Genetic diversity and structure analysis of the endangered plant species
*Horsfieldia hainanensis* Merr. in China

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**ABSTRACT**

The genetic diversity and structure of nine natural populations of *Horsfieldia hainanensis* Merr., an endangered plant endemic to China, were studied using inter-simple sequence repeat markers. Nine primers were selected from 100 primers to evaluate 126 individual plants, from which a total of 136 bands were amplified and 108 bands were polymorphic. Our results demonstrated that the genetic diversity level of *H. hainanensis* was high with a percentage of polymorphic bands, Shannon’s diversity index and Nei’s genetic diversity index at the species level of 79.4%, 0.4787 and 0.3314, respectively, and correspondingly, averages of 40.4%, 0.2615 and 0.1843 at the population level. Significant genetic differentiation was observed among populations, showing that the coefficient of genetic differentiation among populations calculated using Nei’s genetic diversity was 0.4509. The ranges of Nei’s genetic identity and genetic distance among populations were 0.7387–0.8637 and 0.1466–0.3029, respectively. The unweighted pair group method with arithmetic mean clustering based on Nei’s genetic distance indicated that nine natural *H. hainanensis* populations could be classified into two lineages. Collectively, we speculated that habitat fragmentation and disturbance from human activities could be considered the main reasons for the endangerment of *H. hainanensis*, and we propose *in situ* conservation for the existing natural *H. hainanensis* populations, especially at Mengtun, Niandou and Tongbiguan, where the genetic diversity is relatively high.

**Introduction**

*Horsfieldia hainanensis* Merr., belonging to the Myristicaceae family, is an evergreen tree with a narrow domain distribution in China. Trees belonging to this species possess good essential oil from the bark and red wood, which can be used for high-end furniture, decoration and other purposes, which make it an attractive resource [1]. *H. hainanensis* is mainly distributed in Guangxi, Yunnan, Hainan and other regions, in the shady and wet forests of hills and valleys at altitudes of 400–450 m near the borders of Burma, Vietnam and China [2]. In recent years, the natural resources of *H. hainanensis* have been gradually reduced by habitat destruction and illegal logging. This has left limited remaining parents and it is now listed as a second-grade protected plant and an endangered tree species [1]. As the marker species in humid tropical rainforests, *H. hainanensis* is of great value for the study of composition, geographical distribution and ecological characteristics of tropical rainforests, and of the conservation biology of endangered plant species in this region.

Habitat loss and fragmentation are the immediate causes of species becoming endangered. Therefore, the study of the population genetic diversity and the genetic structure of an endangered species is a prerequisite for the development of an effective protection strategy, and has become a core issue of conservation genetics [3]. There are few studies of *H. hainanensis*, and those that have been published mainly focus on breeding technology [4], chemical composition of its volatile oil [2] and population structure characteristics [1]. There has been no study of genetic diversity and structure of its natural populations, and the genetic information is scarce. In the present study, we used the inter-simple sequence repeat (ISSR) marker technique to analyse the genetic diversity and structure of the natural *H. hainanensis* populations in the tropical rainforest regions with relatively concentrated numbers of individuals. The objectives of this
study were to understand the population genetic information, to investigate the mechanisms responsible for species endangerment and to provide a reference for the appropriate protection of genetic resources of *H. hainanensis*.

**Materials and methods**

**Materials**

The field investigation and resource collection of wild *H. hainanensis* were carried out in Guanxi, Yunnan and Hainan provinces from May to June 2015. Populations consisting of more than five adult plants were sampled, and a total of 126 individual plants from nine populations were collected with a distance between maternal plants of over 50 m. Young leaves were placed in a sealed bag with silica, brought back to the laboratory and stored at $-70^\circ$C in a freezer. The sample information is shown in Table 1.

**Experimental method**

**Genomic DNA extraction and PCR amplification**

As described by Zong et al. [5], genomic DNA was extracted from the leaves of *H. hainanensis* using a modified CTAB (cetyl trimethylammonium bromide) method. Polymerase chain reaction (PCR) was run with a 20-$\mu$L reaction system, including 40 ng of template DNA, 1.5 mmol/L Mg$^2+$, 0.5 U of *Taq* polymerase, deoxyribonucleoside triphosphates (dNTP) at 0.2 mmol/L and primers at 0.7 mmol/L. The PCR programme was 94 $^\circ$C for 3 min, then 30 cycles of 94 $^\circ$C for 45 s, annealing for 45 s and 72 $^\circ$C for 90 s, followed by 72 $^\circ$C for 5 min. The amplifications were performed in a Peqstar 96X Universal Gradient thermocycler (PEQLAB Biotechnologie GmbH, Germany). The amplification products were separated in 1.5% agarose gel, using TAE (Tris–acetate–EDTA) buffer and stored at $-20^\circ$C in a freezer. The sample information is shown in Table 1.

**Results and discussion**

**Genetic diversity**

Nine primers with a clear and stable product were selected from 100 ISSR primers published by Columbia University (Table 2), and were used to amplify the 126 individuals from nine natural *H. hainanensis* populations for a total of 136 loci. The PPB for these nine ISSR primers fell in the range of 76.5%–84.6% with an average of 80.0%, and the PPB at the species level was 79.4%. The amplification results obtained using the nine primers are shown in Table 2.

The genetic diversity parameters of the nine studied *H. hainanensis* populations are shown in Table 3. The ranges of $I$, PPB and $H$ were 0.2144–0.3206, 33.1%–49.3% and 0.1509–0.2269, respectively, with corresponding averages of 0.2615, 40.4% and 0.1843, respectively. The population from the Mengtun village in Daxin County, Guanxi exhibited the highest genetic diversity ($I = 0.3206, \text{PPB} = 49.3\%$ and $H = 0.2269$), whereas the lowest

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**Table 1. Sampling information and sample size of *H. hainanensis*.**

| Population | Location | Representative geological coordinate (°) | Altitude (m) | Sample size |
|------------|----------|------------------------------------------|-------------|------------|
| TBG        | Tongbiguan Natural Reserve in Yunnan     | 24.69773’N, 97.58031’E | 345         | 12         |
| ZWY        | Xishuangbanna Botanical Garden in Yunnan | 21.92738’N, 101.25278’E | 566         | 10         |
| ND         | Niandou Village, Daxin County, Guanxi    | 22.75117’N, 106.79873’E | 375         | 20         |
| MT         | Mengtun Village, Daxin County, Guanxi    | 22.77938’N, 106.65725’E | 335         | 22         |
| NG         | Nonggang Nature Reserve in Guangxi       | 22.48478’N, 106.94260’E | 163         | 20         |
| DZ         | Tongzhong forest farm Fangchenggang City, Guangxi | 21.69901’N, 107.56389’E | 470         | 14         |
| BWL        | Bawangling Natural Reserve in Hainan     | 19.11961’N, 109.15078’E | 683         | 8          |
| PLL        | Polong Ridge, Wuzhishan, Hainan          | 18.87444’N, 109.71424’E | 684         | 10         |
| JFL        | National Forest Park, Jianfeng Ridge, Hainan | 18.74311’N, 108.83906’E | 208         | 10         |
one was in Wuzhishan Natural Reserve in Hainan (I = 0.2144, PPB = 33.1% and H = 0.1509). The I and H for H. hainanensis at the species level were 0.4787 and 0.3314, respectively, indicating abundant genetic variation in this species.

The level of genetic diversity of a species reflects its ability to adapt to the environment, so the higher the genetic diversity, the stronger the species’ adaptability to the environment [10,11]. It is believed that the genetic diversity of endangered species, endemic species and narrow-field species is low [12,13]. We demonstrated that although H. hainanensis is an endangered, narrow-field species endemic to China, it has maintained a relatively abundant genetic diversity and strong adaptability to the environment. Its PPB and H were 79.4% and 0.3314, respectively, which were significantly higher than the averages for various plant species (PPB = 71.0% and H = 0.22–0.23) according to Nybom [14]. In addition, the PPB of H. hainanensis was higher than that of many other endemic or endangered plants, including Xylocarpus granatum Koen (58.1%) [15], Neolitsea sericea (Bl.) Koidz. (23.1%) [16], Tetraena mongolica Maxim. (63.3%) [17] and Primula interjacens Chen (75.5%) [18]; but lower than that of Sindora glabra Merr. ex de Wit (93.4%) [19], Ranunculus cabrerensis Rothm. ex de Wit (82.5%) [20] and Primula heterochroma Stapf. (86.2%) [21]. Genetic relationship

The F$_{ST}$ among investigated H. hainanensis populations was 0.450. Wright [22] illustrated that genetic differentiation was great among populations when F$_{ST}$ > 0.25, significant when 0.15 < F$_{ST}$ ≤ 0.25, intermediate with 0.05 ≤ F$_{ST}$ ≤ 0.15, and slight if F$_{ST}$ < 0.05. Thus, H. hainanensis exhibited substantial genetic differentiation among populations with minor gene flow.

The Bayesian analysis of 126 H. hainanensis individuals demonstrated that the log-likelihood value (LnP(D)) increased as the population numbers increased, with no significant inflection point (Figure 1(A)). The change of delta K showed a maximum when K = 2 (Figure 1(B)), indicating that the optimal number of H. hainanensis groups was 2. The genetic structure of 126 H. hainanensis individuals is shown in Figure 1(C). Group 1 (blue) was composed of plants from populations of TBG, ZWY, ND, MT and NG, as well as one individual from DZ; while group 2 (red) consisted of three populations: BWL, PLL and JFL. The individuals originated from a unitary source when the individual Q > 0.6 and so belonged to corresponding groups; however, mixed origins were indicated if Q ≤ 0.6, and in this case the lineage was more complicated [23]. Among the 126 tested H. hainanensis individuals, group 1 accounted for 67.5% (85 plants), group 2 for 22.2% (28 plants) and 10.4% (13 plants) belonged to mixed groups.

The genetic distance and genetic similarity of the nine natural H. hainanensis populations are presented in Table 3. The genetic similarity of the nine natural H. hainanensis populations was in the range of 0.7387–0.8637, with an average of 0.7991, suggesting that the nine populations were phylogenetically close and may share the same origins. Of these, the genetic relationship between JFL and DZ populations was the most distant with a similarity of 0.7387, while NG and MT shared the closest relationship with a similarity of 0.8637. The UPGMA clustering based on the Nei’s genetic distance among populations is shown in Figure 2. The nine

Table 2. ISSR–PCR primers and their amplification.

| Primer | Sequence (S’–F’ | Anneal temperature (°C) | Total bands | Polymorphic bands (%) | PPB (%) |
|--------|----------------|--------------------------|-------------|-----------------------|--------|
| UBC808 | (AG)$_8$C     | 53.6                     | 17          | 13                    | 76.5   |
| UBC809 | (AG)$_8$G     | 51.8                     | 15          | 12                    | 80.0   |
| UBC812 | (GA)$_4$A     | 47.1                     | 14          | 11                    | 78.6   |
| UBC825 | (AC)$_4$T     | 49.2                     | 13          | 11                    | 84.6   |
| UBC826 | (AG)$_4$C     | 51.2                     | 14          | 12                    | 85.7   |
| UBC834 | (AG)$_4$YT    | 52.9                     | 16          | 11                    | 68.8   |
| UBC836 | (AG)$_4$YA    | 50.5                     | 16          | 13                    | 81.3   |
| UBC840 | (GA)$_4$YT    | 52.8                     | 16          | 13                    | 81.3   |
| UBC872 | (GATA)$_4$    | 40.8                     | 15          | 12                    | 80.0   |
| Average|               |                          | 136         | 108                   | 79.4   |

Table 3. ISSR-PCR primers and their amplification results (the population codes are shown in Table 1).

| Population | UBC 808 | UBC 809 | UBC 812 | UBC 825 | UBC826 | UBC 834 | UBC 836 | UBC 840 | UBC 872 | I     | PPB (%) | H   |
|------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|-------|---------|-----|
| TBG        | 0.2649  | 0.3040  | 0.2902  | 0.2511  | 0.3360  | 0.2392  | 0.2769  | 0.3635  | 0.2215  | 0.2830| 44.1   | 0.1987|
| ZYW        | 0.2749  | 0.2130  | 0.2904  | 0.3154  | 0.1863  | 0.2843  | 0.2457  | 0.3064  | 0.2574  | 0.2639| 40.4   | 0.1863|
| ND         | 0.2769  | 0.2577  | 0.2756  | 0.4083  | 0.3352  | 0.2362  | 0.2703  | 0.3853  | 0.2172  | 0.2938| 45.6   | 0.2066|
| MT         | 0.3441  | 0.2583  | 0.2844  | 0.3060  | 0.4289  | 0.1688  | 0.2857  | 0.4586  | 0.3535  | 0.3206| 49.3   | 0.2209|
| NG         | 0.2312  | 0.2463  | 0.1800  | 0.2561  | 0.2919  | 0.1626  | 0.2816  | 0.2805  | 0.3094  | 0.2485| 38.2   | 0.1752|
| DZ         | 0.1144  | 0.3823  | 0.3308  | 0.2527  | 0.2344  | 0.1239  | 0.2447  | 0.2466  | 0.1731  | 0.2303| 35.3   | 0.1625|
| BWL        | 0.1983  | 0.2501  | 0.3047  | 0.3072  | 0.2403  | 0.1257  | 0.3571  | 0.2473  | 0.2381  | 0.2500| 39.0   | 0.1755|
| PLL        | 0.1920  | 0.2170  | 0.2249  | 0.3025  | 0.2333  | 0.1271  | 0.2275  | 0.3075  | 0.2171  | 0.2494| 38.2   | 0.1760|
| JFL        | 0.1123  | 0.3130  | 0.2768  | 0.3483  | 0.1945  | 0.1994  | 0.2792  | 0.2079  | 0.3449  | 0.2494| 38.2   | 0.1760|
| Mean       | 0.2332  | 0.2713  | 0.2733  | 0.3053  | 0.2756  | 0.1852  | 0.2743  | 0.2915  | 0.2690  | 0.2615| 40.4   | 0.1943|
| Species    | 0.4647  | 0.4692  | 0.5170  | 0.5368  | 0.5231  | 0.3915  | 0.4825  | 0.4936  | 0.4498  | 0.4787| 79.4   | 0.3314|
populations were divided into two clusters: cluster 1 consisted of six populations, TBG, ZWY, ND, NG, MT and DZ; and cluster 2 of BWL, PLL and JFL. This was consistent with the model clustering results according to posterior probability in the software of STRUCTURE, and further validates the accuracy of the clustering.

It is generally believed that the genetic structure of a population is the integrative result of its life history, mating system, seed dispersal mode, gene flow and geographical distribution [24,25]. The reproduction system of *H. hainanensis* has not been reported, but the panicles of its male flowers are bright yellow, which is a characteristic to attract pollinators. In addition, according to the average $F_{ST}$ of various plants [14], the $F_{ST} = 0.4509$ for *H. hainanensis* populations is higher than that of outcrossing plants (0.27), lower than that of selfing plants (0.65) and comparable to that of mixed mating plants (0.40). Therefore, we speculate that it has a mixed mating system with mainly outcrossing, which might be one reason for the relatively high genetic differentiation in populations. However, in certain cases, the $F_{ST}$ depends on factors other than the mating system, so it is necessary to deeply study the mating system in *H. hainanensis*.

The fruit of *H. hainanensis* is a capsule, oval and yellow with a large seed, and its $F_{ST}$ is 0.4509, which is substantially higher than that of the animal-dispersed (0.27) and water-dispersed seeds (0.25), but comparable to that of gravity-dispersed seeds (0.45), based on the average $F_{ST}$ values of Nybom [14]. During the field observation and sampling, seedlings of *H. hainanensis* were mostly located underneath mother trees, so we hypothesize that gravity is the major means of seed dispersal for this species, which may explain its small gene flow. Previous studies showed a significant effect of gene flow on the

Table 4. Genetic similarity and distance of nine natural *H. hainanensis* populations (the population codes are shown in Table 1).

| Population | TBG   | ZWY   | ND    | MT    | NG    | DZ    | BWL   | PLL   | JFL   |
|------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| TBG        | 0.8542| 0.8436| 0.8212| 0.8282| 0.7938| 0.8082| 0.7873| 0.7676|       |
| ZWY        | 0.1575| 0.1681| 0.8487| 0.8583| 0.8271| 0.7705| 0.7637| 0.7800|       |
| ND         | 0.1701| 0.2059| 0.1641| 0.8637| 0.8030| 0.7983| 0.7732| 0.7654|       |
| MT         | 0.1970| 0.2297| 0.1528| 0.1466| 0.8033| 0.7754| 0.7928| 0.7397|       |
| NG         | 0.1885| 0.2250| 0.1898| 0.2194| 0.2191| 0.7576| 0.7793| 0.7387|       |
| DZ         | 0.2309| 0.2550| 0.2608| 0.2544| 0.2775| 0.8277| 0.8013| 0.8062|       |
| BWL        | 0.2130| 0.2241| 0.2696| 0.2321| 0.2494| 0.1892| 0.2154|       |
| PLL        | 0.2392| 0.2511| 0.2572| 0.3015| 0.3029| 0.2215|       |       |
| JFL        | 0.2645| 0.2411| 0.2510| 0.3015| 0.3029| 0.2215| 0.2154|       |
genetic differentiation of populations [26]. Gene flow of <1 is insufficient to resist the genetic differentiation caused by population genetic drift [27]. The \( N_m \) of \( H. hainanensis \) was 0.6088, suggesting that the genetic drift in populations would be likely to influence the genetic differentiation.

**The endangering mechanism for \( H. hainanensis \) and protection of its resources**

It is commonly believed that low fertility, little genetic variation within species, weak competitiveness, poor adaptability, excessive logging and habitat destruction are the reasons for plant endangerment [28,29]. Williamson and Werth [30] pointed out that endangered species with narrow field distribution and abundant genetic diversity had not experienced a bottleneck effect, and inbreeding within the population might not necessarily lead to selfing depression; thus, it was likely that fragmentation of native habitats caused by geographical isolation and human disturbance was the cause of their being endangered. Our data showed that the natural \( H. hainanensis \) populations possessed relatively abundant genetic diversity. Therefore, its endangerment does not originate in low potential of population genetic evolution. We speculate that there are three reasons. (1) Historical climate change transformed a large population with a wide distribution and high level of genetic diversity into the current residual and fragmented distribution. (2) \( H. hainanensis \) is distributed in Hainan island of China and the border of Burma, Vietnam and China. The populations are separated and geographically isolated by high mountains and oceans, so the gene flow is blocked. (3) In the past few hundred years, human activities have caused deterioration or even loss of tropical rainforest habitats. The habitat for \( H. hainanensis \) has been greatly disturbed by human activities, and the difficulty of natural population regeneration and gradual reduction of individuals has led to genetic drift.

Species conservation is essential to protect the genetic diversity and evolutionary potential. The higher the genetic diversity, the stronger the species’ adaptability to environments and the greater the evolutionary potential [11,31,32]. Because of the high degree of genetic diversity in \( H. hainanensis \) at the species level, especially within populations, we believe that there is still high adaptability and evolutionary potential in the natural \( H. hainanensis \) population, and thus, \textit{in situ} conservation should be the primary measure. Due to the genetic differentiation among \( H. hainanensis \) populations, the protection priority should be given to MT, ND and TBG populations, whose genetic diversity is relatively abundant. In addition, although genetic diversity is relatively high at both species and population levels, our investigation showed that the natural \( H. hainanensis \) population is very limited with only one or several individuals in most populations, so the decline from inbreeding within these populations and genetic drift will be more significant, and extinction is possible. Thus, in addition to improved protection of current populations, it is necessary to study the biological characteristics of \( H. hainanensis \), to monitor the inbreeding decline, to adopt effective measures to promote population recovery and to expand the population size.

Figure 2. UPGMA clustering of nine \( H. hainanensis \) populations based on Nei’s genetic distance (the population codes are shown in Table 1).
Conclusions

*H. hainanensis* Merr. populations have maintained a relatively abundant genetic diversity. There is still high adaptability and evolutionary potential in the natural *H. hainanensis* populations. Based on the analysis of population genetic information, it could be suggested that the main reasons for the endangerment of *H. hainanensis* were habitat fragmentation and disturbance from human activities. Accordingly, it would be a reasonable and effective measure to carry out *in situ* conservation for the existing natural *H. hainanensis* populations.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Jiang YH, Xiang WH, Jiang Y, et al. [Floristic composition, structure and phytogeographic characteristics of *Horsfeldia hainanensis* Merr. community in Guangxi]. J Beijing Forest Univ. 2016;38(1):74–82. Chinese.

[2] Dang JL, Yang XB, Huang YF, et al. [GC-MS Analysis on the chemical constituents of essential oil from bark of *Horsfeldia hainanensis* Merr.]. Chin Med Mater. 2009;32(5):714–716. Chinese.

[3] Sork VL, Smouse PE. Genetic analysis of landscape connectvity in tree populations. Landscape Ecol. 2006;21:821–836.

[4] He GZ, Cai L, Liang G, et al. [Sowing and breeding technique of *Horsfeldia hainanensis* Merr.]. Pract Forest Technol. 2013;6:35–37. Chinese.

[5] Zong M, Liu HL, Qiu YX, et al. Genetic diversity and geographic differentiation in the threatened species *Dysosma pleiantha* in China as revealed by ISSR analysis. Biochem Genet. 2008;46:180–196.

[6] Nei M. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics. 1978;89:583–590.

[7] Yeh FC, Yang R, Boyle TBJ. POPGENE, the user-friendly shareware for population genetic analysis. Edmonton: University of Alberta; 1997.

[8] Falush D, Stephens M, Pritchard JK. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Mol Ecol Notes. 2007;7:574–578.

[9] Rohlf FJ. NTSYSpc numerical taxonomy and multivariate analysis system version 2.1-user guide. New York (NY): Applied Biostatistics Inc; 2004.

[10] Jump AS, Marchant R, Penuelas J. Environmental change and the option value of genetic diversity. Trends Plant Sci. 2009;14:51–58.

[11] Souza GB, Souza VA, Lima PS. Molecular characterization of *Platonia insignis* Mart. ("bacurizeiro") using inter simple sequence repeat (ISSR) markers. Mol Biol Rep. 2013;40:3835–3845.

[12] Kesseli RV. Population biology and conservation of rare plants applied population biology. Berlin: Springer; 1992.

[13] Qiu YX, Hong DY, Fu CX, et al. Genetic variation in the endangered and endemic species *Changium smyrnioiodes* (Apiaceae). Biochem Syst Ecol. 2004;32:583–596.

[14] Nybom H. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol Ecol. 2004;13:1143–1155.

[15] Jugale SB. Genetic diversity assessment in intra- and inter-populations of *Xylocarpus granatum* Koen.: a critically endangered and narrowly distributed species of Maharashtra. Curr Sci. 2009;97:695–701.

[16] Wang ZS, Liu YH. Genetic structure of the endangered plant *Neolitsea sericea* (Lauraceae) from the Zhoushan archipelago using RAPD markers. Ann Bot. 2005;95:305–313.

[17] Ge XJ, Yu Y, Zhao NX, et al. Genetic variation in the endangered Inner Mongolia endemic shrub *Tetraena mongolica* Maxim. (Zygophyllaceae). Biol Conserv. 2003;111:427–434.

[18] Xue DW, Ge XJ, Hao G, et al. High genetic diversity in a rare, narrowly endemic primrose species: *Primula interjacentis* by ISSR analysis. Acta Bot Sin. 2004;46:1163–1169.

[19] Yang JC, Li QQ, Yu N, et al. Genetic diversity and structure among natural populations of *Sindora glabra* in Hainan Island, China as revealed by ISSR markers. Biochem Syst Ecol. 2016;69:145–151.

[20] Cires E, Cuesta C, Prieto JAF. Genetic diversity and structure in fragmented populations of the endangered species *Ranunculus cabrerensis* (Ranunculaceae): implications for conservation. Biologia. 2013;68(1):30–40.

[21] Noroozisharaf A, Hatamzadeh A, Lahiji HS, et al. Genetic diversity of endangered primrose (*Primula heterochroma* Stapf.) accessions from Iran revealed by ISSR and IRAP markers. Sci Hortic. 2015;190:173–178.

[22] Wright S. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution. 1965;19:395–420.

[23] Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol. 2005;14:2611–2620.

[24] Schaal BA, Hayworth DA, Olsen KM, et al. Phylogeographic studies in plants: problems and prospects. Mol Ecol. 1998;7:465–474.

[25] Nybom H, Bartish IV. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Perspect Plant Ecol. 2000;3:93–114.

[26] Keyghobadi N, Roland J, Strobeck C. Genetic differentiation and gene flow among populations of the alpine butterfly, *Parnassius smintheus*, vary with landscape connectivity. Mol Ecol. 2005;14:1897–1909.

[27] Slarkin M. Gene flow in natural populations. Annu Rev Ecol Syst. 1985;16:393–430.

[28] Gaudeul M, Taberlet P, Till-Bottraud I. Genetic diversity in an endangered alpine plant, *Eryngium alpinum*, L. (Apiaceae), inferred from amplified fragment length polymorphism markers. Mol Ecol. 2000;9:1625–1637.
[29] Li Q, Xu Z, He T. Ex situ genetic conservation of endangered *Vatica guangxiensis*, (Dipterocarpaceae) in China. Biol Conserv. 2002;106(2):151–156.

[30] Williamson PS, Werth CR. Levels and patterns of genetic variation in the endangered species *Abronia macrocarpa*. Amer J Bot. 1999;86:293–301.

[31] Bennett JR, Elliott G, Mellish B, et al. Balancing phylogenetic diversity and species numbers in conservation prioritization, using a case study of threatened species in New Zealand. Biol Conserv. 2014;174:47–54.

[32] Le NT, Mien NT, Tien TV, et al. Genetic diversity of Tsai in North Vietnam detected by inter simple sequence repeat (ISSR) markers. Biotechnol Biotecnol Equip. 2016;30(3):1–6.