African Journal of Biotechnology Vol. 8 (7), pp. 1226-1232, 6 April, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Genetic diversity of *Pogonatherum paniceum* (Lam.) Hack. in Southwest China revealed by AFLP markers

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Accepted 9 March, 2009

Amplified fragment length polymorphism (AFLP) markers were used to estimate the genetic diversity of *Pogonatherum paniceum* (Lam.) Hack. from Sichuan Province, Yunnan Province, Chongqing City and Guangxi Zhuang autonomous Region in China. 10 primer combinations were carried out on 180 different individuals sampled from 22 wild populations. Out of the 485 discernable DNA fragments generated, 441 were polymorphic. The percentage of polymorphic bands (PPB) was 90.93% at the species level. Observed number of alleles was 1.9093 ± 0.2875 and effective number of alleles was 1.4048 ± 0.3342. Nei's gene diversity and Shannon's Information index were 0.2494 ± 0.1657 and 0.3903 ± 0.2186, respectively. Nei's genetic distance among 22 populations ranged from 0.0996 to 0.6952. Genetic identity among populations ranged from 0.4990 to 0.9052, averaging 0.7387. Unweighted pair group method with arithmetic mean (UPGMA) cluster analysis based on Nei's genetic distance indicated that most populations were positioned into the relevant area. The above-mentioned results suggested the level of genetic variation of *P. paniceum paniceum* (Lam.) Hack. was low. The high score of PPB might be caused by low frequent polymorphism distributed in different populations. A significant correlation between genetic and geographic distances of populations was revealed based on Mantel test.

Key words: AFLP marker, differentiation, genetic diversity, *Pogonatherum paniceum* (Lam.) Hack., Southwest China.

INTRODUCTION

Pogonatherum paniceum (Lam.) Hack. is a rock plant, belonging to the *Pogonatherum* genus, which is classified in the family Poaceae.This perennial herbaceous species is distributed in many geographical regions including South China,Central China,Southwestern China Australia and Malaysia. It grows in diverse habitats. Most populations of this species are associated with surface of rocks. The perennial evergreen species is a promising species for gardens and soil conservation since it has a series of outstanding characters such as attractive appearance, strong root system, high density and high tolerance to environmental stresses. Previous studies were focused mainly on population traits (Chen and Wang, 2004; Chen et al., 2005; Peng et al., 2004; Wang et al., 2005) and physiology traits (Bai et al., 2005; Zhuo et al., 2006) of *P. paniceum* (Lam.) Hack., but little information exists on its genetic diversity and genetic variation. A clear understanding genetic diversity of natural populations is urgently required for establishing exploitation programs.

In recent years, a number of polymerase chain reaction (PCR)-based DNA markers had been widely used to investigate population genetic diversity. DNA markers are considered to be the most suitable means for estimating genetic diversity because of their abundant polymorphism and being independent of environment (Renganayaki et al., 2001). In the various molecular markers, the AFLP

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Abbreviations: AFLP, Amplified fragment length polymorphism; **PPB**, percentage of polymorphic bands; **PCR**, polymerase chain reaction; **UPGMA**, unweighted pair group method with arithmetic mean; **TFPGA**, tools for population genetic analysis; **CTAB**, cetyltrimethyl ammonium bromide.

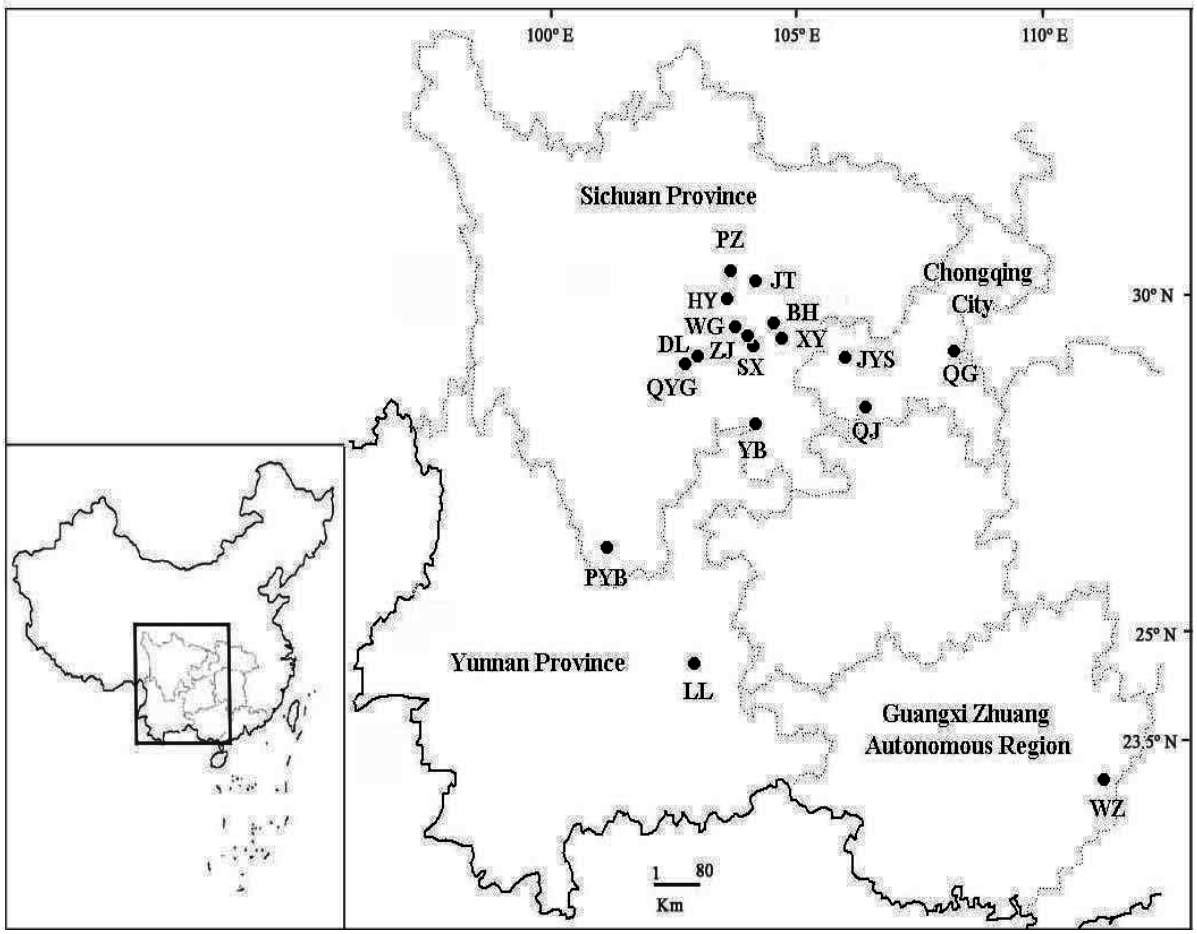

Figure 1. Map showing locations of the population of *Pogonatherum paniceum* (Lam.) Hack. sampled.

technique is a combination of the specificity of restriction enzyme analysis with the relative technical simplicity of the PCR (Vos et al., 1995). The advantages of AFLP, is that it has the capacity to inspect many more loci for polymorphisms than any other currently available technique based on PCR, it does not require previous knowledge of the species genome, is highly sensitive and requires small amounts of DNA. AFLP has been successfully to the study of genetic diversity in many plant species such as bermudagrass (Wu et al., 2006), jackfruit (Schnell et al., 2001), *Allium acuminatum* (Nathan et al., 2006), Texas bluegrass (Renganayaki et al., 2001), Faba bean (Zeid et al., 2003), *Caragana korshinskii* (Wang et al., 2007), *Cicer* (Nguyen et al., 2004), *pepino* (José et al.,2007), *Dimocarpus longan* (Lin et al., 2005), *Trillium tschonoskii* (Li et al., 2005) and *Hedysarum coronarium* (Marghali et al., 2005). However, the genetic diversity of *P. paniceum*(Lam.) Hack. has not been previously studied by AFLP. In this paper, we estimate the genetic diversity of natural populations of *P. paniceum* (Lam.) Hack. in Southwest China by AFLP analysis to provide a guide for effective management and selection of this wild germplasm resource.

MATERIALS AND METHODS

Sample collection

During July 2003 and May 2004, 15 individuals of *P. paniceum* (Lam.) Hack. were randomly chosen for sampling from each of 22 wild populations in the Southwestern China at elevations ranging from 50 to 1936 m in this study (Figure 1). Of these populations, 14 collected in Sichuan Province [PZ, JT, HY, XY, BH, ZJ, SX, WG (including HH, CJ and YD) YB ,DL ,QYG and PYB], 3 in Chongqing City (JYS, QG and QJ), 4 in Luliang county, Yunnan province (YTZG, HZG, YTG and DCB) and 1 in Guangxi Zhuang autonomous region (WZ). Most population are separated geographically by at least 10 km, except for ZJ and SX, which are 5.78 km apart (Table 1). The distance between sample plants within populations was at least 5 m, to increase the likelihood of sampling inter-individual variation within each population. Fresh leaves were collected and preserved in plastic zip-lock bags with silica and stored at -80°C prior to DNA extraction.

DNA extraction and AFLP analysis

DNA was extracted from the young leaves using the modified CTAB (Cetyltrimethyl Ammonium Bromide) method (Zou et al., 2001). After precipitation, DNA was suspended in 100 µL of TE buffer (pH 8.0). The DNA was quantified with an ultraviolet spectrophotometer

Table 1. Characteristics of *P. paniceum* (Lam.) Hack. samples used in this study.

Population	Origin	Altitude (m)	Latitude(N), Longitude(E)	The Types of Soil	Soil pH	Sample size(*N*)
WZ	WuzhouTown, Guangxi Province	50	22^{0}58'12" 111^{0}51'	Yellow earths	6.0	15
QG	BishanTown, Chongqing Province	283	29^{0}49'45.4" 106^{0}24'04.1"	Lateritic red earths	5.13	15
JYS	JinyunMountai, Chongqing Province	309	29^{0}26'25.0" 106^{0}12'12.7"	Yellow earths	4.97	15
QJ	QijiangTown, Chongqing Province	320	29^{0}01'52.9" 106^{0}38'49.3"	Yellow earths	6.46	15
XY	Xiaoyuan,ZiyangTown, SichuanProvince	387	30^{0}00'26.1" 104^{0}52'42.2"	Lateritic red earths	8.43	15
BH	Baohe,ZiyangTown, SichuanProvince	380	30^{0}12'05.1" 104^{0}45'06.8"	Lateritic red earths	8.43	15
PZ	PengzhouTown, SichuanProvince	450	30^{0}59'13.0" 103^{0}56'29.9"	Yellow earths	5.02	15
YB	YibinTown, SichuanProvince	600	28^{0}46'13.6" 104^{0}37"12.2'	Lateritic red earths	6.68	15
HY	Huayang,ShuangliuTown, Sichuan Province	475	30^{0}30'32.5" 104^{0}03'15.3"	Lateritic red earths	8.23	15
JT	Luanshitan ,JintangTown, Sichuan Province	412	30^{0}38'09.9" 104^{0}29'23.1"	Lateritic red earths	8.01	15
DL	Douliang, EmeiTown, SichuanProvince	456	29^{0}36'57.3" 103^{0}27'49.8"	Lateritic red earths	8.12	15
QYG	Qingyinge, EmeiMoutain, SichuanProvince	582	29^{0}35'19.6" 103^{0}24'08"	Lateritic red earths	8.12	15
SX	Sanxi, Renshou town, Sichuan province	393	30^{0}02'17.2" 104^{0}11'58.3"	Lateritic red earths	8.40	15
ZJ	Zhujia, RenshouTown Sichuan province	393	30^{0}02'17.2" 104^{0}11'58.3"	Lateritic red earths	8.43	15
HH	Honghu, renshoutown, Sichuan province	620	30^{0}13'08.6" 104^{0}07'02.8"	Lateritic red earths	8.65	15
CJ	Chuangjiang,RenshouTown SichuanProvince	719	30^{0}12'19.8" 104^{0}06'22.2"	Lateritic red earths	8.36	15
YD	Youding, RenshouTown, SichuanProvince	723	30^{0}12'21.0" 104^{0}06'23.4"	Lateritic red earths	8.45	15
PYB	Huiming, YanbianTown, Sichuan province	1200	26^{0}534'0.3" 101^{0}30'19.6"	Yellow earths	6.38	15
YTZG	Yutouzhonggou, LuliangTown, Yunna province	1912	25^{0}03'27.4" 103^{0}34'16.9"	Yellow-brown earths	5.72	15
DCB	Dongchongba,LuliangTown, Yunnan province	1920	25^{0}03'21.1" 103^{0}34'16.9"	Yellow-brown earths	4.42	15
HZG	Haizigou, LuliangTown, YunnanProvince	1928	25^{0}03'25.3" 103^{0}34'17.6"	Yellow-brown earths	5.29	15
YTG	Yangtougou,Luliang town, YunnanProvince	1936	25^{0}03'27.4" 103^{0}34'15.9"	Yellow-brown earths	4.59	15

and stored at – 20℃ until use.

The AFLP analysis was carried out using the GibcoBRL AFLP analysis system I (Gaithersburg, Md.). Restriction digests of genomic DNA with EcoRI and MseI were carried out at 37℃ for 6 h. Following heat inactivation of the restriction endonucleases, the genomic DNA fragments were ligated to EcoRI and MseI adapters overnight at 18℃ to generate template DNA for amplification. PCR was performed in two consecutive reactions. The template DNA

Table 2. AFLP bands obtained by selective amplification based on ten primer pairs.

Primer pairs codes	Sequence of primer*	No. of bands scored	No. of polymorphic bands
3143	E-AAC/M-CTA	53	46
3239	E-AAG/M-CAA	53	47
3241	E-AAG/M-CAG	39	32
3244	E-AAG/M-CTC	54	49
3246	E-AAG/M-CTT	41	36
3339	E-ACA/M-CAA	63	62
3340	E-ACA/M-CAC	48	46
3344	E-ACA/M-CTC	46	44
3741	E-AGC/M-CAG	50	45
3839	E-AGG/M-CAA	38	34
Average		48.5	44.1
Total		485	441

*E: 5′-GACTGCGTACCAATTC-3′ and M: 5′-GATGAGTCCTGAG TAA- 3′

generated was first preamplified using AFLP primers, none of which contained selective nucleotides. The PCR products of the pre-amplification reaction were then used as templates at a ratio 1:10 dilution for selective amplification using two AFLP primers, containing three selective nucleotides for two primers. The total number of the primer combinations in the analysis was 10 (at the selective amplification stage). The primer sequences are shown in Table 2. The EcoRI primers were used and it is not radioactively labeled. Instead, a modified silver staining method was used. The final PCR products were separated on a 6% denaturing polya-crylamide gel in 1× TBE buffer, followed by silver staining and photographing. AFLP amplified fragments were scored by eye.

Data analysis

Since AFLP markers are dominant, we assumed that each band represented the phenotype at a single biallelic locus (Williams et al., 1990). Amplified fragments were scored for presence (1) or absence (0) of homologous bands. The resulting presence/absence data matrix of the AFLP phenotypes were analyzed using POPGENE version 1.31 (Yeh et al., 1997) to estimate genetic diversity parameters, percentage of polymorphic bands (PPB), effective number of alleles per locus (Ne) and observed number of alleles per locus (Na). Nei's genetic identity (I) and genetic distance (D) between populations was computed using the same program. Genetic diversity was also estimated using Shannon's Information index (I) and Nei's gene diversity (H). To examine the genetic relationship among populations, cluster analysis was constructed by an unweighted paired group method of cluster analysis using arithmetic averages (UPGMA) of NTSYSpc version 2.02c (Rohlf, 1997) (Figure 2). In order to test for a correlation between genetic and geographical distances (in km) among populations, a Mantel test was performed using tools for population genetic analysis (TFPGA) (Miller, 1997), computing 1,000 permutations.

RESULTS

AFLP marker profile

AFLP analysis was performed on the 180 different indivi-duals sampling from 22 wild populations of *P. paniceum* (Lam.) Hack. using 10 primer combinations (Table 2). A total of 485 bands was scored and their molecular weights ranged from approximately 60 to 600 bp, of which 441(90.93%) were polymorphic and 44(9.07%) monomorphic across all samples examined that is, PPB for this species was 90.93%. The average number of bands detected per primer combination was 48.5, with 44.1 being polymorphic. Polymorphism varied remarkably among primer combination. The most infor-mative primer combination was the E-ACA/M-CAA pair (Figure 3), which produced the largest number of bands (63) and accounted for 12.99% of the total bands examined in the study.

Genetic diversity parameters

In the present study, observed number of alleles was 1.9093 ± 0.2875, effective number of alleles was 1.4048 ± 0.3342. Assuming Hardy-Weinberg equilibrium, Nei's gene diversity and Shannon's information index were 0.2494 ± 0.1657 and 0.3903 ± 0.2186, respectively. Nei's genetic identity and Nei's genetic distance are show in Table 3. The values of genetic identity range from 0.4990 to 0.9052 with a mean of 0.7387. Genetic identity between populations XY and PYB was the lowest (0.4990), and between populations YTG and DCB was the highest (0.9052). Nei's genetic distance between 22 populations ranged from 0.0996 (between populations YTG and DCB, both from Yunnan Province) to 0.6952 (between populations PYB and XY, both from Sichuan Province).

Cluster analysis

A dendrogram constructed by the UPGMA method based on genetic distances to show relationships among these

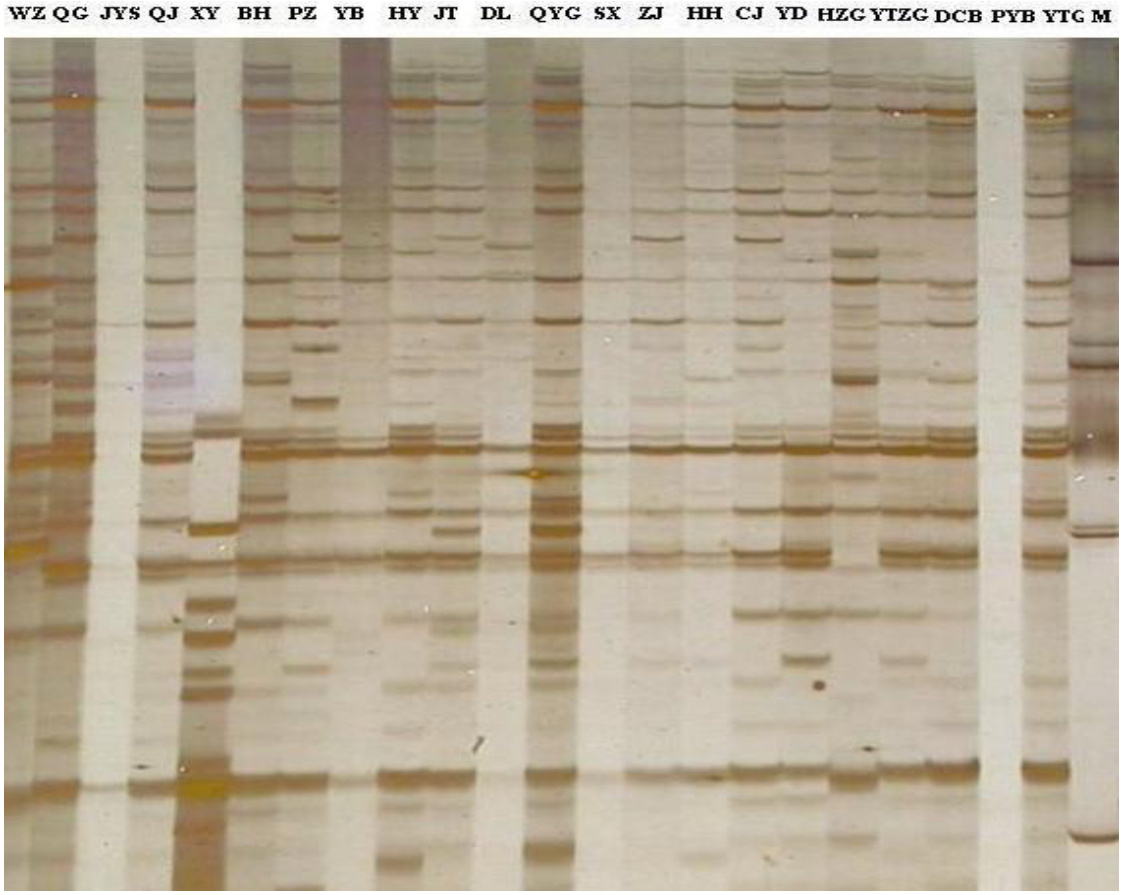

Figure 3. AFLP profiles of 22 populations of *P. paniceum* (Lam.) Hack. With primer combination 3339(based on polypropylene acyl gel-electrophoresis).

populations. Within this dendrogram, seven groups were identified when a transect line was placed at approximately 0.32 on the distance scale (Figure 2). Population YTG and population DCB were genetically most similar and population XY was the most distant from the other populations. Some correlation between geographic and genetic distances was found by the dendrogram. Most populations were cluster according to their distribution region. The cluster of populations from Yunnan Province was shown to have a closer relationship. But two populations (XY and PYB) did not cluster according the region as above, the reasons were to be studied further. The correlation coefficient (r) between genetic and geographical distance using Mantel test for all populations was 0.3408 (p = 0.072). Generally, the dendrogram of UPGMA cluster based on AFLP revealed a similar result that the genetic distances among populations show a spatial pattern corresponding to their geographic locations (Figure 1).

DISCUSSION

In the present study, we examined the genetic diversity of *P. paniceum* (Lam.) Hack. populations with AFLP technique. This result indicated that the PPB was 90.93%, which was higher than that of other monocotyledon turf grass species reported previously with this method [89.95% for Lolium (Ning et al., 2005). 60.8% for *Eremochloan ophiuroides* (Gou et al., 2002); 41% for *Eulaliopsis binata* (Yao et al., 2004)]. Though the PPB from *P. paniceum* (Lam.) Hack. was high, but it was not an appropriate coefficient to assess the level of genetic variation because it was depended on the size of population and the number of DNA markers used. On the contrary, as our research showed, both the average values of Nei's genetic diversity and Shannon index of diversity, two coefficients independent from the size of population, were very low. Shannon index of diversity was 0.390. Nei's gene diversity index showed the similar result, and the value of 0.2494 was low.

Therefore, we thought the above-mentioned results suggested the level of genetic variation of *P. paniceum* (Lam.) Hack. was low, and the high score of PPB might be caused by low-frequent polymorphism distributed in different populations. Under the assumption of Hardy-Weinberg equilibrium, the calculated value of effective number of alleles (1.40) was low, also suggested a contri-

Table 3. Nei's genetic distance (below diagonal) and Nei's genetic Identity (above diagonal) between 22 *P. paniceum* (Lam.) Hack. populations.

ID	WZ	QG	JYS	QJ	XY	BH	PZ	YB	HY	JT	DL	QYG	SX	ZJ	HH	CJ	YD	PYB	YTZG	DCB	HZG	YTG
WZ	***	0.71	0.64	0.69	0.52	0.64	0.63	0.63	0.64	0.61	0.61	0.64	0.65	0.62	0.63	0.63	0.62	0.68	0.62	0.62	0.59	0.61
QG	0.35	***	0.79	0.79	0.51	0.79	0.79	0.76	0.75	0.74	0.72	0.77	0.75	0.76	0.76	0.75	0.72	0.66	0.76	0.74	0.72	0.75
JYS	0.44	0.23	***	0.80	0.53	0.75	0.73	0.76	0.73	0.75	0.76	0.74	0.76	0.76	0.74	0.73	0.73	0.63	0.77	0.72	0.80	0.76
QJ	0.38	0.24	0.23	***	0.57	0.77	0.73	0.74	0.76	0.75	0.71	0.74	0.71	0.72	0.73	0.73	0.74	0.68	0.76	0.74	0.72	0.79
XY	0.65	0.67	0.63	0.56	***	0.56	0.54	0.54	0.55	0.54	0.54	0.53	0.57	0.55	0.53	0.54	0.56	0.50	0.52	0.52	0.51	0.53
BH	0.44	0.24	0.29	0.27	0.59	***	0.81	0.78	0.81	0.79	0.76	0.81	0.77	0.77	0.78	0.80	0.78	0.66	0.76	0.77	0.72	0.77
PZ	0.46	0.23	0.31	0.32	0.61	0.21	***	0.79	0.79	0.78	0.75	0.78	0.77	0.79	0.78	0.79	0.77	0.67	0.77	0.74	0.69	0.73
YB	0.46	0.28	0.27	0.30	0.62	0.26	0.23	***	0.81	0.81	0.80	0.79	0.82	0.82	0.82	0.79	0.78	0.69	0.80	0.76	0.78	0.76
HY	0.44	0.29	0.32	0.28	0.60	0.22	0.23	0.21	***	0.85	0.79	0.83	0.81	0.82	0.80	0.83	0.81	0.68	0.82	0.82	0.77	0.82
JT	0.49	0.31	0.29	0.28	0.62	0.24	0.26	0.21	0.166	***	0.81	0.84	0.80	0.83	0.83	0.82	0.82	0.68	0.83	0.80	0.76	0.80
DL	0.49	0.33	0.28	0.35	0.62	0.28	0.29	0.22	0.23	0.21	***	0.80	0.86	0.82	0.82	0.80	0.79	0.68	0.77	0.76	0.76	0.77
QYG	0.448	0.27	0.30	0.31	0.63	0.22	0.26	0.23	0.19	0.18	0.22	***	0.83	0.82	0.83	0.84	0.80	0.68	0.83	0.80	0.76	0.81
SX	0.43	0.29	0.27	0.35	0.57	0.26	0.27	0.20	0.21	0.22	0.15	0.19	***	0.85	0.85	0.84	0.81	0.68	0.79	0.77	0.77	0.77
ZJ	0.474	0.28	0.28	0.34	0.60	0.27	0.24	0.20	0.20	0.19	0.20	0.20	0.17	***	0.84	0.83	0.84	0.66	0.81	0.78	0.77	0.79
HH	0.46	0.28	0.30	0.32	0.64	0.25	0.25	0.20	0.22	0.18	0.20	0.19	0.16	0.17	***	0.83	0.83	0.70	0.81	0.76	0.76	0.78
CJ	0.46	0.29	0.32	0.31	0.61	0.23	0.23	0.23	0.19	0.20	0.22	0.17	0.18	0.18	0.19	***	0.85	0.69	0.82	0.80	0.76	0.80
YD	0.47	0.33	0.32	0.30	0.59	0.25	0.27	0.26	0.22	0.20	0.23	0.25	0.21	0.18	0.18	0.16	***	0.69	0.80	0.77	0.72	0.77
PYB	0.39	0.41	0.46	0.39	0.70	0.42	0.39	0.37	0.39	0.39	0.38	0.39	0.38	0.41	0.35	0.37	0.37	***	0.67	0.66	0.64	0.67
YTZG	0.48	0.28	0.27	0.27	0.65	0.28	0.26	0.25	0.20	0.19	0.26	0.19	0.24	0.21	0.22	0.20	0.23	0.40	***	0.85	0.83	0.85
DCB	0.47	0.30	0.33	0.30	0.65	0.27	0.30	0.27	0.20	0.23	0.27	0.22	0.27	0.25	0.28	0.21	0.26	0.42	0.16	***	0.83	0.91
HZG	0.53	0.33	0.23	0.32	0.67	0.34	0.37	0.25	0.27	0.28	0.27	0.28	0.26	0.26	0.28	0.28	0.32	0.44	0.19	0.19	***	0.84
YTG	0.49	0.28	0.28	0.24	0.64	0.26	0.31	0.28	0.20	0.22	0.27	0.22	0.27	0.24	0.25	0.23	0.27	0.40	0.17	0.09	0.18	***

bution of low-frequent mutations.

The maintenance of genetic diversity in a plant species is influenced by specific characteristics of the species (Hamrick and Godt, 1989), as well as by its evolutionary history of the species. *P. paniceum* (Lam.) Hack. is a widespread, long-lived, perennial herbaceous species and occurs in a rang of habitats, such as exposed ridges and slops, edge of farmland, and the bank of the rivers and streams even appeared in extreme growth conditions such as arid surface of bare rock. It can also distribute in different soil, including yellow-brown, yellow, purple soil and various sandy rocks, among which, soil pH value rang from 4.21 to 8.54. *P. paniceum* (Lam.) Hack. grows in different elevation from 50 to 2300 m. These factors reveal the adaptability of *P. paniceum* (Lam.) Hack. The combinations of these biological and ecological traits may enable the species to maintain a low-frequent polymorphism distributed in different populations.

In our study, in general, genetic variations were correlated with geographic distances among populations of *P. paniceum* (Lam.) Hack. For example, there were closer geographic distances (Figure 1) while their genetic distances were close among four populations from Luliang county, Yunnan province (Table 2), and there is the largest geographic distances (Figure 1). Between population WZ (from Wuzhou City,Guangxi Zhuang autonomous region) and other populations, accordingly, their genetic distance was larger (from 0.3493 to 0.0.6468). The rest of the populations also showed similar trend. Our results suggested that geographic distances had clear effect on genetic differentiation of this species except for two populations, XY (from Ziyang City, Sichuan) and PYB (from Yanbian county, Sichuan), the reasons were to be studied further.

A significant correlation between genetic and geographic distances of populations was revealed based on Mantel test. These results suggested that geographic isolation might not be one of reasons leading to low level of genetic diversity of *P. paniceum* (Lam.) Hack.

ACKNOWLEDGEMENTS

The authors are grateful to the Key Project of Science and Technology Research of Ministry of Education of China (No. 104255). The authors also acknowledge Prof. Yong Jiong Jia, Dr Ying Xu , Dr LinTang and Associate Prof. Fang Yan for excellent technical assistance. We are also grateful to Dr. Nianhui Zhang for valuable comments on the manuscript.

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