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Relationship between the genetic diversity of *Artemisia halodendron* and climatic factors

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ABSTRACT

Artemisia halodendron (Asteraceae) is a dominant sand-fixing semi-shrub species native to the Horqin Sandy Land of northeastern China. In this study, we evaluated levels of genetic variation within and among sampled *A. halodendron* populations from two different hydrothermal regions of the Horqin Sandy Land using inter-simple sequence repeat (ISSR) markers. We also investigated possible relationships between genetic diversity of this species and climatic factors. Our analysis revealed that *A. halodendron* is highly genetically diverse, with populations from a low hydrothermal level region having higher genetic diversity index values than those from a high hydrothermal level region. An analysis of molecular variation (AMOVA) revealed relatively high levels (>89.83%) of within-population genetic variation. Based on cluster analysis, the 13 studied *A. halodendron* populations can be clustered into two clades. Genetic diversities of all populations have been influenced by many climatic factors, and Nei's genetic diversity (*h*) is strongly correlated with annual temperature range (ART). These results have important implications for restoration and management of degraded ecosystems in arid and semi-arid areas.

1. Introduction

Artemisia halodendron (Asteraceae) is a climax and dominant sand-fixing semi-shrub species native to the Horqin Sandy Land of northeastern China. It is an important component of vegetation rehabilitation efforts in the Horqin Sandy Land because of several highly valuable ecological traits, which include its high drought tolerance, anti-wind erosion utility, sand burial-resistance (Dong et al., 2000; Li et al., 2002; H.L. Zhao et al., 2006), and status as a key species for plant community establishment and landscape formation (Li, 1991). *A. halodendron* is distributed in mobile, semi-mobile and fixed dunes, and lowlands. A special combination of conditions with respect to water fertility and heat in Inner Mongolia, and the Horqin Sandy Land characterizes the main part of the distribution range (Fu, 1993). The life history traits of *A. halodendron* include long-lived, perennial, wind-pollinated, seed reproduction, vegetative propagation and broad ecological amplitude (Fu, 1993). Previous studies on *A. halodendron* have focused on its population distribution patterns (Chao et al., 1999; Cao et al., 2008), biomass allocation (Li et al., 2005), breeding distribution (Li et al., 2005), morphological characteristics and physiological adaptations (Zhou et al., 1999), root longevity (Huang et al., 2009), and establishment (Li et al., 2002) in the Horqin Sandy Land. The relationship between *A. halodendron* genetic diversity and climatic factors has not yet been reported, however.

The Horqin Sandy Land is located in an agro-pastoral transition zone between the Inner Mongolian Plateau and the Northeast Plains (42°41′–45°45′N, 118°35′–123°30′E). It covers an area of approximately 139,300 km^2, of which about 71,884 km^2 is desertified (Wang, 2003; Zhao et al., 2003). The landscape in this area is characterized by sand dunes alternating with gently undulating lowland areas (Li et al., 2005). The region, which is located in the continental temperate zone, experiences a semi-arid monsoon climate with a mean annual temperature of 3–7 °C and mean annual rainfall of 350–500 mm (Zhao et al., 2003). Over recent decades, this region has undergone severe desertification (Li et al., 2000, 2004), a northward-moving phenomenon affecting

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interlocked agro-pastoral areas of northern China during the past few centuries (Zhao et al., 2000, 2002).

In this study, we assessed *A. halodendron* population genetic variation along temperature and humidity gradients in Horqin Sandy Land using inter-simple sequence repeat (ISSR) markers. We addressed the following questions: (1) what is the level of genetic diversity within and among *A. halodendron* populations in different hydrothermal regions? (2) what are the relationships between uncovered genetic diversity and climatic factors, and how are they correlated? These results were interpreted with the aim of providing baseline genetic information for restoration and management of degraded ecosystems in arid and semi-arid regions.

2. Materials and methods

2.1. Population sampling

A total of 290 individuals were sampled from 13 natural *A. halodendron* populations. Populations 1–8 were located in a low hydrothermal synthesis index region (average 25.29), while populations 9–13 were in a high hydrothermal synthesis index region (average 32.8). Hydrothermal synthesis index values were calculated from the formula

$$S = \sum_{t=1}^{12} 0.18 r_t / 1.045^{T_t}$$

where t = month, r_t = monthly rainfall, and T_t = monthly mean temperature (Bailey, 1979). We sampled 19 to 30 individuals from each population in July 2011 (Table 1, Fig. 1). Climatic data were obtained from CMA (China Meteorological Administration) and given in Table 2. The time period used to derive the climatic means was 1971–2000. Annual temperature range was calculated from the formula

$$\mathrm{ART} = (\mathrm{MTWM} - \mathrm{MTCM})$$

where MTWM = warmest monthly mean temperature, MTCM = coldest monthly mean temperature. Warm index values were calculated from the formula

$$WI = \sum_{i=1}^{12} (t_i - 5)$$

where t = above 5 °C monthly mean temperature. Cold index values were calculated from the formula

$$CI = \sum_{i=1}^{12} (5 - t_i)$$

where t = below 5 °C monthly mean temperature. Humidity index values were calculated from the formula

$$HI = AP/WI$$

where AP = mean annual rainfall (Xu, 1983). Young healthy leaves were randomly sampled from plants spaced at least 30 m apart, and immediately stored with silica gel in zip-lock plastic bags for later DNA extraction.

Table 1
Origin of materials and number of samples for 13 populations of *Artemisia halodendron* from the Horqin Sandy Land.

Population	No. of plants	Latitude (°N)	Longitude (°E)	Mean altitude (m)	Habitats
Pop1	30	42°45′46″	120°35′07″	385	Semi-mobile dune
Pop2	19	42°58′11″	120°40′45″	357	Semi-mobile dune
Pop3	21	42°55′45″	119°11′37″	367	Fixed dune
Pop4	22	43°10′10″	119°55′49″	434	Mobile dune
Pop5	20	42°47′12″	120°36′02″	452	Mobile dune
Pop6	19	42°31′06″	120°23′51″	330	Lowland between mobile dunes
Pop7	23	42°39′34″	120°10′23″	495	Mobile dune
Pop8	30	42°54′15″	119°46′47″	479	Mobile dune
Pop9	20	44°30′46″	121°13′44″	346	Semi-fixed dune
Pop10	21	43°41′33″	122°33′19″	253	Fixed dune
Pop11	20	43°15′54″	122°11′22″	177	Fixed dune
Pop12	20	42°51′26″	122°30′32″	175	Fixed dune
Pop13	25	43°15′42″	121°25′11″	251	Fixed dune

2.2. DNA extraction and ISSR-PCR amplification

Total DNA was extracted using an AxyPrep genomic DNA mini kit (Axygen, Beijing, China). DNA was quantified spectrophotometrically; samples yielding high quantities of good quality DNA were used in consecutive experiments. After screening 100 ISSR primers from the University of British Columbia (UBC primer set no. 9) for well-amplified and polymorphic bands among plant populations, we selected 14 primers for use with all individuals. To ensure data quality, we have done some planning when run on gels. Then we will state the specific methods. There were 25 bands in each gel, and included one marker and twenty-four bands came from eight populations (randomized samples three individuals from each population when run on gels) in the same primer, and according to this order analogized.

ISSR amplifications were performed in 25-μL reaction volumes containing 40 ng genomic DNA, 1.0 U Taq polymerase, 3 mM $MgCl_2$, 500 μM of each dNTP, 20 mM Tris–HCl (pH 8.3), 100 mM KCl, and 0.3 μM primer. Amplification conditions consisted of an initial step of 3 min at 94 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at the appropriate annealing temperature (see Appendix S1 for details), and 2 min at 72 °C, and a final 7 min extension step at 72 °C. ISSR reactions were performed at least twice for all individuals and for all the primers to determine the reproducibility of banding patterns. Amplification products along with 100-bp DNA ladder were electrophoretically resolved on 1.8% agarose gels containing ethidium bromide (0.5 μg/mL final concentration) at 100 V for 2 h, and photographed under ultraviolet light.

2.3. Data analysis

During analysis of the resulting gels, only clear and reproducible bands were considered. Amplified fragments were scored for presence (1) or absence (0) of bands, and the data transformed into a 0/1 binary character matrix. The resulting binary data matrix was analyzed using POPGENE Version 1.32 (Yeh and Yang, 1999). Genetic diversity of each population was estimated according to percentage of polymorphic loci (P), observed number of alleles (Na), effective number of alleles (Ne), Nei's genetic diversity (h), and Shannon's diversity index (I). Analysis of molecular variance (AMOVA) was performed to analyze among-population sources of variation using ARLEQUIN with 1000 bootstrap replicates (Schneider et al., 2000). A correlation analysis between genetic diversity indices and climatic factors was conducted using SPSS 17.0.

3. Results

3.1. Genetic diversity

ISSR band profiles revealed high levels of polymorphism in the surveyed *A. halodendron* populations. The 14 selected ISSR primers