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Full Length Research Paper

Population genetic structure and gene flow of *Forsythia suspensa* (Oleaceae) in Henan revealed by nuclear and chloroplast DNA

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Forsythia suspensa **(Thunb.) Vahl, is a climbing plant belonging to Oleaceae, which is widely distributed in China, North and South Korea and Japan. In this study, the genetic diversity of *F. suspensa* was analyzed using two noncoding chloroplast DNA regions (*trn*L-F and *psb*A-*trn*H) and nuclear ribosomal internal transcribed spacer (nrITS) with 60 individuals of Henan Province. A survey of nrDNA and cpDNA variation detected remarkably high levels of genetic diversity (nrDNA: h_T = 0.478; cpDNA: h_T = 0.806). As revealed by the results of AMOVA analysis, genetic differentiation for nrDNA (Φ_{ST} = 0.110) was obviously lower than for cpDNA data (Φ_{ST} = 0.937) in *F. suspensa*, gene flow among populations based on nrDNA (*Nm* = 2.02) was significantly higher than that based on cpDNA data (*Nm* = 0.02). Significant isolation-by-distance (IBD) for cpDNA was detected at the species-wide range (*r* = 0.548, P < 0.05), however, non-significant IBD for nrDNA was detected (*r* = 0.362, P > 0.05). On the basis of the genetic information, we propose LY and LJ populations should be conserved *ex situ*; however, JG and SS populations should be conserved *in situ*.**

Key words: *Forsythia suspensa*, genetic diversity, gene flow.

INTRODUCTION

Forsythia suspensa (Thunb.) Vahl, is a climbing plant belonging to Oleaceae and is recognized as an important Chinese herbal medicine. It is widely distributed in China, North and South Korea and Japan. In China, *F. suspensa* mainly occurs in majority of warm temperate regions and partial of sub-tropical regions (for example, Henan Province, Hebei Province, Shanxi Province, Shaanxi Province, Shandong Province, Anhui Province, Hubei Province and Sichuan Province) at elevations of 300 to 2200 m. *F. suspensa* is sprawling deciduous shrub. The species can reach a height of 3 m, which has launched and vaulted drooping branches. It usually possesses simple leaves, sometimes ternately compound leaves. Twigs are tan and have obvious lenticels and hollow pith. Flowers blossom before leaves grow out in March to May. The flowers are bright yellow and the green calyx has four sepals, fruit is ovoid capsule. The plant has a morphologyical and biological character adapted to cross-pollination and good sexual reproduction (Li et al., 2006). Seeds of *F. suspensa* numerous in each locule, slightly winged. In natural conditions, the seed dispersal distance of *F. suspensa* is short. *F. suspensa* has a wide region of distribution; however, within that region, it mostly appears on the slopes with secondary vegetation.

F. suspensa was used for the treatment of various infectious diseases (Nishibe et al., 1982; Yin et al., 1991; Prieto et al., 2003), which is attributed to its containing important bioactive anti-inflammatory lignans, such as

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Abbreviations: ITS, Internal transcribed spacer; $\mathbf{h_T}$**,** total genetic diversity; $\mathbf{\Phi_{ST}}$**,** genetic differentiation among populations; **Nm,** gene flow among populations; **IBD,** isolation by distance; **cpDNA,** chloroplast DNA; **nrDNA,** nuclear DNA; **AMOVA,** analyses of molecular variance; **MP,** maximum parsimony; **π,** nucleotide diversity; **h,** haplotype diversity; **JG,** Jigong Mountain; **TB,** Tongbai Mountain; **LY,** longyuwan; **LJ ,** Laojieling; **SS,** Songshan; **JL,** Jiulian Mountain.

rutin and forsythin (Li et al., 2002; Qu et al., 2008). The natural resources of *F. suspensa* have been declining in recent years because it was harvested for its fruit. In

Table 1. Details of population locations, sample size, cpDNA and nrDNA variation of *Forsythia suspensa* sampled in Henan. Sample size (N) is indicated for cpDNA and nrDNA analysis separately; *h*: haplotype diversity, π: nucleotide diversity.

Population number and code	Location	Latitude(N)/ Longitude(E)	N	cpDNA			nrDNA		
				Haplotype	$\pi\times10^{-3}$	h	Haplotype	$\pi\times10^{-3}$	h
1 JG	Jigong Mountain, Xinyang	31°50′/114°05′	10	H4	0	0	D, F	0.92	0.442
2 TB	Tongbai Mountain, Nanyang	32°23′/112°50′	10	H2	0	0	A, D, E, F, H, M	2.05	0.516
3 LY	Longyuwan, Luoyang	33°42′/111°45′	10	H1, H3, H6	0.81	0.378	F, H, I, J, L	1.44	0.442
4 LJ	Laojieling, Nanyang	33°45′/111°20′	10	H1	0	0	B, F, K, N	3.38	0.574
5 SS	Songshan, Zhengzhou	34°28′/113°05′	10	H5	0	0	C, F, M	1.85	0.416
6 JL	Jiulian Mountain, Xinxiang	35°35′/113°35′	10	H3	0	0	F, G, M	1.32	0.353
Species mean					1.84	0.806		2.02	0.478

previous studies of *F. suspensa*, the pharmacology and biochemistry attracted more attention (Guo et al., 2007; Piao et al., 2008; Seya et al., 1989) and the genetic diversity of wild population was seldom mentioned. In order to devise adequate conservation and management strategies for this species, it is important to characterize its genetic diversity and understand its population structure.

Chloroplast DNA (cpDNA) is transmitted only through seeds in most angiosperms and often shows a more highly population structure than the nuclear genome. Thus, chloroplast DNA noncoding spacers have been used frequently to survey population variation of plants (Beheregaray, 2008). Unfortunately, the low nucleotide variation of cpDNA hinders the investigation on genetic diversity in many plant species. Nuclear gene can compensate for the weakness for its high levels of genetic variation. However, due to the biallelic nature of nuclear gene, strong gene flow through pollen usually obscures the population genetic structure.

In this study, two noncoding chloroplast regions (*trn*L-F and *psb*A-*trn*H) and one nuclear region (nrITS) were used to assess the extent and structure of genetic variation among and within populations of *F. suspensa* from one mainly production place, Henan Province, China. Our specific objectives were (1) to examine levels and distribution of genetic variability within and among populations of *F. suspensa*; (2) to compare whether there may have the consistence of genetic structure revealed by cpDNA variation and nuclear gene data; (3) to compare the seed-mediated and pollen -mediated gene flow among different populations.

MATERIALS AND METHODS

Population sampling

Ten individuals of *F. suspensa* were randomly chosen for sampling from each of six wild populations in Henan Province, China (Table 1; Figure 1). The distance between samples within populations was at least 10 m, to increase the likelihood of sampling interindividual variation within each population. Young leaves were collected and dried in silica gel and stored at -20°C for DNA extraction. *Forsythia viridissima* was used as the out group.

DNA Isolation, PCR amplification and DNA sequencing

Total genomic DNA was extracted using the modified CTAB method of Doyle and Doyle (1987). DNA concentrations were determined (1) on ethidium bromide-stained 1% agarose gels by comparison with known amounts of λ DNA; (2) by spectrophotometry. Working stocks of DNA were then prepared based on both estimates, and stored in 0.1 × TE buffer. After preliminary screening, polymorphism was observed in two cpDNA uncoding regions (*trn*L-F and *psb*A-*trn*H) and internal transcribed spacer (ITS). These were amplified via polymerase chain reactions (PCRs) using primers of Taberlet et al. (1991; *trn*L-F), Kress et al. (2005; *psb*A-*trn*H) and White et al. (1990; ITS4-ITS5). PCR reactions were carried out in a PTC-200 thermal cycler (MJ Research, Watertown, MA) using a total volume of 30 µl containing 30 ng of genomic DNA, 0.2 mM of each dNTP, 0.3 µM of each primer, 3 µl *Taq* buffer and 1 unit *Taq* polymerase (Takara, Dalian, Liaoning, China). The PCR program was as follows: 4 min initial denaturation at 94°C, followed by 35 cycles of 40 s denaturation at 94°C, 45 s annealing at 50°C, 1 min 30 s elongation at 72°C and 8 min extension at 72°C. PCR products were purified using E.Z.N.A.® gel extraction kit (Omega Bio-Tek, Winooski, VT) and directly sequenced in both directions by standard methods using a *Taq* dye deoxy terminator cycle sequencing kit (Applied Biosystems, ABI; Foster City, CA) with the same primers used in the original amplification. Sequences were generated with an ABI 377XL DNA sequencer and edited using SEQUENCHER™ (version 4.0; Gene Codes Co., Ann Arbor, MI).

Data analysis

The DNA sequences were edited manually based on the chromatograms and aligned by CLUSTAL_X (version 1.81;

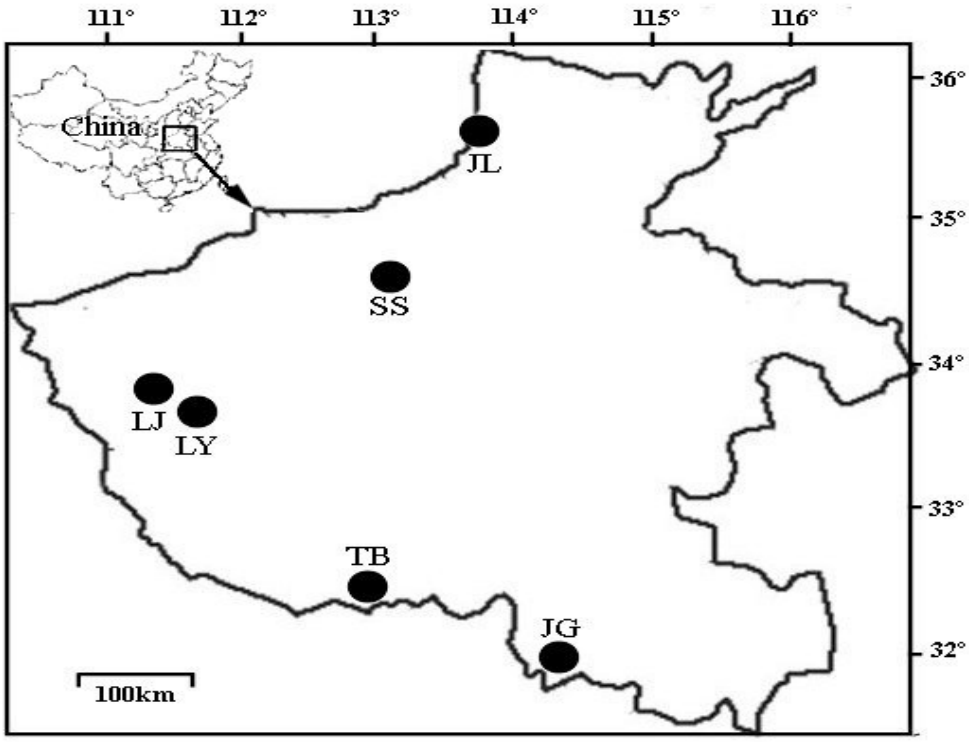

Figure 1. Locations of *F. suspensa* populations sampled for this study. Population codes as in Table 1.

Thompson et al., 1997). Because *F. suspensa* are diploid (2n=28), we counted the 2 alleles of each sampled individual and treated a site as polymorphic if there was a "double peak" in the chromatogram. Haplotypes of nuclear gene were inferred via "haplotype subtraction" (Zhou et al., 2007; Clark, 1990. Haplotype diversity (*h*) and nucleotide diversity (*n*) were calculated for each population (h_S, π_S) and at the species level (h_T, π_T) using DNASP (version 4.0; Rozas et al., 2003). Gene flow (*Nm*) (Hudson et al., 1992) among populations was also calculated by DNASP. Analyses of molecular variance (AMOVAs) were used to calculate genetic variance components and their significance levels among populations and within populations by ARLEQUIN (version 3.1; Excoffier et al., 2005). Genetic relationships among 6 populations of *F. suspensa* were illustrated by the procedures NEIGHBOR and CONSENSE of the program PHYLIP (version 3.63; Felsenstein, 2004). Phylogenetic relationships between cpDNA and nrDNA haplotypes of *F. suspensa* were assessed under Maximum Parsimony (MP) in PAUP* (version 4.0 beta10; Swofford, 2002) using *F. viridissima* as an out group.

To see if the obtained cpDNA and nrDNA sequences satisfied the assumption of neutrality, we calculated Tajima's *D* (Tajima, 1989) and Fu and Li's *D** (Fu and Li 1993) for the entire species and groups of populations, using DNASP. Statistical significance of *D* and *D** was estimated with coalescent simulations as implemented in this program. In general, significant negative departures of these statistics from zero indicate deviation from neutrality, but might also be taken as evidence of recent demographic expansions or population bottlenecks when markers are otherwise assumed to be independent of selection (Tajima, 1989; Fu, 1997). To further infer demographic processes, we explicitly tested the null hypotheses of a spatial expansion and of a pure demographic expansion in DNASP by comparing observed and expected distributions of pairwise sequence differences (mismatch distributions).

For both cpDNA and nrDNA data, tests of isolation-by-distance (IBD) were performed by regressing values of F_{ST} against the geographic distance (*Km*) with the Mantel permutation procedure as implemented in IBD (Jensen et al., 2005; Isolation by distance, web service. BMC genetics 6: 13. v.3.16 http://ibdws.sdsu.edu/). Finally, a pollen/seed migration ratio (*r*) was calculated using a modified equation of Ennos (1994) following Petit et al. (2005) with AMOVA-derived Φ_{ST} values taken as estimators of population differentiation: $r = m_p/m_s = [(1/\Phi_{ST(n)}-1)-2(1/\Phi_{ST(c)}-1)]/(1/\Phi_{ST(c)}-1)$, where, m_p is the pollen migration rate, m_s is the seed migration ratio, $\Phi_{ST(n)}$ is the nuclear (nrDNA) Φ_{ST}, and $\Phi_{ST(c)}$ is the cytoplasmic (cpDNA) Φ_{ST}.

RESULTS

cpDNA diversity and population structure

Out of the two cpDNA regions sequenced in *F. suspensa* (60 individuals, 6 populations), both of which showed no length variation, *trn*L-F and *psb*A-*trn*H was uniformly 783 and 396 bp, respectively. When combined, these sequences were aligned with a consensus length of 1179 bp, containing eight nucleotide substitutions. The haplotype diversity and nucleotide diversity was $h_T = 0.806$ and $\pi_T = 1.84\times10^{-3}$, respectively. Gene flow among populations based on combined cpDNA data was extremely low (*Nm* = 0.02). Nucleotide diversity (π) among 6 populations ranged from 0 to 0.81×10^{-3} and haplotype diversity (*h*) varied between 0 and 0.378. Highest nucleotide diversity

Table 2. Variable sites of the aligned sequences of two chloroplast DNA fragments in 6 haplotypes of *F. suspensa*.

Nucleotide position	trnL-F		psbA-trnH					
	177	477	803	925	950	1010	1111	1151
H1	G	C	C	C	G	C	T	C
H2	G	T	C	C	G	C	T	C
H3	G	C	C	C	G	C	C	C
H4	G	C	C	A	G	C	C	A
H5	A	C	T	A	G	C	C	C
H6	G	C	C	A	A	T	C	C

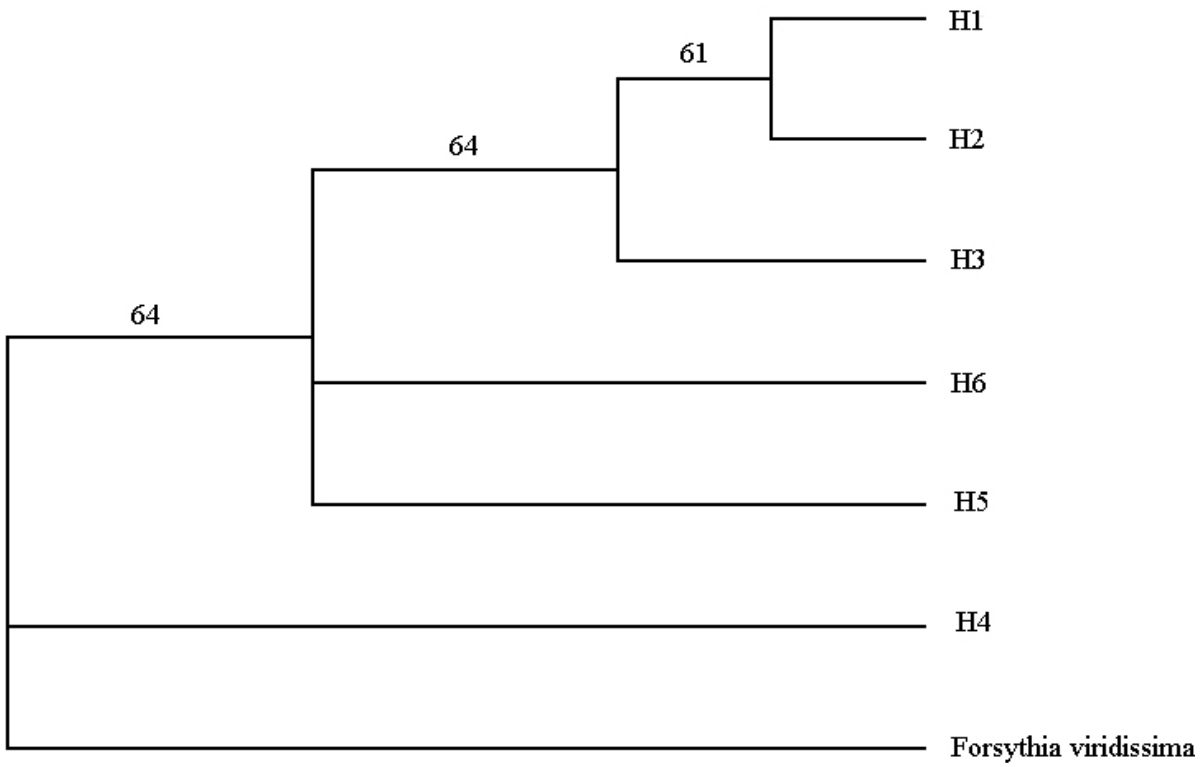

Figure 2. Maximum parsimony (MP) clustering of six chloroplast haplotypes sequences of *F. suspensa*. The bootstrap confidence values (%) are indicated on the branches.

and haplotype diversity were found in population LY (Table 1). Based on these polymorphisms, six cpDNA haplotypes (H1-6) were identified across the material surveyed (Table 2). The sequences of three *trn*L-F and five *psb*A-*trn*H haplotypes have been deposited in GenBank database under accession numbers (HQ658062-HQ658064) and (HQ658065-HQ658069), respectively. The *trn*L-F and *psb*A-*trn*H sequences of the out group (*F. viridissima*) deposited in GenBank database were under accession numbers HQ664452 and HQ664453. Maximum Parsimony tree of haplotypes revealed that H1-3, 5-6 formed a monophyletic group, H4 was closer to the outgoup (Figure 2). However, neighbor joining tree of population suggested that populations LY, LJ, TB and JL were more closely related, which formed a monophyletic group, populations JG and SS were also closely related (Figure 3).

AMOVA revealed that 93.7% of the total cpDNA variance was distributed among populations and only 6.3% was apportioned within populations (Table 3). Significant IBD for cpDNA was detected at the species-wide range (r = 0.548, $P < 0.05$). Tajima's D and Fu and Li's D^* statistics for deviation from neutrality were non-significant for each geographic region and the whole species (all $P > 0.10$). Furthermore, the observed mismatch distributions of haplotypes from the whole species did not differ significantly from mismatches expected under models of both spatial and sudden demographic expansion (r =

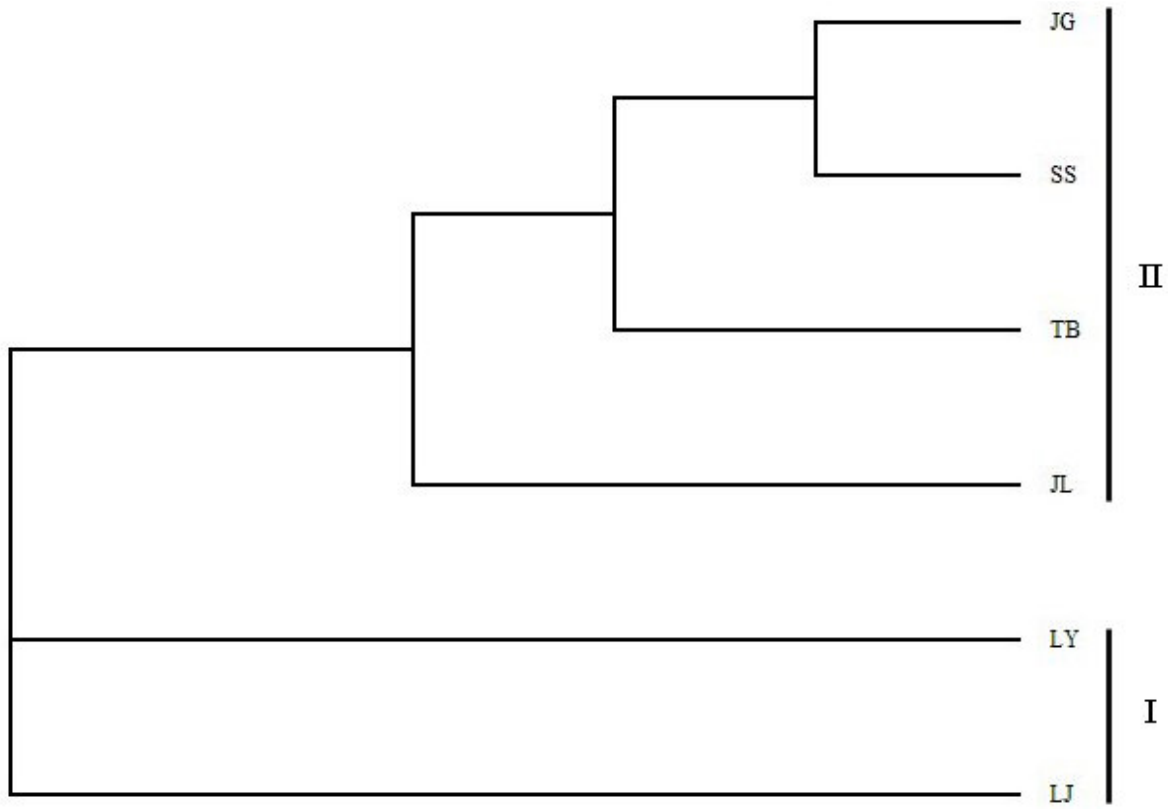

Figure 3. Neighbor-joining (NJ) clustering of 6 populations of *F. suspensa* based on Φ_{ST} of cpDNA markers among populations. Population numbers are identified in Table 1.

Table 3. Analysis of molecular variance (AMOVA) for populations of *F. suspensa* based on cpDNA and nrDNA data.

Source of variation	d.f.	SSD	Variance component	Percentage of variation
cpDNA				
Among populations	5	59.583	1.18370	93.70**
Within populations	54	4.300	0.07963	6.30**
nrDNA				
Among populations	5	7.633	0.05436	11.01**
Within populations	114	50.100	0.43947	88.99**

(d.f., degree of freedom; SSD, sum of squares); **P < 0.001

0.040, P > 0.05).

nrDNA diversity and population structure

The aligned sequences of ITS were 481 bp in length. The haplotype diversity and nucleotide diversity were h_T = 0.478, π_T =2.02×10^{-3}. Nucleotide diversity (π) among 6 populations ranged from 0.92×10^{-3} to 3.38×10^{-3} and haplotype diversity (h) varied between 0.353 and 0.574. Highest nucleotide diversity and haplotype diversity were found in population LJ (Table 1). Thirteen substitutions constituted fourteen haplotypes (A-N, Table 4). The sequences of fourteen ITS haplotypes have been deposited in GenBank database under accession numbers (HQ658070- HQ658083). The outgroups (*F. viridissima*) sequences of ITS deposited in GenBank database under accession numbers (HQ664454). Gene flow among populations based on nrDNA (Nm = 2.02) was obviously higher than that based on cpDNA. Maximum Parsimony tree of haplotypes revealed that haplotype D-L formed a monophyletic group, A-C, M and N was close to outgroup (Figure 4). Neighbor joining tree of population suggested that populations LY, LJ, JL and

Table 4. Variable sites of the aligned sequences of internal transcribed spacer (ITS) in 14 haplotypes of *F. suspensa*.

Nucleotide position	ITS												
	43	53	60	65	75	108	118	195	298	300	350	358	448
A	C	T	C	C	C	G	C	G	C	C	G	G	G
B	C	C	C	T	C	G	C	G	C	C	G	G	G
C	C	C	C	C	C	G	C	G	C	C	C	G	G
D	C	T	T	C	C	G	C	G	C	C	C	G	G
E	C	T	T	C	C	C	C	G	C	C	C	G	A
F	C	T	T	C	C	G	C	G	T	C	C	G	G
G	C	T	T	C	C	G	T	G	T	C	C	G	G
H	C	T	T	C	C	G	C	G	T	T	C	G	G
I	T	T	T	C	C	G	C	G	T	T	C	G	G
J	C	T	T	C	T	G	C	G	T	T	C	G	G
K	C	T	T	T	C	G	C	G	T	C	C	A	G
L	C	T	T	T	C	G	C	G	T	T	C	G	G
M	C	T	C	C	C	G	C	G	C	C	C	G	G
N	C	T	C	T	C	G	C	A	C	C	C	G	G

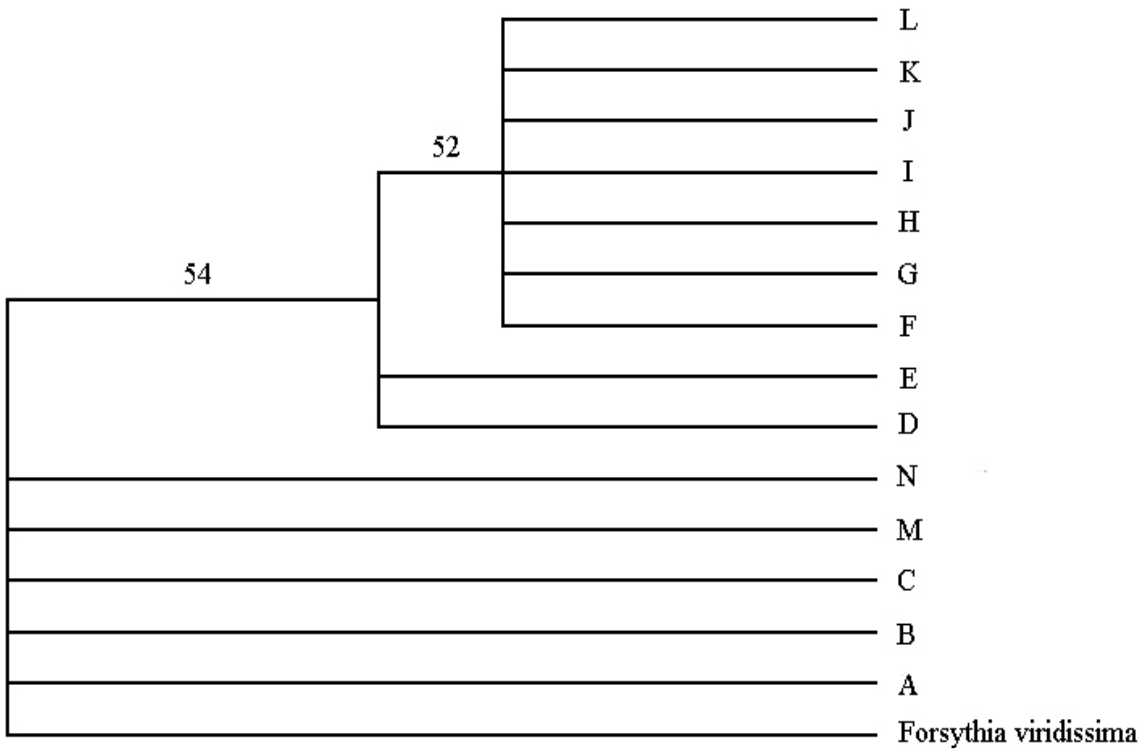

Figure 4. Maximum parsimony (MP) clustering of fourteen nuclear gene haplotypes sequences of *F. suspensa*. The bootstrap confidence values (%) are indicated on the branches.

SS has more closely phylogenetic relations, JG and TB were also closely related populations (Figure 5).

AMOVA revealed that only 11.01% of the total nrDNA variance was distributed among populations, 88.99% was apportioned within populations (Table 3). No significant IBD for nrDNA was detected at the species-wide range (r = 0.362, $P > 0.05$). Tajima's D and Fu and Li's D^* statistics for deviation from neutrality were non-significant for each geographic region and the whole species ($0.10 > P > 0.05$). The observed mismatch distributions of haplotypes from the whole species did not differ significantly from mismatches expected under models of both

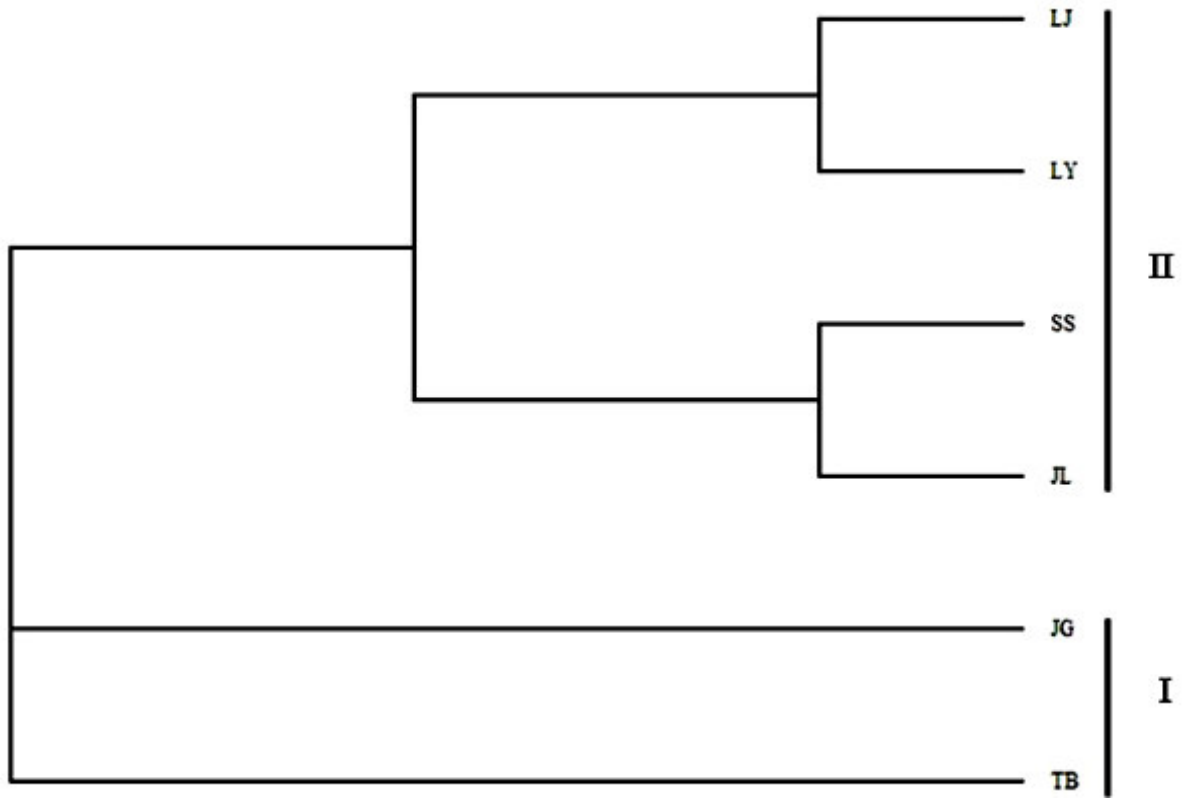

Figure 5. Neighbor-joining (NJ) clustering of 6 populations of *F. suspensa* based on Φ_{ST} of nrDNA markers among populations. Population numbers are identified in Table 1.

spatial and sudden demographic expansion (r = 0.163, P > 0.05).

Using the nrDNA-derived $\Phi_{ST(n)}$ value of 0.110 across the 6 populations surveyed (aforementioned), and their corresponding value for cpDNA, $\Phi_{ST(c)}$ = 0.937, the pollen/seed migration ratio (r) was calculated as 117.3, indicating significantly the high level of pollen flow when compared with seed flow.

DISCUSSION

Genetic diversity

In recent years, the natural resources of *F. suspensa* decrease dramatically because *F. sus*pensa was traditionally harvested for its fruit, but there was little know about the genetic diversity of *F. sus*pensa. The results of using both cpDNA and nrDNA indicate that there are high levels of genetic diversity in all 6 investigated natural populations of *F. suspensa.* Total levels of cpDNA haplotype diversity in *F. suspe*nsa (h_T = 0.806) were significantly higher than other seed plants for maternally inherited markers (Huang et al., 2002; Gao et al., 2007). For nrDNA markers, species-wide levels of haplotype diversity in *F. suspe*nsa (h_T = 0.478) were also high by comparison with other woody plant species with similar life history traits and geographical range reviewed by Hamrick et al. (1992) (mean h_T = 0.211, for outcrossing, animal-pollinated species). Genarally, geographic distribution, breeding system and size of population are all associated with genetic diversity in plant species (Hamrick and Godt, 1989). Species with out-crossing and mixed breeding have significantly higher diversity than species with self-crossing, geographically widespread species usually have higher genetic diversity than limited distributions, large size of population will higher than small (Hamrick and Godt, 1989, 1996; Hamrick et al., 1992; Nybom, 2004). As a dominant species in this region, *F. sus*pensa is an outcrossing, animal-pollinated species and which has large populations size, although, their population size decrease dramatically, the undetected responses in genetic diversity may because insufficient time has elapsed since the reduction.

Among the populations investigated, for cpDNA makers, the population LY(h = 0.378, $\pi = 0.81\times10^{-3}$) had the highest nucleotide diversity and haplotype diversity; for nrDNA marker, the population LJ (h = 0.574, $\pi = 3.38\times10^{-3}$) had the highest nucleotide diversity and haplotype diversity. LY and LJ were adjacent populations, both of which belong to Funiu Mountains. In this region, plants resources are well protected due to the nature

reserves have been established. However, LY located on the north slope of Funiu Mountains, LJ located on the south slope. This discrepancy of cpDNA data and nrDNA data was probably related the asymmetrical gene flow between North slope and South slope.

Gene flow

A high level of pollen-mediated (*Nm* = 2.02) and low level of seed-mediated (*Nm* = 0.02) gene flow among populations is suggested by our results. Hence, cpDNA data suggest a substantial amount of population isolation for chloroplast reduced levels of contemporary intersite gene flow via seed, but high gene flow via pollen. The stated results seem to accord with a predominantly outcrossing breeding system and wind dispersed mechanism of *F. suspensa.* Although, seeds of *F. suspensa* are small and with wing-like structures and they were dispersed by wind, the dispersal distance was short and most of them were spread in the same population; however, pollen was transmitted by insects, dispersal distance was longer than seeds.

Interestingly, the pollen to seed migration ratio (*r*) obtained for *F. suspensa* (*r* = 117.3) is obviously higher than the corresponding average value reported for seed plant species (median $r \approx 17$, estimated over 93 species, Petit et al., 2005; Hodgins and Barrett, 2007). The spatial pattern of maternally inherited chloroplast differentiation has indicated the presence of obvious genetic structure, gene flow through long-distance dispersed pollen in insect pollinated plants usually erodes the genetic signature of isolation by distance. Our results therefore, represent another example of this situation, and provide evidence for efficient pollen-mediated gene flow among the isolated populations. Because of the effects of such long-distance, pollen-mediated gene flow, forest fragmentation and habitat isolation among populations may not have played an important role in nuclear genomic diversification and speciation at least in insect-pollinated taxa. A significant isolation-by-distance pattern registered by cpDNA ($r = 0.548$, $P < 0.05$), suggested restricted gene flow among populations of *F. suspensa*. In contrast to cpDNA, an analysis of the degree of genetic isolation with increasing geographic distance (IBD) was statistically non significant revealed by nrDNA($r = 0.163$, $P > 0.05$), suggesting high level gene flow among *F. suspensa* populations has obscured population genetic structure.

Genetic structure

cpDNA data demonstrated significantly population differentiation within *F. suspensa* ($\Phi_{ST} = 0.937$), this was due to the fixation of particular haplotypes in single populations. In contrast to the significant population genetic structure obtained with cpDNA, there was a moderate level of genetic differentiation for nrDNA haplotype ($\Phi_{ST} = 0.110$). Limited seed flow and high pollen flow among populations was considered to be the explanation for the high population differentiation in *F. suspensa*. Although, this result is not strictly comparable to our case in nrDNA because of the different molecular markers, studies indicated limited potential for long-distance dispersal. Tajima's *D* and Fu and Li's *D** statistics together with the multimodal mismatch distribution also suggested that population expansion did not occur in *F. suspensa*, which is consistent with the limited dispersal hypothesis.

The rooted network illustrates the relationships between the 6 cpDNA haplotypes and 14 nrDNA haplotypes (Figures 2 and 4). Maximum parsimony tree of haplotypes revealed that H1-3, 5-6 formed a monophyletic group, haplotype D-L also formed a monophyletic group. H1, H3, H6 were all presented in one population (LY), H5 was also found in the adjacent population SS. Both LY and SS belong to Funiu Mountains, indicating that closely related haplotypes were more likely to co-occur in the same region. For nrDNA markers, D-H were all presented in one population (TB); H-L were all presented in populations LJ and LY, which had the shortest geographic distance in all sampled populations. The three populations were all adjacent, also supported closely related haplotypes were more likely to co-occur in the same region. Two groups were obviously subdivision based on neighbor-joining tree of 6 populations and cpDNA haplotyes, including I (LY and LJ) versus II (JG, TB, SS and JL). As LY and LJ have most short geographic distance, the seed dispersal between the two populations was easier than populations of long-distance. The divergence of this population genetic group apparently coincides with the seeds dispersal mechanism of *F. suspensa*. However, two groups were also subdivision based on neighbor-joining tree of 6 populations and nrDNA haplotyes, including I (JG and TB) versus II (LY, LJ, SS and JL). This result also indicated the populations of short-distance (3 pairs: JG and TB versus LY and LJ versus SS and JL) almost had similar genetic components. Although, the pollen flow was very strong compare to seed flow, the gene flow might play a main role in the adjacent populations.

Guidelines for conservation

The estimate of genetic diversity and population genetic structure provide a basis for conservation and utilization of *F. suspensa.* It will help us in determining what to conserve and how to conserve this species.

In this study, the results show that there was high genetic diversity at the species level, although, over collection has caused the natural population dramatically decrease. *In situ* conservation is first recommended to

protect its original habitat from further destruction. Among the populations investigated, the JG and SS populations are critically endangered because of their small population size and habitats lose. Therefore, both populations should be protected immediately. For *ex situ* conservation, we suggest that the population which has high gene diversity should be selected as the conservation unit. The LY and LJ populations, which revealed higher level of genetic diversity in each region, are the best candidates for *ex situ* conservation.

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