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Genetic variation within the sand-fixation species *Caragana microphylla* (Leguminosae) in Horqin sandy land detected by inter-simple sequence repeats analysis

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ABSTRACT

Caragana microphylla is the most dominant and constructive shrub species in the Horqin sandy land in the northeast of China. We evaluated it's the level of genetic variation within and among populations sampled from two different populations types in Horqin sandy land by using inter-simple sequence repeat polymorphism (ISSR) molecular markers. The results showed that eight ISSR primers generated 106 bands, of which 87 were polymorphic. At the species level, genetic diversity was relatively high ($P = 82.08\%$, $h = 0.2831$, $I = 0.4233$). Genetic variation in natural populations ($h = 0.2152$, $I = 0.3169$) was more than that in plantation populations ($h = 0.2021$, $I = 0.3040$). Based on Nei's G_{ST} value, more genetic differentiation among plantation populations was detected ($G_{ST} = 0.7787$). Six populations of *C. microphylla* clustered into two clades. These results have important implications for restoring and managing the degraded ecosystem in arid and semi-arid areas.

1. Introduction

Caragana microphylla is a widely used shrub for vegetation rehabilitation in Horqin sandy land, North-eastern China (Cao et al., 2000; Chen et al., 2009a). It is used in rehabilitation projects because it is drought tolerance, "anti-wind erosion-rephrase" and has N_2-fixation capacity (Zhao, 2005; Zhang et al., 2009; Han et al., 2011); it is widely planted throughout severely desertified sites to control land desertification in North China (Su et al., 2005; Zhang et al., 2006); it is also an important forage and medicinal plant in Horqin sandy land (Fu, 1993; Jia and Qu, 2001). Previous studies on *C. microphylla* focused on aspects of population distribution pattern and ecological adaptations (Zhao, 2005), morphological characteristics and variations (Li et al., 2008), physiological adaptations (Ma et al., 2003; Li et al., 2008), nutrient absorption (Li et al., 2013), and genetic diversity (H.Y Guo et al., 2008). However, systematic comparison of genetic diversity of *C. microphylla* from natural populations and plantation populations have not been investigated.

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The Horqin sandy land is located in the agro-pastoral transitional zone between the Inner Mongolian Plateau and the Northeast Plains (42°41′–45°45′N, 118°35′–123°30′E), and is one of the four major sandy areas in northern China. It covers an area of approximately 43,000 km^2, and is an important component of Inner Mongolian grassland resources (Liu et al., 1996). Landscape in this area is characterized by sand dunes alternating with gently undulating lowland areas (Li et al., 2005). This area belongs to the continental semi-arid monsoon climate in the temperate zone. However, over recent decades, especially in the last 30 years, this region has undergone severe desertification (Li et al., 2000, 2004), and has displayed the northern moving phenomenon of the interlocked agro-pasturing area of north China in the most recent hundred years (Zhao et al., 2000, 2002).

This investigation is to assess the level of population genetic variation in *C. microphylla* between natural and plantation populations in Horqin sandy land using ISSR marker. The aims are to: (1) reveal the genetic diversity of *C. microphylla* within and among populations; (2) investigate the correlation between the genetic diversity and population types of *C. microphylla*; (3) provide some suggestions for future conservation and breeding programs for this species.

2. Material and methods

2.1. Population sampling

We sampled 104 individuals from six *C. microphylla* populations. Populations 1–3 were from the natural woodland, while populations 4–6 were from the plantation (planting age >15 years). For each population, 15–20 individuals (spaced 30 m apart) were sampled (Table 1). Young-healthy leaves were randomly sampled from individual plants and immediately stored with silica gel until DNA extractions were carried out.

2.2. DNA extraction procedure

Total DNA was extracted using AxyPrep Genomic DNA Mini Kits following the manufacture's instructions. DNA was quantified by spectrophotometrically and samples yielding good high quantity DNA were used for next step experiment.

2.3. ISSR amplifications strategies

One hundred primers from the University of British Columbia (UBC primer set no. 9) were initially screened for well-amplified and polymorphic bands among plant populations. Eight ISSR primers were finally selected for the full screening of all individuals. ISSR PCRs were performed in a 25 μL reaction volume containing 40 ng of genomic DNA, 1.0 U of Taq polymerase (Axygen Inc., Beijing, China), 3 mM $MgCl_2$, 500 μM each dNTP, 20 mM Tris–HCl (pH 8.3), 100 mM KCl, 0.3 μM primer. The amplification conditions were: an initial denaturation temperature of 3 min at 94 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at an appropriate annealing temperature (see Table 2 for details), 2 min at 72 °C, and a final 7 min extension step at 72 °C. ISSR reactions were performed at least twice to determine the reproducibility of banding patterns. The amplification products were electrophoretically resolved in 1.8% agarose gels containing ethidium bromide (final concentration of 0.5 μg/ml) at 100 V for 2 h, along with 100 bp DNA ladder (Axygen Inc., Beijing, China) and photographed under ultraviolet light.

2.4. Statistical analysis

On the gel analysis, we only considered distinct and reproducible bands. The amplified fragments were scored for presence (1) or absence (0) of bands and 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 for each population was measured by the percentage of polymorphic loci (*P*), the observed number of alleles (Na), the effective number of alleles (Ne), Nei's gene diversity (*h*), and Shannon's information index (*I*). Genetic differentiation among populations was estimated by Nei's gene diversity statistic (Nei, 1973). Pairwise genetic distance between populations (Nei, 1972) was calculated to construct a UPGMA dendrogram using the software NTSYS pc2.02 (Rohlf, 1998).

Table 1
Origin location, abbreviation, the number of plants, population types of the 6 populations of *Caragana microphylla*.

Population	No of plants	Latitude (°N)	Longitude (°E)	Altitude (m)	Population types
Subp1	16	42°55′55″	120°42′40″	358	Natural population
Subp2	19	43°10′10″	120°37′50″	434	Natural population
Subp3	20	42°47′12″	120°36′02″	452	Natural population
Subp4	15	43°26′48″	121°01′26″	368	Plantation population
Subp5	15	42°57′31″	120°41′25″	350	Plantation population
Subp6	19	43°15′42″	121°25′11″	251	Plantation population

Table 2
Primers sequence, melting temperature and percentage of polymorphism in ISSR analyses of *Caragana microphylla*.

Primers	Sequences (5′→3′)	Tm (°C)	No. bands	No. polymorphic loci	Percentage of polymorphic loci	Amplified band size (bp)
UBC807	$(AG)_8T$	54	12	11	91.67	200–800
UBC826	$(AC)_8C$	54	9	6	66.67	300–1250
UBC827	$(AG)_8G$	55	12	11	91.67	250–1750
UBC840	$(GA)_6YT$	58	14	13	92.86	300–1750
UBC857	$(AC)_8YG$	58	17	14	82.35	150–1750
UBC864	$(ATG)_6$	50	12	9	75.00	300–1750
UBC880	$(GGAGA)_3$	57	18	14	77.78	200–2500
UBC889	$DBD(AC)_7$	52	12	10	83.33	250–900

Note: $y = c/t$; $b = c/g/t$; $d = a/g/t$.

3. Results

3.1. ISSR profile

The profile of ISSR bands showed high polymorphism in *C. microphylla* populations. The 8 selected ISSR primers generated a total of 106 clear and distinguishable band fragments, 87 bands (82.08%) were polymorphic. The size of the amplified fragments ranged from 150 to 2500 bp, with an average of 10.875 fragments generated per primer. The highest number of bands was generated with primers UBC880 and the higher polymorphism was obtained with primer UBC857 and UBC880 (Table 2).

3.2. Genetic diversity and differentiation

The genetic diversities among the six populations of *C. microphylla* are given in Table 3. The number of polymorphic loci (n) ranged from 10 to 20 and the percentage of polymorphic bands (P) ranged from 9.43% to 18.87%. The observed number of alleles (Na) ranged from 1.0943 to 1.1887. The effective numbers of alleles (Ne) were from 1.0647 to 1.1490. The range of gene diversity (h) and Shannon's information index (I) was 0.0371–0.0802 and 0.0545–0.1152, respectively. At the species level, Nei's genetic diversity (h) was 0.2831 and the Shannon's information index (I) was 0.4233, respectively (Table 3). At the population level, the Nei's genetic diversity (h) and the Shannon's information index (I) between two population types varied between 0.2152 (natural populations) and 0.2021 (plantation populations), and between 0.3169 (natural populations) and 0.3040 (plantation populations), respectively. Compared with the plantation populations, natural populations had high genetic diversity indices. Plantation populations of *C. microphylla* were highly differentiated. Nei's gene differentiation (G_{ST}) was 0.7787, indicating that approximately 77.87% of the genetic variation was among plantation populations. Natural populations had less differentiation than plantation populations, with 72.37% of the genetic variation among populations.

Distances of natural population pairs ranged from 0.1590 to 0.4037, with a mean of 0.2920. Plantation population distances ranged from 0.0756 to 0.4204, with a mean of 0.3004 (Table 4). Distances between natural and plantation populations ranged from 0.0756 to 0.4843, with a mean of 0.3444. On the basis of Nei's (1972) genetic distances, a dendrogram of the six populations was generated using UPGMA cluster analysis. The six populations were divided into two main groups: all natural populations including population 4 from plantation populations in clade I, others plantation populations (pop5 and 6) formed clade II (Fig. 1).

Table 3
Genetic diversity indices of *Caragana microphylla* populations.

Population	Sample size	n	P (%)	Na (±SD)	Ne (±SD)	h (±SD)	I (±SD)
Natural populations							
pop1	16	15	14.15	1.1415 ± 0.3502	1.1074 ± 0.2833	0.0590 ± 0.1507	0.0853 ± 0.2152
pop2	19	20	18.87	1.1887 ± 0.3931	1.1490 ± 0.3328	0.0802 ± 0.1735	0.1152 ± 0.2458
pop3	20	10	9.43	1.0943 ± 0.2937	1.0647 ± 0.2156	0.0371 ± 0.1191	0.0545 ± 0.1728
Overall	55	60	56.6	1.5660 ± 0.4980	1.3743 ± 0.3833	0.2152 ± 0.2087	0.3169 ± 0.2984
Plantation populations							
pop4	15	16	15.09	1.1509 ± 0.3597	1.1050 ± 0.2711	0.0595 ± 0.1473	0.0873 ± 0.2127
pop5	15	14	13.21	1.1321 ± 0.3402	1.0860 ± 0.2389	0.0501 ± 0.1343	0.0742 ± 0.1958
pop6	19	15	14.15	1.1415 ± 0.3502	1.1022 ± 0.2683	0.0575 ± 0.1469	0.0837 ± 0.2113
Overall	49	62	58.49	1.5849 ± 0.4951	1.3373 ± 0.3503	0.2021 ± 0.1954	0.3040 ± 0.2821
All populations							
Average	17	15	14.15	1.1415 ± 0.3479	1.1024 ± 0.2683	0.0573 ± 0.1453	0.0834 ± 0.2089
Species level	104	87	82.08	1.8208 ± 0.3854	1.4867 ± 0.3641	0.2831 ± 0.1852	0.4233 ± 0.2538

n = the number of polymorphic loci; p = the percentage of polymorphic loci; Na = observed number of alleles; Ne = effective number of alleles; h = nei's gene diversity; i = shannon's information index.

Table 4
Nei's genetic identity (*above diagonal*) and genetic distance (*below diagonal*) between *Caragana microphylla* populations.

Populations	pop1	pop2	pop3	pop4	pop5	pop6
pop1	****	0.8530	0.7310	0.7342	0.6288	0.6161
pop2	0.1590	****	0.6678	0.7058	0.6845	0.6604
pop3	0.3134	0.4037	****	0.8602	0.6570	0.6582
pop4	0.3090	0.3484	0.1505	****	0.6668	0.6568
pop5	0.4640	0.3790	0.4201	0.4053	****	0.9272
pop6	0.4843	0.4149	0.4182	0.4204	0.0756	****

4. Discussion

Genetic variation is non-randomly distributed among populations and species (Nevo, 1998). This distribution of alleles and genotypes in space or time is often referred to as the genetic structure of a population (Chen et al., 2009b). In Horqin sandy land *C. microphylla* is one of the most important shrub species in North China. The systematic comparison of *C. microphylla* at the level of genetic diversity within and among populations in different population types has not been investigated.

4.1. Genetic diversity

Desert plants tend to present high levels of genetic diversity (Xu et al., 2003; Wang et al., 2004; Wan et al., 2008; Huang et al., 2011). At the species level, the genetic diversity indices ($P = 82.08\%$, $h = 0.2831$, $I = 0.4233$) showed that genetic variation in *C. microphylla* was relatively high. On the basis of *C. microphylla* life history traits, this species is a long-lived, perennial shrub and the combination of these life history traits should enable the species to maintain a high level of genetic diversity. At the population level, the results revealed that plantation populations (planting age >15 years) showed no significant genetic diversity loss compared with the natural populations. Therefore, we propose that the planting of *C. microphylla* would greatly assist in restoring the degraded ecosystem. Our data revealed remarkable genetic differences in different population types. This may reflect that the natural populations were older than the plantation populations. Several reports have indicated that the genetic diversity of natural populations was higher compared with other population type (Xue et al., 1998; Chen et al., 2009a).

4.2. Population genetic structure

The population genetic structure of a species is affected by a number of evolutionary factors including mating systems, gene flow, seed dispersal, mode of reproduction and natural selection (Hamrick and Godt, 1989). Evidence suggests that genetic differentiation would have increased between the natural and plantation populations and among the plantation populations because of the founder effect and the environment (Wang et al., 2009). Our study showed that the plantation populations displayed larger genetic differentiation than the natural populations in *C. microphylla*. This may occur because the population size of plantation shrubs was bigger than natural populations and individual shrubs were more concentrated than natural populations, leading to pollen exchange. The results of differentiation were found among natural populations and among plantation populations, which were significantly higher compared with earlier findings from the RAPD analysis (Q. Guo et al., 2008). This may occur because of the use of different genetic markers. The use of more reliable genetic markers may resolve this conflict.

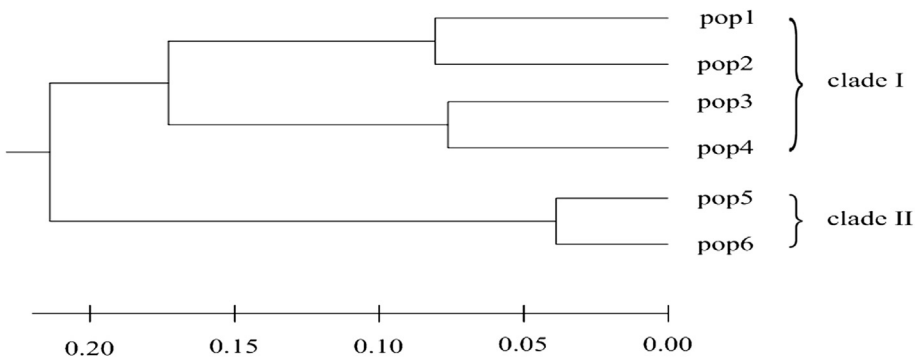

Fig. 1. UPGMA dendrogram of *Caragana microphylla* populations based on Nei's (1972) genetic distances from ISSR data.

4.3. Genetic relationships

In our study, the genetic distances among the six studied populations ranged from 0.0756 to 0.4843. The shortest distance existed between populations 5 and 6. This would suggest both have similar genetic backgrounds or underwent similar genetic adaptations. The largest genetic distance was observed between populations 1 and 6. This is from the population age differences. Population 1 was fenced for 40 years, but population 6 was planted only 19 years ago. Our data showed that the relationship between populations in *C. microphylla* does not agree with populations types. This phenomenon is similar to studies investigating relationships between populations in a species with a large distribution area (Díaz et al., 1999; Qiu et al., 2004).

In conclusion, our data show that genetic variation in *C. microphylla* is associated to population types in the Horqin sandy land. Genetic references are necessary when restoring and managing a degraded ecosystem in arid and semi-arid areas.

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