Analysis of the genetic diversity of the coastal and island endangered plant *Elaeagnus macrophylla* by conserved DNA-derived polymorphism markers

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Abstract

The genetic diversity and genetic structure of five natural populations of the island and coastal endangered plant *Elaeagnus macrophylla* were analyzed by conserved DNA-derived polymorphism molecular markers. A total of 289 discernible loci were obtained from 102 individuals using fifteen primers, and 100% of the loci were polymorphic. The observed number of alleles was 1.9654, and the effective number of alleles was 1.2604, the average of Nei's genetic diversity index was 0.1724, and Shannon's information index was 0.2869, indicating that *Elaeagnus macrophylla* had levels of genetic diversity lower than those reported for continental relatives and other continental species. The average percentage of polymorphic loci was 42.1%, and the maximum and minimum values were 80.97% and 14.88%, belonging to the Nanji Island and Liugong Island populations, respectively. Populations of *Elaeagnus macrophylla* were highly differentiated. Cluster analysis revealed that the similarity between the tested samples was related to their geographical locations, samples from the same island tended to cluster together, and there was no cross-clustering between the samples. The Nanji Island and Da Rushan populations differentiated into two subpopulations. We detected no correlation between genetic

distance and geographic distance between populations (Pearson's correlation coefficient r = 0.256579, p-value = 0.8309).

Introduction

Elaeagnus macrophylla is an endangered evergreen shrub species of East Asian coastal areas and islands. It is distributed in China's Shandong, Zhejiang, and Jiangsu Provinces, mainly on offshore islands and in coastal lowlands (Chinese Flora, 1983). Because of its unique geographical distribution pattern, *E. macrophylla* has great value for studying coastal flora and can be widely used in coastal greening due to its tolerance of sea breeze, salinity, drought and thin soil (Zang, 2016). It also has potential economic value; for example, it can be used to produce fruit juice and wine (Zang, 2016). In recent years, with the rapid development of the economy and coastline, the intensification of human interference, and a continuous reduction in suitable environments, the number, and size of natural populations have decreased sharply, causing the species to become endangered.

The genetic diversity of island species is generally lower than that of terrestrial species, and the risk of extinction is higher for island species (Raven, 1998). From the 17th century to the 20th century, 384 species of vascular plants went extinct worldwide, 139 of which were island plants. Moreover, forty percent of vulnerable or endangered vascular plant species are island species (Reid & Miller, 1989). Human disturbances, such as habitat destruction and invasion by alien species, are considered to be the main factors threatening island species (Wolf & Harrison, 2001). Studies of *Ilex integra* based on inter simple sequence repeat (ISSR) molecular markers (Leng et al., 2005) and *Neolitsea sericea* based on random amplified polymorphic DNA (RAPD) molecular markers (Wang et al., 2004) concluded that the geographical isolation of islands had a significant impact on the genetic differentiation of island populations and that the genetic diversity of island relatives was lower than that of close relatives on continents. However, studies of the genetic diversity of the island plant *E. macrophylla* have not been conducted.

The analysis of conserved DNA-derived polymorphism (CDDP) is based on a single primer amplification reaction, with primers designed to target conserved sequences of plant functional genes−mostly transcription factors such as WRKYs, MYBs, MADs, ERFs, KNOXs, and ABP1. Because of the strong conservation of some sequences of plant DNA, CDDP molecular marker technology can be used across different species. Studies of rice (*Oryza sativa*) have shown that CDDP molecular markers have many advantages, including convenience, low cost, and rich polymorphism, and can effectively produce markers related to target traits (Collard & Mackill, 2009). Compared with traditional DNA molecular methods, CDDP is practical because the primers used in CDDP are specific for conserved DNA sequences of genes. Via the amplification of these conserved sequences, which tend to be linked with phenotypic traits, CDDP can provide advantages in plant genetic diversity assessment (Andersen & Lübberstedt, 2003). Since Poczai first successfully used CDDP molecular markers and in-targeting markers to investigate the genetic diversity and group structure of *Solanum dulcamara* (Poczai et al., 2011), CDDP

molecular markers have proven useful in the analysis of several other plant species, such as *Chrysanthemum* (Li et al., 2014), *Paeonia suffruticosa* (Li et al., 2013), *Vaccinium vitis-idaea* (Fang et al., 2016), and *Rosa rugosa* (Jiang & Zang, 2018). However, CDDP markers have not yet been used to study *E. macrophylla*.

In this study, CDDP molecular markers were used to analyze the genetic diversity and genetic relationships among major natural populations of *E. macrophylla* in China, and the study's aims were to reveal the level of genetic diversity and degree of genetic differentiation, assess the relationships between populations, examine the influence of geographical isolation and human factors on the genetic structure, and provide a scientific basis for the protection and rational utilization of *E. macrophylla*.

Materials and Methods

Plant materials

A total of 102 individual leaf samples were collected from 7 islands and offshore sites (Fig. 1 and Table 1) from April to July 2018; sampling was conducted within the natural distribution area of *E. macrophylla*. Interval sampling was applied except for small populations (such as the Liugong Island population, where samples from all individual plants found were collected). Only one individual was found on each of Lingshan Island and Putuo Island. After collection, silica gel was used to quickly dry the specimens, after which they were stored at -20°C.

DNA extraction and PCR amplification

Total DNA was extracted from *E. macrophylla* via the modified cetyltrimethylammonium bromide (CTAB) method (Doyle & Doyle, 1987). The quality and purity of DNA were determined by 2% agarose gel electrophoresis, and a spectrophotometer (Thermo Fisher Scientific Inc., USA) was used to ensure DNA quantification. All DNA samples were stored at -20°C for later use.

The DNA from one sample per population was selected to screen 21 CDDP primers (Collard & Mackill, 2009) (synthesized by Sangon Biotech, China). According to the results, 15 primers with clear and reproducible amplification bands were screened out (Table 2). PCR was conducted in a total reaction volume of 20 μl containing 10 μl 2× Ex Taq MasterMix (dye), 7.5 μl double-distilled H_2O, 1 μl 30 ng/μl DNA template, and 1.0 μl 10 pmol/μl primer (Sangon Biotech, China). A standard PCR cycle (RT-PCR 7500, Thermo Fisher Scientific Inc., USA) was used: an initial denaturation step at 94°C for 3 min; 35 annealing cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 2 min; and a final extension of 5 min at 72°C. PCR products were stored at 4°C. The PCR products were electrophoresed on a 2% agarose gel at a voltage of 110 V and current of 110 mA for 1.5-2 h; a DL2000 marker was used as a size marker. The electrophoresis

results were photographed and recorded by a gel imaging system. All amplification procedures were repeated at least twice to ensure the repeatability of the experiment.

Data statistics and analysis

We used POPGEN v.1.32 (Yeh, Yang & Boyle, 1999) to compute the following: observed allele number (Na), effective allele number (Ne), Nei's genetic diversity index (H), Shannon's information index (I), polymorphic loci, percentage of polymorphic loci (PPL, %), total genetic diversity (Ht), genetic diversity within populations (Hs), the genetic differentiation coefficient (Gst) and gene flow (Nm) between populations. The estimates were also calculated within sampling localities when significant differences among specimens were found.

A dendrogram was generated using the unweighted pair group method with arithmetic mean (UPGMA) clustering procedure in NTSYS-pc 2.10e software (RohlF, 1994). The relationship between geographic distance and genetic distance was analyzed with Pearson's correlation coefficient in R.

We further assessed the genetic structure of populations using the Bayesian clustering approach implemented in STRUCTURE v.2.3.4 (Pritchard, Stephens & Donnelly, 2000). The number of potential genetic clusters (K values) was set from 1 to 10, with 10 independent runs for each K. The contribution to the genotypes of the accessions was calculated based on a 10^5 iteration burn-in and 10^5 iteration sampling period. Then, the optimal number of clusters K was identified according to the method of Evanno, Regnaut & Goudet (2005).

Results and Analysis

Population- and species-level diversity of *E. macrophylla*

The DNA of 102 samples was amplified with 15 primers to obtain 289 bands, and the fragment length was between 500 and 2000 bp (Fig. 2). The number of amplified bands ranged from 11 to 30, and the average number of amplified bands was 19.3. The number of Pr2 and Pr6 amplification bands was the highest, at 30, and the number of Pr7 and Pr8 amplification bands was the lowest, at 11. The percentage of polymorphism reached 100% (Table 2), which indicated that the genomic DNA polymorphism of *E. macrophylla* was high.

At the population level, the PPL ranged from 14.88% to 80.28%, with an average of 48.928%, whereas it was 96.54% at the species level. The Na ranged from 1.1488 to 1.8028, while the Ne varied from 1.0739 to 1.2410. H varied from 0.0446 to 0.1580, with an average of 0.1149, and I ranged from 0.0690 to 0.2613, with an average of 0.1848. At the species level, H and I were 0.1724 and 0.2869, respectively (Table 3). The Na, Ne, H, I and PPL were consistent among populations, with the NJD population exhibiting the largest values and the LGD population exhibiting the lowest values, all of which were lower than those observed at the species level.

Genetic differentiation of the populations of *E. macrophylla*

The Ht and Hs were 0.1706 and 0.1149, respectively, as calculated with POPGEN v.1.32 software. The Gst was 0.3263, indicating that 67.37% of the variation was within the populations and 32.63% of the variation existed among the populations. A certain degree of genetic differentiation was observed among the populations. The Nm was 1.0325, indicating that there was some (albeit limited) genetic communication between populations.

Genetic differentiation between populations can be further analyzed based on Nei's genetic distance and genetic identity. For the five populations of *E. macrophylla*, the genetic distance was between 0.0490 and 0.1443 (Table 4), with a mean of 0.08127, and Nei's genetic identity was between 0.8656 and 0.9588, with a mean of 0.9226.

UPGMA cluster analysis

The applied measure of genetic similarity was used to construct UPGMA dendrograms (Fig. 3). The clustering map shows that the five populations can be divided into three groups. One group represented the LGD population; the populations DRS, NJD, and LS formed a second group, indicating that these three populations are closely related; and the third group represented the DGD population. Populations with similar geographical distances were not clustered into the same group, indicating that the genetic distance between the populations of *E. macrophylla* was not related to geographical distance. Consistent with these results, the Pearson correlation coefficient test revealed no significant correlation between geographic and genetic distance (Pearson's correlation coefficient r = 0.256579, p-value = 0.8309).

The UPGMA clustering map provided a clearer division of 102 samples (Fig. 4). Notably, cross-clustering was observed between samples from different populations, and samples from the same island tended to be clustered together. The groups in the clustering results were generally consistent with the regional sources of the samples. As clearly shown by the clustering map, all the samples from LGD formed a small branch and then formed another branch with all samples from DRS. Among the samples, Nos. 9-18 from DRS were more closely related to the samples from LGD than to Nos. 19-28 from DRS; thus, differentiation within the DRS population was observed between Nos. 9-18 and Nos. 19-28. All samples from LS formed a group, all samples from DGD formed another group, and the two groups formed a large branch. A sample from PTD formed a separate group. Samples from the NJD population composed of two subpopulations. Based on the sampling location and latitude and longitude, samples 29-40 were collected in the northwestern part of NJD (121°3'24-121°3'8, 27°27'53-27°28'21), and samples 41-61 were collected in the southeastern part of NJD (121°5'52-121°6'11, 27°26'54-27°27'12). Twenty-one samples (Nos. 41-61) formed a group, 12 samples (Nos. 29-40) formed another group, and the two groups formed different branches. Therefore, the samples could be easily divided into 5 groups based on the clustering results.

Population structure analysis

The results of the Bayesian clustering analysis of genetic structure showed that the populations of *E. macrophylla* best fit three genetic groups, and when K=5, the value of delta K was also large (Fig. 5A). When K=3 (Fig. 5B), the LGD population and half of the DRS samples were clustered into the first groups, further indicating that the two populations were closely related. Most samples from LS, half of the DRS samples and the samples from the NJD population

clustered into the second group, and a portion of the samples from LS and DGD formed the third group. When K=5 (Fig. 5C), the LGD population and half of the DRS samples were grouped into the first group, half of the DRS samples and the yellow part of the NJD samples were grouped into the second group, the blue part of the NJD samples formed the third group, all samples from the LS population were clustered into the fourth group, and all samples from the DGD population were clustered into the fifth group. The LGD, LS, and DGD population pedigrees were simple, and samples from the islands tended to cluster together. The NJD and DRS populations were differentiated. The NJD population formed two subpopulations when K=5: a northwestern group (yellow part) and a southeastern group (blue part). The DRS population was differentiated into two subpopulations when K=3 and K=5: Nos. 9-18 and Nos. 19-28.

Gst and Nm of the two subpopulations of NJD and DRS

The Nm between subpopulations of the NJD and DRS populations was 2.6084 and 2.0843, respectively. The Gst between subpopulations of the NJD and DRS populations was 0.1609 and 0.1935, respectively. Subpopulations exhibited strong gene flow but high genetic differentiation.

Discussion

Frankham (1997) compared and analyzed the allelic diversity of 202 groups of land and island populations of various species, including mammals, birds, fish, reptiles, insects and plants: in 165 cases (81.7%), the genetic diversity of island populations was lower than that of terrestrial populations, with an average decrease of 29%. The average PPL of the CDDP molecular markers detected in five populations of the island plant *E. macrophylla* was 48.928%, Ne was 1.1801, H was 0.1149, and I was 0.1848. All these values are far below those of populations of the continental species *Camellia japonica* (PPL=86.11%, Ne=1.4775, H=0.2940, and I=0.4459) (Juan, 2018) and *Paeonia suffruticosa* (PPL=72.1%, Ne=1.2389, H=0.1623, I=0.2682) (Li, 2013) estimated based on CDDP molecular markers. Moreover, compared with the rich genetic diversity of a species within the same genus (*Elaeagnus mollis*) (Qin, Zhang & Yan, 2006), the genetic diversity of *E. macrophylla* is low, which is consistent with the results of Frankham. As an endangered coastal plant, *E. macrophylla* exhibits lower genetic diversity than its continental relatives and other continental species. For island species, breeding characteristics, dispersal capability, and effective population size are often considered important factors affecting genetic diversity (Frankham, 1997; Weller, Sakai & Straub, 1996). *E. macrophylla* is a typical bisexual flowering plant with a small flower diameter, produces nectar, and relies mainly on small insects as pollinators for cross-pollination (Zang, 2012). The Ht of *E. macrophylla* was lower than the average value of insect-pollinated plants (Ht=0.2019; Hanwick & Godt, 1990), indicating that its insect-borne pollination has been affected to some extent. Owing to the large sea breeze on the island, insects can only live in groups, which affects their range of activities. As a result, pollen transmission is limited to a very small range, and random fixation of alleles and limited gene exchange leads to poor population expansion, thus reducing genetic diversity (Hamrick & Nason, 1996; Hamilton & Miller, 2002). As another important carrier of gene flow, seeds are also essential for the natural regeneration and expansion of plant populations and for increasing the genetic diversity of populations (Hamilton & Miller, 2002). According to previous studies, *E. macrophylla* can produce fruit naturally in the wild, and the fruit is sweet (Zang, 2016). The fruit

is heavily favored by birds, and some birds that feed on these fruits can spread the seeds. Unfortunately, owing to the influence of insect pollination, the seed setting rate in the wild is low. Moreover, owing to increasing human activities, especially the vigorous development of tourism, island birds are becoming increasingly rare, which further restricts the spread of seeds and affects the genetic diversity of populations. Compared to *Camellia japonica* and *Elaeagnus mollis*, the distribution of populations of *E. macrophylla*, an island species, is small. *E. macrophylla* occupies a fragile habitat and has a narrow distribution range, making it more vulnerable to extinction than terrestrial species (Francisco-Ortega et al., 2000). Island segregation means fragmentation of habitats, maintaining small populations in fragmented habitats; as such, genetic diversity of endangered species may be lost because genetic drift causes allele loss and because inbreeding frequencies are increasing (Zhang & Jiang, 1999; Emerson, 2002).

For this species, the Ht and Hs were 0.1706 and 0.1149, respectively. Compared to endangered and Chinese secondary protected plants (Fu & Jin, 1992), including *Rosa rugosa*, in previous CDDP-based (Ht=0.2770, Hs=0.1522) studies (Jiang & Zang, 2018), *E. macrophylla* in the present study showed low diversity. Similarly, the diversity values in the present study were lower than those estimated using CDDP markers for the plant species *Camellia japonica* (Ht=0.2874, Hs=0.2518; Juan, 2018). Gst is calculated as the ratio of between-population genetic variance to the total variance among populations (Wright, 1965). The Gst of the five populations of *E. macrophylla* was 0.3263, indicating that 32.63% of the variation existed among the populations. Genetic differentiation of *E. macrophylla* populations was significant based on Nei's Gst classification criteria for genetic differentiation (low, Gst < 0.05; medium, Gst = 0.05~0.15; and high, Gst > 0.15) (Nei, 1978). Its genetic differentiation value was higher than the average value of 23 species (28.06%) of the Carinla Islands (Francisco-Ortega et al., 2000). Furthermore, the genetic differentiation between *E. macrophylla* populations was relatively high, and the prevention of gene flow, genetic drift, and inbreeding was the main cause of genetic differentiation among populations (Starkin, 1987; Ouborg, Piquot & Groenendael, 1999; Manel, 2003).

Nm refers to the process by which a biological individual disperses from its place of origin, followed by the exchange of genes between populations. Such exchange may occur between biological populations of the same species or between different species and is essential to the evolution of many plant populations (Grant, 1991; Gerber et al., 2014). The populations of *E. macrophylla* displayed little gene flow (Nm=1.0325), and the UPGMA clustering analysis of samples showed no hybridization among individuals from different localities. Structure analysis (K=5) revealed that most of the populations had a simple pedigree, and the genetic communication between each pair of populations was low. These results were mainly due to the geographical isolation of the islands (mainly barriers posed by seawater), which limited the range of dispersal by pollen- and seed-dispersing birds (Kwon & Morden, 2002). For the *E. macrophylla* populations, the shortest distance is between the populations of LS and DGD (8100 m); it is difficult for small pollinators to spread pollen across islands separated by vast seas. In addition to pollen, seeds play an important role in the spread of gene flow. One important aspect of seed movement is its role in the initial founding of a population (Chung, Chung & Oh, 2002). In a study of *Neolitsea sericea*, owing to seeds being dispersed over long distances by birds, numerous seedlings and juveniles (mostly aged < 5 yr) of *Neolitsea sericea* were dispersed within a range of approximately 480 m (Hakdongri on Keojae Island) and 680 m (Naechori on Oenaro Island, respectively) from maternal plants (Chung, Chung & Oh, 2002). The fruit of *E.*

macrophylla is a drupe-shaped nut with a seed and rich flesh (Zang, 2012). After birds feed and digest their food, they excrete in their feces. This type of transmission is called intra-animal transmission, which carries seeds far away and promotes gene exchange (Petit et al., 2003). It is difficult to directly observe how far birds can spread seeds in the wild, but the retention time of seeds in the digestive tract of fruit-eating birds can be used to determine the potential propagation distance and the ability to reach the appropriate breeding ground (Manson & Stiles, 1998). The retention time of fruits of *Sorbus pohuashanensis* in the digestive tract of the birds was found to be approximately 20 min. The first stopping point after feeding was mostly located between 5 and 10 m from the female plants, but the birds could have many various landing points within 20 min, which might spread the seeds to distant areas (Zhang et al., 2010). However, birds are affected by factors such as seed size, feed intake, digestion, and excretion; thus, it is very difficult to distribute seeds between distances over sea areas greater than 8000 m or even farther. Last, water currents are also a major medium of genetic communication between islands (Kwon & Morden, 2002). Zhang et al. (2007) studied the differences in genetic variation between the species *E. emarginata*, *Ilex integra* and *Machilus thunbergii*. Clustering analysis revealed that the reason for the intermixing of individuals among *E. emarginata* populations was that seeds floating with ocean currents promoted gene exchange among populations, while those of *Ilex integra* and *Machilus thunbergii* did not. Therefore, the genetic differentiation of *E. emarginata* is lower than that of *Ilex integra* and *Machilus thunbergii*. The fruits of *E. macrophylla* fall into the sea due to sea breezes, but the spread of seeds by ocean currents to allow genetic communication between populations is a rare event, as submersion quickly reduces the germination capability after a few days (Angélique & Debussche, 2000). Seeds of *Ilex integra* also presented a germination rate close to zero when soaked in seawater (Leng et al., 2005). Our sample clustering map shows no hybridization among individuals, indicating that the gene communication between the populations is limited. Therefore, it is very difficult for *E. macrophylla* to achieve gene communication via seeds floating by currents. For *E. emarginata*, the reason for success may be that the distance between the islands is short, and the seeds do not lose their ability to germinate after floating.

The results of the Bayesian clustering analysis showed that when K=3, the same gene pool exists between different populations. Based on limited gene flow and high genetic differentiation among populations, we speculate that the reason for this may be that *E. macrophylla* inherits the gene pool of its ancestors, such that populations with distant geographic distances also contain the same gene pool.

As opposed to the high genetic differentiation among different island populations, individuals on islands are often grouped into a single group, indicating that individuals in each population have relatively close kinship, which may be related to the small island range, similar habitats within the population, and strong gene flow (Sahuquillo & Lumaret, 1995; Carlos, Emerson & Oromi, 2000); however, the DRS and NJD populations are exceptions. Based on the UPGMA clustering and STRUCTURE analysis results, the DRS population differentiated into two subpopulations: Nos. 9-18 and Nos. 19-28. Gene flow (Nm=2.0843) was strong between the two subpopulations, but there was high genetic differentiation (Gst=0.1935). Because gene flow was not blocked and the habitats were similar, we speculate that the cause of this result was that the DRS population contains two gene pools, and complete gene introgression has not yet occurred in the two subpopulations under the condition of gene flow of 2.0843. When K=3, the NJD population did not exhibit differentiation; when K=5, the NJD population differentiated into two subpopulations: a northwestern group and a southeastern group. The gene flow between the two

subpopulations was determined to be 2.6084, indicating strong gene communication between the two subpopulations of NJD. However, there is still some genetic differentiation between the two subpopulations (Gst = 0.1609). After excluding explanations such as blocked gene flow, the reason may be differences in habitats within the population. The NJD nature reserve has numerous islands and reefs with meandering shorelines, headlands, and numerous bays. There are many types of coastal beaches, such as mudflats, gravel beaches, and rocky reefs, and NJD is at the intersection of the Taiwan Warm Current and the Jiangsu and Zhejiang Coastal Currents. The flow system is complex, so the habitat is complex (Xiao, 2007). The marine shellfish algae in this area are not only abundant in species but also have the characteristics of temperate zones and tropical zones. Moreover, there is an obvious regional "fracture distribution" phenomenon. It is rare that three species with different temperature properties of tropical, subtropical and temperate zones coexist in the NJD sea area at the same time; for example, typical tropical species such as *Oliva emicator* can survive in the NJD above 27°N, which is sufficient to demonstrate the particularity of the NJD habitat (Xiao, 2007). The two subpopulations are located in the northwestern and southeastern regions of NJD, respectively, and may have some genetic differentiation due to habitat differences. However, our current evidence is insufficient, and additional research is needed.

In the comparison of the genetic diversity index of 22 endemic plants on the Canary Islands, the average genetic diversity index of island species with relatively large populations (number of individuals > 2500; H=0.1460) was significantly greater than that of those with relatively small populations (number of individuals < 100; H=0.0970) (Francisco-Ortega et al., 2000). The average diversity index of each population of *E. macrophylla* was (from large to small) NJD (0.1580)>DRS (0.1290)>DGD (0.1222)>LS (0.1208)>LGD (0.0446), with an average value of 0.1149. The average genetic diversity index of the NJD population is much higher than that of the LGD population. The values of the other three populations are relatively similar. The NJD population is the largest, the DRS, DGD, and LS populations are similar in size, and the LGD population shows a population decline. Human disturbances such as excessive logging, habitat destruction, and the introduction of exotic species are considered to be the main causes of endangerment of island species (Atkinson, 1989; Frankham, 1997; Raven, 1998), which are manifested mainly as effective population decline, increased frequencies of inbreeding, loss of genetic diversity, decline in survival competitiveness, etc. (Ferson & Burgman, 1995; Frankham, 1997; Mengens, 1998). NJD is far from mainland China, is relatively closed, and experiences relatively limited exchange with the mainland, so there is little man-made damage. Moreover, the island area is large, and the genetic background is complex, so the island shows high genetic diversity and a large population. LGD is a famous tourist destination in China, and the coastline is developing rapidly. At the same time, LGD has a small area, and its populations have similar genetic backgrounds and a single habitat; as such, loss of genetic diversity and declines in populations have occurred.

Conservation of *E. macrophylla* diversity

The in situ conservation method was proposed as it is of paramount urgency that sufficient natural population numbers and sizes be conserved to prevent a reduction in genetic diversity. The best strategy for in situ conservation of genetic diversity during an endemic is the preservation of natural habitat (Francisco-Ortega et al., 2000). In this study, the NJD population displayed relatively high genetic diversity and should, therefore, be a priority for in situ

conservation. The LGD population had the lowest genetic diversity and the smallest population size; the site of this population should be protected as the most urgent site. Furthermore, natural protection areas should be established to conserve and restore the habitat and populations, the awareness of local residents and tourism management personnel should be increased, and the populations should be increased by artificial cultivation and subsequent management; for example, seeds collected from other populations could be sown, branches could be collected for cuttings, gene barriers could be broken by appropriate species regression, and genetic diversity of populations could be increased. Moreover, to achieve effective conservation of germplasm resources, efforts are needed to carefully plan and construct pollen banks and gene banks for *E. macrophylla*.

Conclusions

The present study is the first genetic investigation of *Elaeagnus macrophylla* using conserved DNA-derived polymorphism markers to investigate this species' distribution and genetic variation. The results showed that conserved DNA-derived polymorphism molecular markers can be effectively used to study the genetic diversity of *Elaeagnus macrophylla* populations and revealed that *Elaeagnus macrophylla* populations have low genetic diversity and high genetic differentiation. The low levels of gene flow among populations are the main cause of the high levels of genetic differentiation. Based on these findings, some conservation measures for *Elaeagnus macrophylla* are proposed.

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