Front. Biol. China 2007, 2(1): 39–45
DOI 10.1007/s11515-007-0007-5

RESEARCH ARTICLE

Genetic structure and differentiation of *Psathyrostachys huashanica* populations detected with RAPD markers

WANG Li[1,2], YANG Juan[2], GUO Jing[2], ZHAO Guifang (✉)[2]

1 College of Biological Science and Technology, Central South University , Changsha 410013, China
2 School of Life Science, Biodiversity Research Center of Qinling Mountains, Northwest University, Xi'an 710069, China

© Higher Education Press and Springer-Verlag 2007

Abstract *Psathyrostachys huashanica* Keng is a perennial grass and belongs to genus *Psathyrostachys* under Triticeae. *sathyrostachys* is found in the center of Middle Asia and the Caucasus Mountain, while *P. huashanica*, a species endemic to China, is only located in Mt. Hua in the Shaanxi province, China. At present, the population of this species is decreasing, and reaching the edge of extinction. Due to the limitation in distribution and the importance as breeding material for germplasm storage, it has been considered as first class among the national protected rare plants. For this reason, the present study is significant in probing plant flora, origin and evolution of Triticeae, and crop breeding. Randomly amplified polymorphic DNA (RAPD) markers were used to analyze the genetic structure and differentiation of *P. huashanica* populations sampled in three valleys (Huangpu, Xian, and Huashan Valleys) in Mt. Hua. One hundred and twenty-two RAPD fragments were obtained in all 266 individuals with 20 primers with a mean of 6.1 (2–10) fragments per primer. The percentage of polymorphic loci (*PPB*) was 60.66% in Huangpu Valley, 90.98% in Xian Valley, 95.08% in Huashan Valley, and the total *PPB* was 95.08%, which indicated a highly genetic variability of *P. huashanica*. The Shannon's Information index and G_{ST} were 0.3306 and 0.3263, respectively, indicating that there were more genetic variations within the subpopulations than those among the subpopulations. The gene flow among the subpopulations of *P. huashanica* ($Nm = 1.0322$) was much less than that of the common anemophytes ($Nm = 5.24$). Mean genetic distance is 0.1 571 (range: 0.0022–0.2901). The highest value of genetic distance was found between the subpopulation (hp1) of Huangpu Valley and the highest altitude subpopulation (h8) of Huashan Valley. Correlation analysis detected significant correlation between genetic distance and vertical distance of altitude. Clustering analysis and principal coordinate analysis revealed the genetic differentiation among the populations of *P. huashanica*. Differentiation mainly occurred between the higher altitude subpopulations and the lower altitude subpopulations, suggesting that altitude might be the major factor that restricted the gene flow between different altitude subpopulations and resulted in differentiation of subpopulations.

Keywords *Psathyrostachys huashanica*, randomly amplified polymorphic DNA, genetic structure, genetic diversity

1 Introduction

Psathyrostachys huashanica Keng is a perennial grass belonging to genus *Psathyrostachys* under Triticeae. *Psathyrostachys* is found in the center of Middle Asia and Caucasus Mountains., while *P. huashanica*, a species endemic to China, is only located in Mt. Hua in the Shaanxi province, China (Instituto Botanico Boreali-occidentali Academiae Sinicae, 1976). *Psathyrostachys* is phylogenetically related to *Triticum* (Lu, 1995), and its study will be helpful to the analysis of the origin and evolution of *Triticum*. Botanists, especially plant breeders, have made many studies and have paid attention to *P. huashanica* for its early maturity, resistance to drought, disease, and salinity (Chen et al., 1991; Sun et al., 1992; Ding et al., 1997; Hou et al., 1997; Zhang et al., 2002). At present, it has been listed as an endangered and imperatively protected wild species (Chinese Forest Bureau and Agriculture Ministry, 1999). Genetic structure and intraspecific genetic polymorphisms in natural populations of *P. huashanica* have been detected with allozymes (Liu et al., 2001a). Its endangered status and reproductive strategy have been argued (Yue et al., 2001). Nevertheless, application of randomly amplified polymorphic DNA (RAPD) markers for

Translated from *Acta Ecologica Sinica*, 2005, 25(4): 719–726 [译自: 生态学报]

E-mail: guifang@nwu.edu.cn

the analysis of genetic structure and population differentiation in *P. huashanica* has not been conducted. For this reason, the present study is significant in probing plant flora, origin, and evolution of Triticeae and crop breeding.

2 Materials and methods

2.1 Plant material

Samples were collected in April and May from 2000 to 2002. At that time, the species can be distinguished accurately by spike florescence. Materials were collected along Huangpu, Huashan, and Xian valleys (populations) in Huashan Mountain. Sampling locations (subpopulations) were according to altitude (Table 1). Individual samples were at least 1 m apart from each other because *P. huashanica* has rhizome and clonal growth. Healthy leaf samples were desiccated by silica gel and kept at −80°C.

Table 1 Altitude and number of individuals sampled in 13 subpopulations of three valleys

Location		Serial number	Average altitude / m	Number of individuals
Huangpu Valley		hp1	370	21
		hp2	560	20
Xian Valley		*x*1	350	21
		*x*2	400	20
		*x*3	530	20
Huashan Valley	Yuquanyuan	h1	483 (460–510)	20
	Wuliguan	h2	684 (640–710)	22
	Qingkeping	h3	930 (850–1 030)	21
	Huixinshi	h4	1 265 (1 230–1 280)	21
	Baichixia	h5	1 350 (1 310–1 400)	20
	Beifeng	h6	1 559 (1 550–1 610)	20
	Dongfeng	h7	2 000	20
	Nanfeng	h8	2 140	20

2.2 DNA isolation and primer selection

Approximately 5 g of leaves from about 20 plants of each subpopulation were used for the extraction. Leaves were grounded with quartzose sand, little PVP and Vc, incubated at 60°C for 1–2 h. DNA was isolated with 2 × CTAB (Wang et al., 1998). An equal volume of chloroform/isoamyl alcohol (24:1) was added and mixed well. The mixture was centrifuged at 12 000 g for 10 min; this procedure was re-peated several times. The supernatant was precipitated with an equal volume of ice-cold 100% ethanol. The precipitate was hooked out by sterile pipettes, washed in 70% ethanol and air-dried. The DNA was suspended in 200 μL 0.1 × TE buffer and kept at −20°C.

A total of 100 RAPD random primers purchased from Bosia Company were used. Twenty of 100 primers produced clear and repeatable results, and were used to amplify 266 *P. huashanica* individuals from 13 subpopulations (Table 2).

2.3 PCR amplification and product detection

PCR was carried out in a 15 μL solution containing: 1.5 μL 10 × buffer (100 mM KCl, 80 mM $(NH_4)_2SO_4$, 100 mM Tris–HCl, pH = 9.0, NP-40), 1.13 μL $MgCl_2$ (25 mM), 0.45 μL dNTP (dATP, dTTP, dGTP, dCTP, 10 mM each), 1.5 μL template DNA (10 ng/μL), 6 μL primers (0.7 μM), 0.3 μL *Taq* polymerase (5 U/μL, Sangon), 4.1 μL ddH_2O. All RAPD amplifications were performed on T Gradient (Biometra®, Whatman. Techne 〈Cambridge〉 Limited, United Kingdom) Thermal Cycler, and commenced with 5 min 30 seconds at 96°C, followed by 40 cycles of 1 min 10 seconds at 94°C, 1 min at 40°C, and 1 min 30 seconds at 72°C, and ended with 10 min at 72°C.

Amplification products were analyzed with electrophoresis (TAE electrophoresis) on 1.8% agarose gel stained with ethidum bromide at 55V for 2 h, and the gel images were analyzed with the Kodak Scientific Imaging Systems 120 (Estman Kodak Company).

2.4 Data analysis

Randomly amplified polymorphic DNA (RAPD) bands were scored as binary presence (1) or absence (0) characters, to assemble the matrix of the RAPD phenotypes. Genetic diversity was measured by the percentage of polymorphic bands (*PPB*), Shannon information index, Nei's gene diversity, the genetic distances between the subpopulations, the value of gene flow (*Nm*) and subpopulation differentiation (G_{ST}) by using POPGEN32 (Yeh et al., 1999). Cluster analysis was performed based on unweighted pair-group method with arithmetic average (UPGMA). Principal Components Analysis (PCA) and the correlation between genetic distance and altitude distance of *P. huashanica* were calculated by using SPSS 11.0.

3 Results

3.1 The percentage of polymorphic loci (*PPB*)

One hundred and twenty-two loci were detected in 266 individuals of 13 subpopulations using 20 RAPD random primers. One hundred and sixteen loci were polymorphic. The percentage of polymorphic loci (*PPB*) is 95.08% across all subpopulations. Among the three valleys, the *PPB* in Huashan Valley is the largest ($PPB = 0.9508$) than that in Xian Valley ($PPB = 0.9098$), and the *PPB* in Huangpu Valley is the smallest ($PPB = 0.6066$). The order based on the *PPB* from the experiments was: h4 > h5 > *x*1 > *x*3 > h3, *x*2 > h2 > h6 > h7 > h8, hp1 > h1 > hp2 (Table 3, Fig. 1).

Table 2 Random primers used in this study

Serial number	Sequence 5′–3′	Serial number	Sequence 5′–3′	Serial number	Sequence 5′–3′	Serial number	Sequence 5′–3′
BA0017	AGGGAACGAG	BA0031	CAATCGCCGT	BA0047	TTGGCACGGG	BA0079	GTTGCCAGCC
BA0027	GAAACGGGTG	BA0033	CAGCACCCAC	BA0048	GTGTGCCCCA	BA0080	ACTTCGCCAC
BA0028	GTGACGTAGG	BA0039	CAAACGTCGG	BA0051	AGCGCCATTG	BA0123	CCTGATCACC
BA0029	GGGTAACGCC	BA0040	GTTGCGATCC	BA0068	TGGACCGGTG	BA0379	CACAGGCGGA
BA0030	GTGATCGCAG	BA0042	GGACCCAACC	BA0075	GACGGATCAG	BA1020	GGAAGGTGAG

Table 3 Polymorphic loci detected with twenty primers for thirteen subpopulations of *P. huashanica* (proportion of polymorphic loci)

Primer	hp 1	hp2	h1	h2	h3	h4	h5	h6	h7	h8	*x*1	*x*2	*x*3	hp	h	*x*	Total loci
BA0017	1(0.17)	1(0.17)	3(0.50)	1(0.17)	0(0.00)	1(0.17)	0(0.00)	3(0.50)	2(0.33)	3(0.50)	2(0.33)	5(0.83)	5(0.83)	1(0.17)	6(1.00)	6(1.00)	6(1.00)
BA0027	5(0.63)	2(0.25)	4(0.50)	3(0.38)	3(0.38)	6(0.75)	5(0.63)	8(1.00)	8(1.00)	7(0.86)	6(0.75)	3(0.38)	5(0.63)	5(0.63)	8(1.00)	7(0.88)	8(1.00)
BA0028	1(0.50)	0(0.00)	0(0.00)	1(0.50)	1(0.50)	1(0.50)	1(0.50)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(0.50)	1(0.50)	1(0.50)	1(0.50)	1(0.50)	1(0.50)
BA0029	4(0.80)	3(0.60)	3(0.60)	4(0.80)	4(0.80)	5(1.00)	2(0.40)	4(0.80)	4(0.80)	3(0.60)	3(0.60)	4(0.80)	5(1.00)	4(0.80)	5(1.00)	5(1.00)	5(1.00)
BA0030	8(1.00)	8(1.00)	8(1.00)	5(0.63)	7(0.88)	8(1.00)	6(0.75)	5(0.63)	5(0.63)	6(0.75)	8(1.00)	8(1.00)	8(1.00)	8(1.00)	8(1.00)	8(1.00)	8(1.00)
BA0031	6(0.86)	5(0.71)	2(0.29)	6(0.86)	7(1.00)	7(1.00)	7(1.00)	4(0.57)	4(0.57)	3(0.43)	2(0.29)	6(0.86)	6(0.86)	6(0.86)	7(1.00)	7(1.00)	7(1.00)
BA0033	1(0.20)	2(0.40)	2(0.40)	4(0.80)	2(0.40)	4(0.80)	3(0.60)	2(0.40)	1(0.20)	0(0.00)	1(0.20)	2(0.40)	3(0.60)	2(0.40)	4(0.80)	4(0.80)	4(0.80)
BA0039	3(0.43)	4(0.57)	3(0.43)	4(0.57)	5(0.71)	7(1.00)	6(0.86)	6(0.86)	5(0.71)	7(1.00)	7(1.00)	2(0.29)	7(1.00)	4(0.57)	7(1.00)	7(1.00)	7(1.00)
BA0040	7(1.00)	7(1.00)	7(1.00)	5(0.71)	6(0.86)	7(1.00)	7(1.00)	4(0.57)	4(0.57)	5(0.71)	7(1.00)	6(0.86)	7(1.00)	7(1.00)	7(1.00)	7(1.00)	7(1.00)
BA0042	5(0.83)	5(0.83)	5(0.83)	4(0.67)	4(0.67)	5(0.83)	6(1.00)	5(0.83)	5(0.83)	5(0.83)	5(0.83)	5(0.83)	5(0.83)	6(1.00)	6(1.00)	6(1.00)	6(1.00)
BA0047	0(0.00)	0(0.00)	0(0.00)	2(0.40)	2(0.40)	4(0.80)	4(0.80)	1(0.20)	1(0.20)	3(0.60)	1(0.20)	1(0.20)	2(0.40)	0(0.00)	4(0.80)	2(0.40)	4(0.80)
BA0048	5(0.71)	4(0.57)	5(0.71)	7(1.00)	7(1.00)	7(1.00)	5(0.71)	4(0.57)	4(0.57)	6(0.86)	7(1.00)	5(0.71)	5(0.71)	50.71)	7(1.00)	7(1.00)	7(1.00)
BA0051	3(0.38)	3(0.38)	5(0.63)	7(0.88)	7(0.88)	6(0.75)	7(0.88)	6(0.75)	6(0.75)	2(0.25)	7(0.88)	6(0.75)	5(0.63)	3(0.38)	7(0.88)	7(0.88)	7(0.88)
BA0068	0(0.00)	0(0.00)	1(0.20)	3(0.60)	3(0.60)	3(0.60)	4(0.80)	4(0.80)	4(0.80)	1(0.20)	4(0.80)	2(0.40)	1(0.20)	0(0.00)	4(0.80)	4(0.80)	4(0.80)
BA0075	0(0.00)	2(0.20)	5(0.50)	6(0.60)	7(0.70)	7(0.70)	8(0.80)	5(0.50)	5(0.50)	2(0.20)	8(0.80)	8(0.80)	8(0.80)	2(0.20)	10(1.00)	8(0.80)	10(1.00)
BA0079	1(0.33)	1(0.33)	2(0.67)	2(0.67)	1(0.33)	2(0.67)	3(0.67)	1(0.33)	1(0.33)	0(0.00)	2(0.67)	2(0.67)	1(0.33)	1(0.33)	2(0.67)	2(0.67)	2(0.67)
BA0080	8(1.00)	6(0.75)	1(0.13)	8(1.00)	8(1.00)	8(1.00)	7(0.88)	6(0.75)	6(0.75)	6(0.75)	8(1.00)	6(0.75)	7(0.88)	8(1.00)	8(1.00)	8(1.00)	8(1.00)
BA0123	4(0.80)	4(0.80)	4(0.80)	5(1.00)	4(0.80)	4(0.80)	5(1.00)	5(1.00)	5(1.00)	3(0.60)	5(1.00)	5(1.00)	5(1.00)	4(0.80)	5(1.00)	5(1.00)	5(1.00)
BA0379	2(0.50)	3(0.75)	4(1.00)	3(0.75)	3(0.75)	3(0.75)	4(1.00)	1(0.25)	1(0.25)	0(0.00)	3(0.75)	3(0.75)	3(0.75)	3(0.75)	4(1.00)	4(1.00)	4(1.00)
BA1020	4(0.67)	3(0.50)	2(0.33)	2(0.33)	3(0.50)	2(0.33)	5(0.83)	4(0.67)	4(0.67)	6(1.00)	6(1.00)	3(0.50)	2(0.33)	4(0.67)	6(1.00)	6(1.00)	6(1.00)
Total loci / %	68(55.74)	63(51.64)	66(54.10)	82(67.21)	83(68.03)	97(79.51)	94(77.05)	78(63.93)	75(61.48)	68(55.74)	92(75.41)	83(68.03)	91(74.59)	74(60.66)	116(95.08)	111(90.98)	116(95.08)

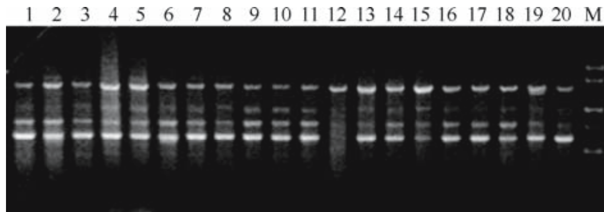


Fig. 1 Amplified products of subpopulation hp2 of *P. huashanica* using primer BA0029

3.2 Genetic structure of *P. huashanica* natural populations

Shannon information index showed that the level of genetic diversity in subpopulation h1 is the lowest (0.2609), and that in subpopulation h4 is the highest (0.3891) (Table 4). The total genetic diversity (H_{SP}) is 0.4849, the ratio of genetic diversity within subpopulations (H_{POP}/H_{SP}) is 0.6694, and that among subpopulations (H_{SP}-H_{POP}/H_{SP}) is 0.3306 (Table 4).

Table 4 Genetic diversity index analysis of *P. huashanica* 13 subpopulations

Shannon diversity index	Mean	Nei's gene diversity index	Mean
hp1	0.307 6	hp1	0.208 6
hp2	0.275 9	hp2	0.1859
h1	0.260 9	h1	0.173 9
h2	0.301 3	h2	0.196 6
h3	0.299	h3	0.194 5
h4	0.389 1	h4	0.259 9
h5	0.354 9	h5	0.231 9
h6	0.344 6	h6	0.232 6
h7	0.313 6	h7	0.209 5
h8	0.311 9	h8	0.211 7
*x*1	0.369 9	*x*1	0.244 7
*x*2	0.337 2	*x*2	0.223 2
*x*3	0.353 9	*x*3	0.231 9
H_{POP}	0.324 6	H_S	0.215 8
H_{SP}	0.484 9	H_T	0.319 8
H_{POP}/H_{SP}	0.669 4	H_S/H_T	0.674 7
$(H_{POP}$-$H_{SP})/H_{SP}$	0.330 6	G_{ST}	0.326 3

Nei's gene diversity in population (H_T) is 0.3198 and was 0.2158 within subpopulations (H_S), and the genetic differentiation (G_{ST}) of this species was 0.3263 (Table 4). At the level of the subpopulation, the gene diversity of subpopulation h1 was the lowest (0.1739), while subpopulation h4 was the highest (0.2599), which was in accordance with the results measured by the Shannon information index. The order on the basis of gene diversity was: h4 > *x*1 > h6 > h5, *x*3 > *x*2 > h8 > h7 > hp1 > h2 > h3 > hp2 > h1.

The *PPB*, the observed number of alleles (na), the effective number of alleles (ne), Nei gene diversity index (*h*), Shannon information index (*I*) and genetic differentiation (G_{ST}) in Huangpu Valley were the lowest among the three populations, while those in Huashan Valley were the highest (Table 5).

Table 5 Genetic diversity of P. huashanica in three valleys

POP	Sample Size	PPB	na	ne	h	I	GST
Huangpu Valley /hp	41	60.66	1.606 6	1.381 2	0.219 6	0.326 2	0.100 6
Huashan Valley /h	164	95.08	1.950 8	1.500 0	0.304 1	0.464 4	0.298 6
Xian Valley /*x*	61	90.98	1.909 8	1.501 1	0.294 8	0.444 9	0.208 6
Total	266	95.08	1.950 8	1.530 2	0.319 8	0.484 9	0.326 3

3.3 Genetic distance of *P. huashanica* between subpopulations and cluster analysis (UPGMA)

Based on Nei's unbiased measure of genetic distance and genetic identity, the range of genetic distance among 13 subpopulations was 0.0022–0.2901 with average value as 0.1571. The largest genetic distance ($D_{max} = 0.2901$) was between the subpopulation of hp1 in Huangpu Valley and the subpopulation of h8 in Huashan Valley, while the lowest ($D_{min} = 0.0022$) between the subpopulation of h1 and h3 in Huashan valley.

The genetic identity ($GI_{min} = 0.7482$) between the subpopulation of hp1 in Huangpu Valley and the subpopulation of h8 in Huashan Valley was the lowest, whereas the largest ($GI_{max} = 0.9978$) between the subpopulation of h2 and h3 in Huashan valley, which was consistent with the results of genetic distance analysis (Table 6).

Cluster analysis was performed using UPGMA (Fig. 2). The 13 subpopulations were divided into four groups: group one contained the subpopulations of hp1 and hp2 in Huangpu Valley; group two uas the six subpopulations in Huashan Valley including the lower altitude subpopulations (h2, h3, h4, h5) and the higher altitude subpopulations (h6, h7); group three uas the three subpopulations in Xian Valley and the lowest altitude subpopulation of h1 in Huashan Valley; group four uas the highest altitude subpopulation of h8 in Huashan Valley. The subpopulation h1 and the subpopulations in Xian Valley clustered together. The result from the cluster analysis was in accordance with the actual distribution of *P. huashanica*.

3.4 Principal Components Analysis (PCA)

The former factors of Principal Components Analysis are main symbols that reflect the effect of sort order. The results in this study showed that the former three factors contained 55.99% information. Factor 1 contained 26.19% information (*x* axes), Factor 2 contained 19.08% (*y* axes), and Factor 3 contained 10.72% (*z* axes).

Most of *P. huashanica* species distributed the region of −2–1 at *x* axes (Fig. 4a), and higher altitude subpopulation h6, h7, and h8 focused on 1–3 region. *P. huashanica* plants in Huashan Valley and Xian Valley distributed the −1–2 region at *y* axes, while those in Huangpu Valley was in the −3 – −1 region, which displayed the special distribution of three populations. *P. huashanica* species in Xian Valley was in the −2 – −1 region (Fig. 4b), those in Huangpu Valley in the −1–1

Table 6 Analysis of genetic distance and genetic identity among 13 subpopulations of *P. huashanica* based on Nei's gene diversity index (Genetic distances below diagonal)

Spop	hp1	hp2	h1	h2	h3	h4	h5	h6	h7	h8	*x*1	*x*2	*x*3
hp1	****	0.945 1	0.776 5	0.859 2	0.853 0	0.871 0	0.866 8	0.820 9	0.829 7	0.748 2	0.818 9	0.848 7	0.828 5
hp2	0.056 4	****	0.778 3	0.883 9	0.878 8	0.882 7	0.873 8	0.847 7	0.856 7	0.758 6	0.848 9	0.870 1	0.839 2
h1	0.253 0	0.250 6	****	0.849 1	0.852 0	0.820 4	0.846 6	0.825 2	0.819 3	0.759 9	0.872 0	0.824 1	0.890 8
h2	0.151 8	0.123 4	0.1635	****	0.997 8	0.927 6	0.909 6	0.871 2	0.879 4	0.779 1	0.887 2	0.865 7	0.888 8
h3	0.159 0	0.129 2	0.160 2	0.002 2	****	0.925 6	0.906 7	0.867 9	0.876 0	0.782 1	0.887 9	0.867 9	0.887 0
h4	0.138 1	0.124 8	0.198 0	0.075 1	0.077 3	****	0.955 4	0.892 4	0.897 9	0.805 6	0.843 3	0.858 1	0.881 8
h5	0.142 9	0.134 9	0.166 5	0.094 7	0.098 0	0.045 6	****	0.912 9	0.919 6	0.815 5	0.871 0	0.858 5	0.897 7
h6	0.197 4	0.165 3	0.192 2	0.137 9	0.141 6	0.113 9	0.091 2	****	0.995 7	0.813 0	0.848 8	0.830 5	0.835 7
h7	0.186 7	0.154 6	0.199 3	0.128 6	0.132 4	0.107 8	0.083 8	0.004 3	****	0.811 3	0.849 2	0.833 0	0.837 3
h8	0.290 1	0.276 3	0.274 6	0.249 7	0.245 8	0.216 2	0.203 9	0.207 0	0.209 2	****	0.760 9	0.768 6	0.787 3
*x*1	0.199 8	0.163 8	0.137 0	0.119 7	0.118 9	0.170 4	0.138 1	0.164 0	0.163 5	0.273 2	****	0.880 7	0.890 5
*x*2	0.164 1	0.139 2	0.193 4	0.144 2	0.141 7	0.153 1	0.152 6	0.185 7	0.182 7	0.263 2	0.127 0	****	0.868 3
*x*3	0.188 2	0.175 3	0.115 6	0.117 9	0.120 0	0.125 8	0.107 9	0.179 5	0.177 6	0.239 1	0.116 0	0.141 3	****

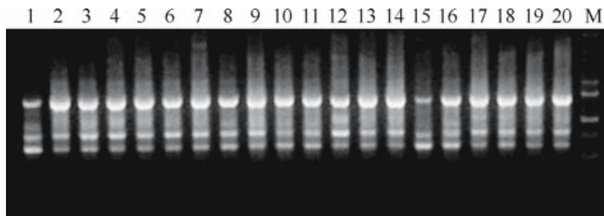


Fig. 2 Amplified products of subpopulation h8 of *P. huashanica* using primer BA0033

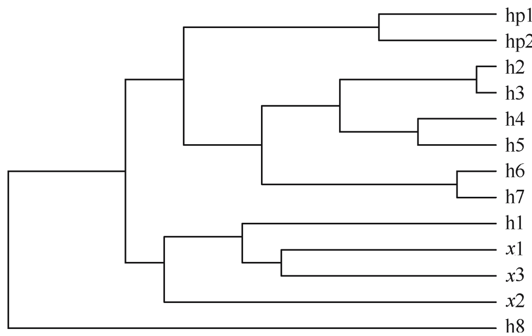
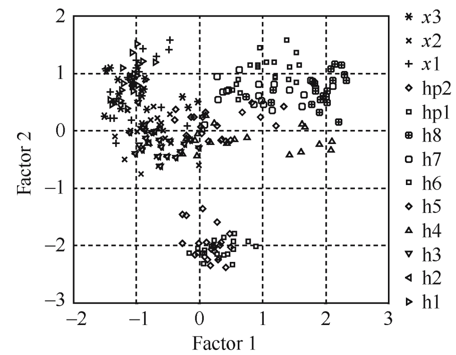


Fig. 3 Cluster analysis of 13 subpopulations of *P. huashanica* based on Nei's genetic distance



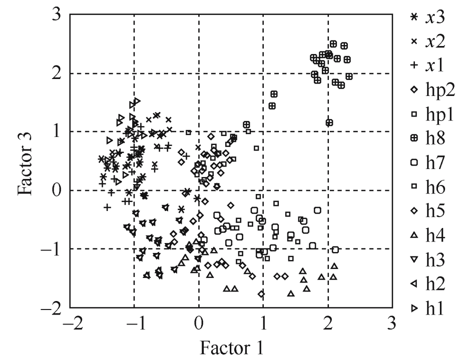


Fig. 4 The 2D[$x-y$ (a) and $x-z$ (b)] distribution of PCA analysis of total individuals of thirteen subpopulations of *P. huashanica*

region at *x* axes. Most of *P. huashanica* species in Huashan Valley were in the −2–0 region at *z* axes except subpopulation h8, while those in Xian Valley and Huangpu Valley were in the 0–2 region, which displayed the trend of differentiation along altitudes. The results from PCA were consistent with those from cluster analysis (Fig. 3).

3.5 Analysis of correlation between genetic diversity and altitude

The results of correlation analysis indicated that the *PPB*, the observed number of alleles (na), the effective number of alleles (ne), Nei gene diversity index (*h*), Shannon information index (*I*) had no significant correlation with altitude.

But there was significant correlation between their genetic distance and altitude distance. Pearson correlation coefficient was 0.504** ($P < 0.01$), which indicated that genetic distance and altitude had significant correlation.

4 Disscussion

4.1 Genetic diversity of *P. huashanica* natural populations

The total *PPB* of *P. huashanica* estimated by RAPD is 95.08%, the *PPB* in Huangpu Valley (60.66%) is lower than those of

the other two populations, while the *PPB* in Huashan Valley is the highest (95.08%). The genetic diversity is lower in Huangpu Valley population, which is different from the other two populations. A possible reason is that Huangpu valley has recently became a cable railroad for tourists to climb Huashan Mountain. Road building and human activity have gradually disrupted the habitat of *P. huashanica*, which resulted in the reduction of *P. huashanica* population density. The chance for gene flow between different subpopulations is limited since the seed spreading by wind cannot find appropriate environment for surviving. The increasing of *P. huashanica* individuals in Huangpu Valley mainly depends on vegetative propagation, leading to the decrease of genetic variability of *P. huashanica* population in Huangpu Valley. The *PPB* of *P. huashanica* (95.08%) is higher than the mean value of all populations (34%) (Hamrick et al., 1989), also higher than that of outbreeding plants (51%) (Ozenda, 1985). Previous studies show that the *PPB* of most of Gramineae species are higher than 51%, such as 81.4% in *Triticum dicoccoides* (Fahima et al., 1999), 63% in *Hordeum vulgare* (Fernández et al., 2002), 80.2% in *Agropyron mongolicum* (Xie et al., 2002), 96.1% in *Leymus chinensis* (Cui et al., 2001). This indicates that the reason for the rare *P. huashanica* is not the lack of genetic diversity. The data from allozymes analysis also shows a high level of genetic diversity of *P. huashanica* (Liu et al., 2001a). Furthermore, the *PPB* observed from RAPD is higher than that from allozymes, consistent with the research in *Triticum dicoccoides* (Fahima et al., 1999).

4.2 Genetic structure and differentiation of *P. huashanica*

The results indicate that genetic variations within the subpopulations (67.47%) are higher than those among the subpopulations (32.63%). The genetic variations vary with the populations in three valleys. Shannon information index (0.3262) and subpopulation differentiation (0.1006) are lowest in Huangpu Valley population and the highest in Huashan Valley population (0.4644 and 0.2986, respectively) in the three valleys. Huashan Valley is the biggest valley and its natural environments are relatively complicated among the three valleys. *P. huashanica* is widely distributed from its entrance (altitude: 460 m) to its peak (altitude: 2 160 m). As a result, the species has a high level of genetic variation compared to the subpopulations in the other two valleys.

Dendrograms clusters 13 subpopulations into four groups. The two subpopulations in Huangpu Valley form one group with larger genetic distance from the other two groups, showing that *P. huashanica* in this valley become a relatively independent population. The altitude difference between subpopulation h1 and the subpopulations in Xian Valley is not significant. *P. huashanica* at lower altitude distributes in the entrance of Huashan Valley conjoin those in Xian Valley, and they have same pollination phenology; the chance of gene flow between them is frequent. Therefore, subpopulation h1 and the subpopulations in Xian Valley cluster together. Except subpopulation h8, the six subpopulations in Huashan Valley cluster into one group which is further subdivided into lower altitude subpopulations (h2, h3, h4, h5) and higher altitude subpopulations(h6, h7). The difference in altitude may affect the genetic diversity of *P. huashanica* in Huashan Valley. Increase in altitude causes the decrease in temperature. Decreasing temperature postponed the phenology (Ozenda, 1985), that is, delayed bloom and condensed development. Based on the pollination phenology data observed in May 2002, the plant is at the maturation phase for the *P. huashanica* in Yuquanyuan in Huashan Valley (460 m), while the species is in florescence in Xiannvfeng (940 m) and Qingkeping (960 m), in the heading stage in Dongfeng (1 900 m) where the plant can get enough sunshine, and in the booting stage in Nanfeng (2 160 m). Flowering is delayed 2 to 3 days for every two hundred meters of increase in elevation. Phenological periods do not overlap between subpopulations in which the difference in elevation where they are distributed is greater than 500 m (Liu et al., 2001). The gene flow of *P. huashanica* natural populations is low ($Nm = 1.0322$), consistent with allozymes ($Nm = 2.77$) (Liu et al. 2001a), which is much lower than the Nm value (5.24) of anemophytes (Hamrick et al. 1995). This suggests that the gene flow of *P.huashanica* might be in a critical level, and genetic differentiation might occur in natural population of this species.

Acknowledgements This research was supported by the National Natural Science Foundation of China (No. 39770087) and Shaanxi Natural Science Foundation (No. 2001SM20).

References

Chen S Y, Zhang A J, Fu J (1991). Hybridization between wheat and *Psathyrostachys huashanica*. Acta Genetica Sinica, 18(6): 508–512 (in Chinese)

Chinese National Forest Bureau and Agriculture Ministry (1999). The list of Chinese urgently protected wild plants (The first group). Plant Journal, 5: 7 (in Chinese)

Cui J Z, Zu Y G, Zheng X D (2001). Genetic differentiation in *Leymus chinensis* populations revealed by RAPD markers: variation on the frequencies of amplified fragments. Bulletin of Botanical Research, 21(2): 272–277

Ding C B, Zhou Y H (1997). Fertilization and embryo development in hybridization between wheat and *Psathyrostachys huashanica*. J. Sichuan Agric.Univ., 15(1): 18–20 (in Chinese)

Fahima T, Sun G L, Beharav A, Krugman T, Beiles A (1999). Nevo E. RAPD polymorphism of wild emmer wheat populations, *Triticum dicoccoides*, in Israel. Theor. Appl. Genet., 98: 434–447

Fernández M E, Figueiras A M, Benito C (2002). The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with know origin. Theor. Appl. Genet., 104: 845–851

Hamrick J L, Godt M J W, Sherman Broyles S L (1995). Gene flow among plant populations: Evidence from genetic markers. In: Peter C H, Stephenson A G, eds. Experimental and molecular approaches to plant biosystematics. St. Louis: Missouri Botanical Garden Press, 215–232

Hamrick J L, Godt M J (1989). Allozyme diversity in plant species. In: Brown A H D, Clegg M T, Kaher A L, et al, eds. Plant population genetics, breeding and germplasm resources. Sunderland: Sinauer, 43–63

Hou W S, Zhang A J, Yang Q H, Fu J, Chen S Y (1997). Breeding and cytogenetic study of *Triticum aestivum-Psathyrostachys huashanica* alien substitution Lines. Acta Bot. Boreal.-Occid. Sin., 17(3): 368–373 (in Chinese)

Instituto Botanico Boreali-occidentali Academiae Sinicae (1976). Flora Tsinlingensis. Beijing: Chinese Scientific Press, 1(1): 99

Liu Z L, Li S, Yan G Q, Yan G Q, Song Y, Zhao G F (2001). Genetic structure and intraspecific genetic polymorphisms in natural populations of *Psathyrostachys huashanica*. Acta Genetica Sinica, 28(8): 769–777 (in Chinese)

Liu Z L, Song Y, Li S, Zhao G F (2001). Pollination phenology and an indirect estimate of gene flow in natural populations of *Psathyrostachys huashanica*. Acta Phytoecologica Sinica, 25(4): 426–430 (in Chinese)

Lu B R (1995). Diversity and conservation of the triticeae genetic resources. Chinese Biodiversity, 3(2): 63–68 (in Chinese)

Ozenda P (1985). La Végétation de la chaine alpine dans I'espace montagnard europeen. Masson, France

Sun G L, Yan J, Yang J L (1992). Production and cytogenetic study of intergenetic hybrid between *Triticum aestivum* and *Psathyrostachys huashanica.* Acta Genetica Sinica, 19(4): 322–326 (in Chinese)

Wang G L, Fang H J (1998). Plant Gene Engineering Principles and Techniques. Beijing: Science and Technology Press, 370–375 (in Chinese)

Xie X M, Yun J F (2002). RAPD analysis of genetic diversity of *Agropyron mongolicum*. Acta Bot. Boreal.-Occid. Sin., 22(1): 56–62 (in Chinese)

Yeh F C, Yang R C (1999). POPGENE v 1. 31. University of Albert and Center for International Research

Yue M, Zhang L J, Ma K, Zhao G F (2001). Endangering reason and reproductive strategy of *Psathyrostachys huashanica* population. Acta Ecologica Sinica, 21(8): 1314–1320 (in Chinese)

Zhang H Q, Zhou Y H, Zheng Y L, Yang R W, Ding C B (2002). Morphology and cytology of intergeneric hybrids between *Hystrix duthiei* ssp. *longearistata* and *Psathyrostachys huashanca* (Poaceae: Triticeae). Acta Phytotaxonomica Sinica, 40(5): 421–427 (in Chinese)

Zhao L F, Li S, Pan Y, Yan G Q, Zhao G Q (2001). Population differentiation of *Psathyrostachys huashanica* along an altitudinal gradient detected by random amplified polymorphic DNA. Acta Bot. Boreal.-Occid. Sin., 21(3): 391–400 (in Chinese)