Biochemical Systematics and Ecology 40 (2012) 25–33

Contents lists available at SciVerse ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Genetic diversity and differentiation of the endangered tree *Elaeagnus mollis* Diels (Elaeagnus L.) as revealed by Simple Sequence Repeat (SSR) Markers

Yiling Wang [a], Yongyan Qin [a,b], Zhen Du [a,c], Guiqin Yan [a,*]

[a] College of Life Sciences, Shanxi Normal University, Linfen 041004, PR China
[b] Department of Biology and Chemistry, Changzhi College, Changzhi 016011, PR China
[c] Northwest University, Xi'an 710069, PR China

ARTICLE INFO

Article history:
Received 21 December 2010
Accepted 22 September 2011
Available online 26 October 2011

Keywords:
Elaeagnus mollis Diels
Simple sequence repeats (SSR)
Genetic diversity
Genetic differentiation
Conservation strategy

ABSTRACT

Elaeagnus mollis Diels (Elaeagnus L.) is an economically and ecologically important but endangered tree in China. Understanding its genetic diversity is essential for its conservation and sustainable utilization. Simple Sequence Repeat (SSR) markers were used to assess the genetic diversity and differentiation in 7 wild populations of *E. mollis*. Ten primer pairs yielded a total of 163 alleles. Allelic richness (Ar = 12.3), Nei's gene diversity ($H = 0.69$) and observed heterozygosity ($H_O = 0.63$) indicated high species-level genetic diversity. Analysis of molecular variance (AMOVA) revealed limited genetic differentiation, with 16.8% of total genetic variability partitioned among populations. Genetic distances were not significantly associated with geographic distances (Mantel test, $r = 0.3085$, $P = 0.1258$), indicating disconformity to the isolation-by-distance model. UPGMA cluster analyses and Bayesian clustering supported the grouping of the populations into 2 groups. The present genetic structure of *E. mollis* may be explained by its life-history traits, breeding system, unique biological traits and anthropogenic disturbance. Considering the high intraspecific genetic diversity, the endangerment of *E. mollis* has more likely resulted from anthropologic effects. A proper conservation strategy is proposed for this plant.

1. Introduction

The long-term survival of an endangered species is critically dependent on the maintenance of sufficient genetic variation to adapt to long-term environmental changes; therefore, the maintenance of genetic variation within and between populations of such species is important for any conservation program (Gitzendanner and Soltis, 2000; Cote, 2003; Chen et al., 2009). Assessing the level and distribution of genetic diversity and differentiation are crucial for the management and development of effective conservation strategies for endangered species. Because of hyper-variability, codominance and high reproducibility, Simple Sequence Repeat (SSR, or microsatellite) markers are superior for genetic studies of population structure (Dayanandan et al., 1998) compared with the commonly used Random Amplified Polymorphic DNA (RAPD), Inter-Simple Sequence Repeat (ISSR) and Amplified Fragment Length Polymorphism (AFLP) markers (Wang and Szmidt, 2001). Numerous studies based on SSR markers have proven that they are highly efficient for the assessment of genetic variation within and between populations of plants (e.g., Guo et al., 2010; Mehmet et al., 2011; Wang et al., 2011).

* Corresponding author. Tel.: +86 0357 2051009.
E-mail address: guiqiny@dns.sxnu.edu.cn (G. Yan).

0305-1978/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bse.2011.09.009

Elaeagnus mollis Diels (Elaeagnus L.) originates from the Tertiary Period, is a relic species of the Quaternary glaciations and is an important endangered, endemic species to China. It is distributed mainly on the hills and lower mountains in southern Shanxi Province and at the northern foot of the Qinling Mountains in Shaanxi Province (Xie and Ling, 1997; Shangguan and Zhang, 2001). *E. mollis* is regarded as an important economic plant; its seeds contain a large percentage of oil, such as oleic acid, linoleic acid and palmitic acid (Zhang, 2008). It also plays an important role in traditional Chinese medicine. For example, fruits of *E. mollis* can be used medicinally for hypertension and hyperlipemia, and its seeds can be used for anti-senile and anti-oxidative treatments (Zhang, 2008). In addition, it is a food plant as its leaves make a healthy tea. However, the life span of seeds is short. When stored for one year, seeds almost lose their ability to germinate (Shangguan and Zhang, 2001). The seed's characteristics may be the cause of its vulnerability (Shangguan and Zhang, 2001). It has been listed as a second-class, state-protected plant and needs urgent protection and restoration.

To date, previous studies of *E. mollis* have been mainly focused on its morphology and anatomy (Liu et al., 2008), reproductive biology (Zhang et al., 2006), population ecology (Zhang and Shangguan, 2000) and pharmacognosy (Zhang, 2008). No study has been reported on its genetic diversity and differentiation, although such information is essential to the formulation of effective conservation strategies for this endangered species. In the present study, the specific SSR primers developed for *Hippophae rhamnoide*s (Sun et al., 2007) were employed to assess allelic variations in wild populations of *E. mollis*.

The major objectives of this study were to address the following questions: (i) How is the genetic diversity partitioned within and between populations of this endangered species? (ii) Are the genetic patterns closely linked to the geographical distributions? (iii) What factors have affected its genetic structure? (iv) Based on the genetic structure, what measures should be adopted in the future for the conservation and propagation of this medically important species?

2. Materials and methods

2.1. Plant sampling

In 2007, 2008, two field studies were conducted across the geographic range of *E. mollis*. A total of 110 individuals, which corresponded to seven natural populations of *E. mollis*, were sampled from Shanxi and Shaanxi Provinces (Fig. 1, Table 1). Leaves of 12–19 plants were randomly collected from each population, depending on the accessibility and the population sizes (Table 1). The neighboring individuals were at least 50 m apart, to avoid resampling from the same individuals. The locations of the studied populations are listed in Fig. 1 and Table 1. Latitude, longitude and altitude were recorded using a handheld GPS (Garmin, Taiwan). Following collection, the leaves were dried and stored in a silica gel. The voucher specimens were deposited in the Shanxi Normal University Herbarium.

2.2. DNA extraction and PCR amplification

Total DNA was extracted from the silica gel-dried leaves using the modified 2 × CTAB procedure (Doyle and Doyle, 1987). DNA quality was checked by electrophoresis on 0.8% agarose gels, and DNA concentration was determined using an Eppendorf biophotometer protein nucleotide analyzer (Eppendorf China Ltd., Germany). The DNA samples were diluted to 10 ng μL^{-1} and stored at −20 °C for subsequent use.

A total of 46 *Hippophae rhamnoides* SSR primer pairs were used (Sun et al., 2007) on *E. mollis* of the family Elaeagnaceae. Ten of 46 SSR primer pairs produced clear, stable, reproducible and polymorphic bands and were used in the present study to amplify *E. mollis* individuals. DNA amplifications were performed in 20 μL reaction volumes containing 1 × reaction buffer (Diguo Biotechnology, Beijing), 25 ng genomic DNA, 0.2 mmol L^{-1} dNTPs, 0.4 μmol L^{-1} of each primer, 2.0 mmol L^{-1} Mg^{2+}, and 1 U Taq DNA polymerase (Diguo Biotechnology, Beijing). The PTC-200 Thermal Cycler (BIO-RAD, USA) PCR was programmed according to the following profile: 94 °C for 1 min, 1 cycle; 94 °C for 45 s, 54–58 °C for 45 s, 72 °C for 105 s, 36 cycles; 72 °C for 10 min, 1 cycle; and 4 °C indefinitely. The PCR products were separated on a 6% denaturing polyacrylamide gel and visualized by silver staining (Yan et al., 2008). A 50-bp molecular weight ladder (Diguo Biotechnology, Beijing) was used to identify alleles.

2.3. Data analysis

The genetic diversity at the SSR loci in each population was quantified in terms of the number of alleles per locus (A), allelic richness (Ar) (El-Mousadik and Petit, 1996), the mean observed heterozygosity (*Ho*) and genetic diversity (*H*) (Nei, 1987) using the program FSTAT v2.9.3 (Goudet, 1995). In addition, G_{ST} (Nei, 1973), F_{ST} (Wier and Cockerham, 1984) and R_{ST} (Slatkin, 1995; Rousset, 1997) were also estimated using FSTAT. G_{ST} is the proportion of the total genetic diversity that occurs among populations. F_{ST} is the coefficient of the genetic differentiation among populations under an infinite allele model (IAM). R_{ST} is also a coefficient of the genetic differentiation among populations, but is defined under a stepwise mutation model (SMM) for SSRs.

Deviations from Hardy–Weinberg expectations were determined through χ^2 analysis, inbreeding coefficients (F_{IS}, Wier and Cockerham, 1984) calculated using Genepop web Version 3.4 (Raymond and Rousset, 1995; http://genepop.curtin.edu.au/genepop) and their significance ($F_{IS} \neq 0$) was tested by 1000 permutations. The significance of the differentiation at each locus was tested by the log-likelihood (G)-based exact test (Goudet et al., 1996) using a Markov chain method in Genepop.