrDNA ITS sequences. The smallest genetic distance value of 0.0018 was registered between 'Yao Mugua' and 'Ornamental Mugua' germplasms characterized by great similarities at the ITS sequence, while germplasms between 'Chuan Mugua' and 'Smooth-surfaced Mugua' were the most divergent since they exhibited the highest genetic distance of 0.0918. Moreover, 'Smooth-surfaced Mugua' germplasm had the largest divergence compared with all the others. All the remaining germplasms showed intermediate levels of similarities.

Table II. Length and G + C contents of ITS sequences of germplasms of Mugua. 1–8, see Table I.

Germplasm	Length [bp]	G + C content (%)
1	596	65.27
2	589	65.70
3	596	65.27
4	577	65.68
5	593	65.60
6	570	64.56
7	593	65.09
8	534	73.03

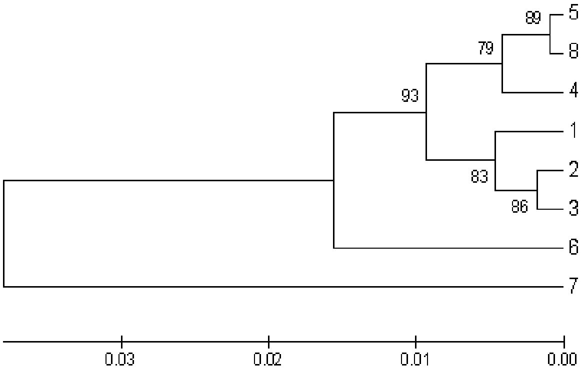

Fig. 3. Neighbour-joining phenogram of eight germplasms of Mugua based on nrDNA ITS sequences. 1–8, see Table I.

The derived neighbour-joining phenogram is illustrated in Fig. 3. This tree classifies Mugua cultivars organization into three main clusters. The first group is only composed of 'Smooth-surfaced Mugua' germplasm. 'Chuan Mugua' germplasm belongs to the second group. The rest Mugua germplasms constitute the third cluster that comprised two sub-groups. The first sub-group is composed of 'Ziqiu Mugua' and 'Luohanqi Mugua' germplasms; while the remaining germplasms constitute the second sub-cluster including 'Apple-shaped Mugua', 'Yao Mugua' and 'Ornamental Mugua'. In addition, this tree branching was made independently either from the geographic origin of the cultivars or from their denominations.

Table III. Genetic distances matrix among germplasms of Mugua based on ITS sequences data calculated by the formula of Kimura-2 parameter, corresponding to the smallest and the highest genetic distances. 1–8, see Table I.

Germplasm	5	8	6	2	4	3	1	7
5		0.0018	0.0077	0.0056	0.0035	0.0060	0.0064	0.0113
8	0.0018		0.0075	0.0054	0.0039	0.0059	0.0061	0.0111
6	0.0338	0.0319		0.0073	0.0069	0.0069	0.0075	0.0125
2	0.0185	0.0167	0.0319		0.0043	0.0025	0.0040	0.0112
4	0.0074	0.0092	0.0280	0.0111		0.0049	0.0053	0.0111
3	0.0223	0.0204	0.0281	0.0037	0.0148		0.0039	0.0113
1	0.0242	0.0223	0.0338	0.0092	0.0167	0.0092		0.0113
7	0.0753	0.0733	0.0918	0.0713	0.0713	0.0733	0.0753	

Discussion

Misapplication of Ch. sinensis as Mugua

Chaenomeles is a genus within the sub-family Maloideae comprising five species, which are all distributed in East Asia. But, there are significant differences between *Ch. sinensis* (Thouin) Kochne and the other four species. The discriminative features of *Ch. sinensis* (Thouin) Kochne lies in anacanthous branches and simple flowers which blossom with full-developed leaves. In addition, *Ch. sinensis* (Thouin) Kochne is a tree, while the other four species are shrubs (Editorial Committee for Flora of Chinese Academy of Sciences, 1974). Therefore, Wu *et al.* suggested that *Ch. sinensis* (Thouin) Kochne should be classified as a new taxon called *Pseudocydonia*. Moreover, the fruits of *Ch. sinensis* (Thouin) Kochne significantly differ from those of *Ch. speciosa* (Sweet) Nakai and *Ch. cathayensis* (Hemsl.) Schneid. in the chemical compositions of the volatile oil: the former one contains less than 20% organic acids and anhydride; the latter two contain more than 40% organic acids. Their diversity mainly manifests in the contents of oleanolic acid and ursolic acid: the former one contains only up to 0.09%; the latter two contain more than 0.48% (Zhang *et al.*, 2009). Based on the above analysis, the fruits of *Ch. sinensis* (Thouin) Kochne, known as 'Smooth-surfaced Mugua', are inappropriately classified as Mugua.

This study showed that *Ch. sinensis* (Thouin) Kochne's genetic distances range from 0.0713 to 0.0918 to all the others. The derived neighbour-joining phenogram showed that the first group is only composed of *Ch. sinensis* (Thouin) Kochne. Based on the experimental results, we support the standpoint that the germplasms of Mugua do not include *Ch. sinensis* (Thouin) Kochne.

Relationship between Ch. speciosa (Sweet) Nakai and Ch. cathayensis (Hemsl.) Schneid.

Ch. cathayensis (Hemsl.) Schneid. differs from *Ch. speciosa* (Sweet) Nakai in its young leaves being covered with thick brown floss (Editorial Committee for Flora of Chinese Academy of Sciences, 1974). Both 'Xuan Mugua' and 'Ziqiu Mugua' are derived from the dried fruit of *Ch. speciosa* (Sweet) Nakai, while 'Chuan Mugua' is derived from the dried fruit of *Ch. speciosa* (Sweet) Nakai and *Ch. cathayensis* (Hemsl.) Schneid. Meanwhile, Bing-zhang Cao, a famous traditional Chinese medicine expert who lived in the Qing Dynasty (Cao, 2004), believed firmly that the quality of 'Chuan Mugua' is inferior to that of 'Xuan Mugua' and 'Ziqiu Mugua'. Why did he believe so? We think the reason for the poor quality of 'Chuan Mugua' is that their germplasm is closely related with 'Smooth-surfaced Mugua' germplasm.

The fruits of *Ch. cathayensis* (Hemsl.) Schneid. largely differ from the fruits of *Ch. speciosa* (Sweet) Nakai in chemical compositions: only the latter onecontains anhydride; and the esters content of the latter one is 7–10 times than that of the former one (Zhang *et al.*, 2008). The big genetic distance mean value of 0.0338 and 0.0333 was registered between 'Chuan Mugua' and 'Xuan Mugua'. 'Chuan Mugua' and 'Ziqiu Mugua' germplasms were characterized by significant divergence in the ITS sequence. The derived neighbour-joining phenogram showed that the second group was composed only of *Ch. cathayensis* (Hemsl.) Schneid. The third group was composed of germplasms of 'Xuan Mugua' and 'Ziqiu Mugua'. Therefore, we support the standpoint that the germplasm of Mugua comes form *Ch. speciosa* (Sweet) Nakai, excluding *Ch. cathayensis* (Hemsl.) Schneid.

Relationship between 'Yao Mugua' and 'Ornamental Mugua'

'Xuan Mugua' has been classified into three farmer varieties such as 'Yao Mugua', 'Apple-shaped Mugua', and 'Luohanqi Mugua'. 'Yao Mugua' significantly differs from the other two farmer varieties, however, it is similar to the 'Ornamental Mugua' in being a small shrub with bright red flowers. This study shows that the smallest genetic distance value of 0.0018 is registered between 'Yao Mugua' and 'Ornamental Mugua' germplasms characterized by great similarities in the ITS sequence.

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