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# Environmental determinants of genetic diversity in *Salix gordejevii* (Salicaceae) in three Sandy Lands, northern China

Wenda Huang[a,b,*], Xueyong Zhao[a,c], Xin Zhao[b], Yulin Li[a], Jing Feng[a], Na Su[a], Chengchen Pan[a], Yayong Luo[a]

[a] Naiman Desertification Research Station, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Donggang West Road 320, Lanzhou City, Gansu Province, 730000, China
[b] Key Laboratory of Stress Physiology and Ecology in Cold and Arid Regions, Gansu Province, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou, 730000, Gansu, China
[c] Urat Desert-grassland Research Station, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Donggang West Road 320, Lanzhou City, Gansu Province, 730000, China

## 1. Introduction

Genetic differentiation in desert species is related to the spatial scale of environmental heterogeneity and to the balance between selection and gene flow (Xu et al., 2003). Genetic variation among populations of desert species for quantitative traits is well established (Scheiner, 1993), and this variation often occurs along different climatic gradients, such as changes in temperature and precipitation (Lovejoy and Hannah, 2005), suggesting strong local adaptation to climate (Sork et al., 2010). Understanding the environmental parameters that drive adaptation among populations is important in predicting how species may respond to global climatic changes and how gene pools might be managed to conserve adaptive genetic diversity (Bradbury et al., 2013).

Globally, desertified lands occupy approximately $3.6 \times 10^7$ km$^2$ in area, which amounts to about 24.1% of Earth's land surface and desertification affects about one-sixth of the world's population (Wang et al., 2012). Land degradation and desertification have become increasingly severe environmental and socio-economic problems throughout the world, while China is one of the most seriously affected countries (Wu and Ci, 2002). There are four large Sandy Lands in the agro-pastoral transitional zone in northern China, from east to west are Hulunbel Sandy Land, Horqin Sandy Land, Hunshandak Sandy Land and Mu Us Sandy Land, area reached 10,000 km$^2$, 139,300 km$^2$, 21,400 km$^2$ and 40,000 km$^2$ (Wu and Ci, 2002). There are environment gradient changes in the four Sandy Lands. There are different water and heat conditions in every Sandy Land, from east to west, mean annual temperature reached 0–2.5 °C, 3–7 °C, 1–3 °C and 5–9 °C, mean annual rainfall reached 280–400 mm, 350–500 mm, 100–200 mm and 200–400 mm (Wang et al., 2014; Cheng et al., 2004; Wang, 2003; Zhao et al., 2003). Horqin Sandy Land, Hunshandak Sandy Land and Mu Us Sandy Land were three of the four largest sandy areas in China and provided a source of sand for sandstorms occurring in Northern China (Li et al., 2005). So it is an important exploration of the genetic diversity level and genetic variation in typical sand-fixation plants in this region.

*Salix gordejevii* Chang et Skv. (Salicaceae) is widely distributed in the Sandy Land in northern China (Su et al., 2005; Zhang et al., 2006). It has an important role in restoring the degraded ecosystem. In desertification control, it also serves as the pioneer species for vegetation re-establishment and moving sand fixation because it has several highly valuable ecological traits, including high drought tolerance, anti-wind erosion utility and sand burial-resistance (Wu, 2003; Zhang et al., 2006; Cui et al., 2011). *S. gordejevii* inhabits semi-fixed and fixed dunes. In terms of life history traits, *S. gordejevii* is long-lived, perennial, insect-pollinated, reproduces by seed and cottage propagation, and has a broad ecological amplitude (Fu, 1993). Previous studies on *S. gordejevii* have focused on aspects of its root morphological characteristics and variations (Huang et al., 2010; Liu et al., 2016), root ecology (Huang et al., 2008; Liu et al., 2014; Cui et al., 2011), physiological adaptations (Su et al., 2009; Ma et al., 2015; Liu et al., 2003), nutrient absorption (Yuan et al., 2005), and water use efficiency (Niu et al., 2006; Yue et al., 2013). The relationship between the genetic diversity of *S. gordejevii* and different Sandy Lands has not yet been reported, however.

The association between genetic and environmental gradients is well-established evidence of natural selection (Endler, 1986; Mannel et al., 2010). Climate is one of the most important drivers of local adaptation in desert species. Standing levels of genetic diversity and structure within and among natural populations of desert species are determined by the interplay between climatic heterogeneity and the balance between selection and gene flow. To investigate this hypothesis, we assessed *S. gordejevii* population genetic variation in different Sandy Lands in Northern China using inter-simple sequence repeat

* Corresponding author. Naiman Desertification Research Station, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Donggang West Road 320, Lanzhou City, Gansu Province, 730000, China.
*E-mail address:* huangwenda2008@163.com (W. Huang).

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**Table 1**
Origin of materials and number of samples for 21 populations of *Salix gordejevii* from three Sandy Land.

| Population | No of plants | Latitude (°N) | Longitude (°E) | Altitude (m) | Habitats | Voucher |
|---|---|---|---|---|---|---|
| pop1 | 15 | 39°22′30″ | 109°39′21″ | 1402 | Fixed sand dune | Huang130101-(1-15) |
| pop2 | 13 | 38°47′02″ | 109°08′47″ | 1414 | Fixed sand dune | Huang130102-(1-13) |
| pop3 | 13 | 39°17′30″ | 109°40′02″ | 1326 | Fixed sand dune | Huang130103-(1-13) |
| pop4 | 13 | 38°25′45″ | 108°45′37″ | 1350 | Lowlands between fixed sand dunes | Huang130104-(1-13) |
| pop5 | 15 | 38°58′55″ | 108°59′05″ | 1410 | Fixed sand dune | Huang130105-(1-15) |
| pop6 | 15 | 39°35′27″ | 110°18′34″ | 1384 | Fixed sand dune | Huang130106-(1-15) |
| pop7 | 15 | 38°49′32″ | 18°52′20″ | 1341 | Fixed sand dune | Huang130107-(1-15) |
| pop8 | 15 | 42°59′20″ | 116°51′04″ | 1327 | Semi-mobile sand dune | Huang130201-(1-15) |
| pop9 | 14 | 43°03′10″ | 117°02′18″ | 1315 | Mobile sand dune | Huang130202-(1-14) |
| pop10 | 14 | 42°42′13″ | 115°09′50″ | 1245 | Lowlands between Fixed sand dunes | Huang130203-(1-14) |
| pop11 | 15 | 42°44′55″ | 115°10′08″ | 1256 | Fixed sand dune | Huang130204-(1-15) |
| pop12 | 15 | 43°01′25″ | 116°31′28″ | 1250 | Lowlands between Fixed sand dunes | Huang130205-(1-15) |
| pop13 | 15 | 42°55′40″ | 114°89′32″ | 1260 | Lowlands between Fixed sand dunes | Huang130206-(1-15) |
| pop14 | 15 | 43°14′22″ | 115°28′33″ | 1270 | Fixed sand dune | Huang130207-(1-15) |
| pop15 | 14 | 43°07′15″ | 122°14′59″ | 224 | Lowlands between fixed sand dunes | Huang130301-(1-14) |
| pop16 | 14 | 43°12′09″ | 122°14′44″ | 216 | Lowlands between mobile sand dunes | Huang130302-(1-14) |
| pop17 | 12 | 44°13′21″ | 120°22′48″ | 362 | Mobile sand dune | Huang130303-(1-12) |
| pop18 | 12 | 43°40′34″ | 120°28′26″ | 310 | Lowlands between mobile sand dunes | Huang130304-(1-12) |
| pop19 | 15 | 43°09′36″ | 122°14′47″ | 220 | Mobile sand dune | Huang130305-(1-15) |
| pop20 | 15 | 43°40′34″ | 120°28′26″ | 325 | Lowlands between mobile sand dunes | Huang130306-(1-15) |
| pop21 | 15 | 43°15′42″ | 121°25′11″ | 251 | Mobile sand dune | Huang130307-(1-15) |

(ISSR) markers. Our specific aims in this study were to (1) characterize the level of genetic diversity of *S. gordejevii* within and among populations along with Mu Us Sandy Land, Hunshandak Sandy Land and Horqin Sandy Land. (2) Test for correlation and pattern between genetic diversity of *S. gordejevii* and climatic factors in three Sandy Lands. (3) Search for the important climatic factors affect genetic diversity of *S. gordejevii* populations across three Sandy Lands. 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. Material and methods

### 2.1. Sampling

A total of 299 individuals were sampled from 21 natural *S. gordejevii* populations. Populations 1–7 were located in Mu Us Sandy Land, populations 8–14 were in Hunshandak Sandy Land, and populations 15–21 were in Horqin Sandy Land (Table 1, Fig. 1). We sampled 12 to 15 individuals from each population in July 2013 (Table 1, Fig. 1). Climatic data were obtained from CMA (China Meteorological Administration) and are shown in Table 2. The time period used to derive the climatic means was 1971–2000. Annual temperature range was calculated from the formula

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

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

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

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

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

where $t$ = below 5 °C monthly mean temperature. 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). *S. gordejevii* was important sand-fixation plant in Northern China. There are many cultivated populations in Sandy Land, however we only sample randomly young healthy leaves from natural populations. In order to reduce and prevent the effect of the vegetatively reproduction of *S. gordejevii* on research results, sampled individuals growing at intervals of at least 30 m, and immediately stored with silica gel in zip-lock plastic bags for later DNA extraction.

### 2.2. DNA extraction and ISSR-PCR amplification

Total DNA was extracted using an AxyPrep genomic DNA mini kit (Axygen, Beijing, China). The DNA was quantified spectrophotometrically; samples yielding high quantities of good quality DNA were used in subsequent 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 fifteen primers for use with all individuals.

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 Table 3 for details), and 2 min at 72 °C, with a final 7 min extension step at 72 °C. ISSR reactions were performed at least twice for all individuals and primers to determine the reproducibility of banding patterns. Amplification products along with a 100-bp DNA ladder were electro-phoretically 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 gels, only clear and reproducible bands were considered. Amplified fragments were scored for presence (1) or absence (0) of bands, and the data were transformed into a 0/1 binary character matrix. The resulting binary data matrix was analyzed using POPGENE Version 1.32 (Yeh and Yang, 1999). The genetic diversity of each population was estimated according to the percentage of polymorphic loci (*P*), observed number of alleles (*Na*), effective number of alleles (*Ne*), Nei's genetic diversity (*h*), Shannon's diversity index (*I*), total gene diversity ($H_t$), gene diversity in population ($H_s$), gene