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Full Length Research Paper

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

Zeng-Bin Luo[1#], Guo-Qing Zhuang[2#], Dan-Wei Ma[3] and Fang Chen[1]*

[1]College of Life Science, Sichuan University, Chengdu 610064, China.
[2]Sichuan Academy of Forestry, Chengdu 610081, China.
[3]College of Life Science, Sichuan Normal University, Chengdu 610066, China.

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Inter-simple sequence repeats 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. 100 primers were carried out on 22 wild populations, 14 could produce highly reproducible inter-simple sequence repeats markers′ bands. Out of the 239 discernable DNA fragments, 227 were polymorphic. The percentage of polymorphic bands was 94.98% at the species level. Nei's gene diversity and Shannon information index were 0.312 and 0.471, respectively. This indicated that the genetic diversity of *P. paniceum* (Lam.) Hack. was low. The values of genetic identity ranged from 0.548 to 0.820 with a mean of 0.673. Nei's genetic distance between 22 populations ranged from 0.198 to 0.601. Unweighted pair group method with arithmetic mean cluster analysis based on Nei's genetic distance showed that most populations were positioned into the relevant areas. Significant correlation between genetic and geographic altitude distances among populations was found by Mantel test. The high score of percentage of polymorphic bands might be caused by low frequent polymorphism distributed in different populations.

Key words: *P. paniceum* (Lam.) Hack., ISSR, genetic diversity, southwest China.

INTRODUCTION

Pogonatherum paniceum (Lam.) Hack, one of the species of the *Pogonatherum* genus, has a series of outstanding characters such as evergreen, attractive appearance and strong root system. This perennial herbaceous specie is a promising species for gardens and soil conservation. It is distributed widely in geographical regions including South China, Central China, Southwestern China, Australia and Malaysia.

To date, previous studies of *P. paniceum* (Lam.) Hack. were focused mainly on population and physiological traits (Wang et al., 2005, 2007), but little information (Zhuang et al., 2009) about its genetic diversity and genetic variation is known, although such information is essential to the formulation of effective exploit on this wild germplasm resource. Since genetic diversity in natural populations can significantly affect the long-term survival and evolution of species or populations in changing environments (Futuyma, 1986), an accurate estimate on the genetic diversity levels and patterns of *P. paniceum* (Lam.) Hack. is essential to the formulation of effective management and selection of this wild germplasm resource and can only be addressed by detailed population genetic analyses (Hamrick and Godt, 1996a).

The present study was based on previous study by Zhuang et al. (2009). Thus in this study, inter-simple sequence repeat (ISSR) markers were employed to investigate the genetic diversity and differentiation in *P. paniceum* (Lam.) Hack. in southwestern and southern China. This microsatellite-derived marker system has established wide and successful applications in population genetic studies (Feyissa et al., 2007; Laura et al.,

*Corresponding author. E-mail: lifeisword@sina.com.cn. Tel/Fax: 86-28-8540-3278.

Abbreviations: ISSR, inter-simple sequence repeats; **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; **AFLP,** amplified fragment length polymorphism.

#These authors contributed equally to this paper

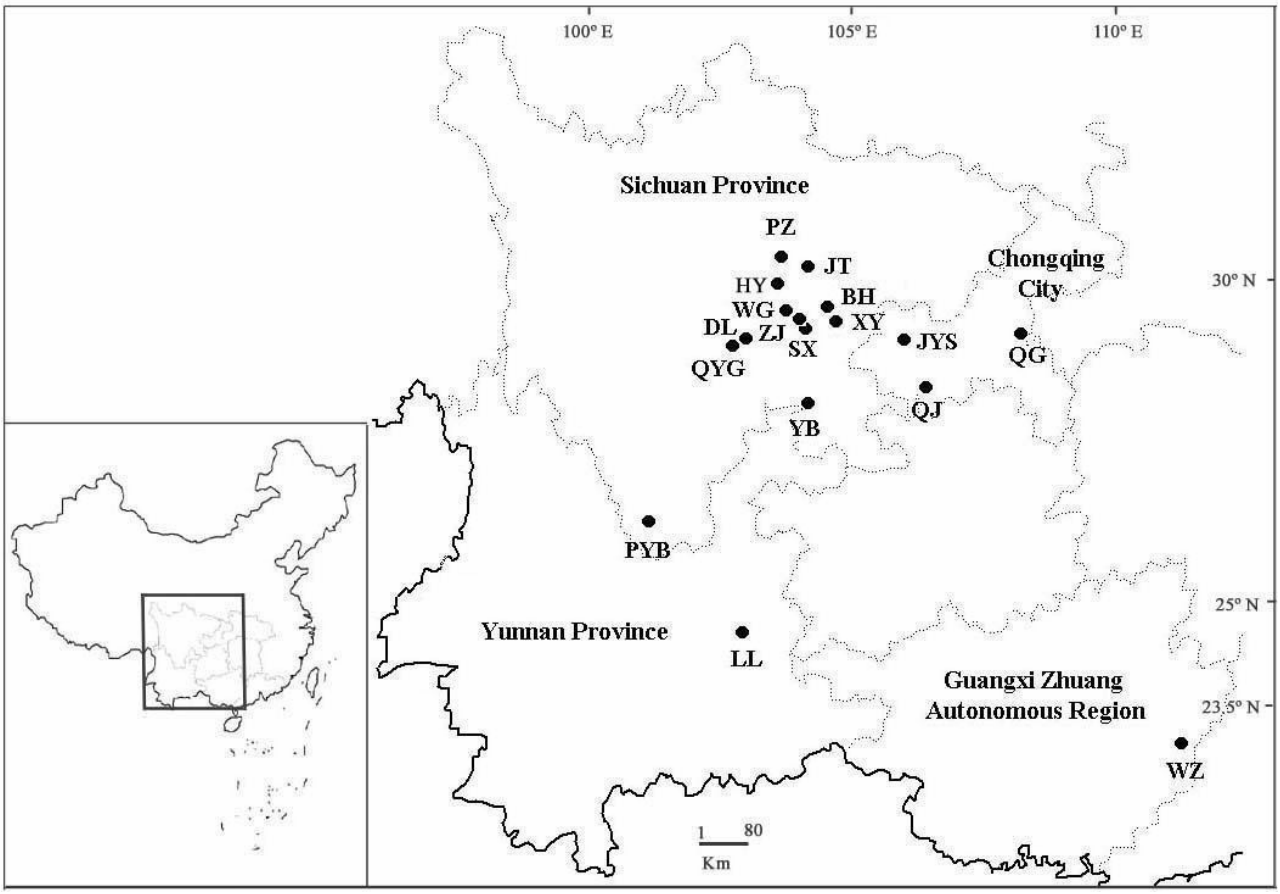

Figure 1. Map showing locations of the population of *P. paniceum* (Lam.) Hack. sampled.

2008). The objectives of this paper were to characterize the level of genetic diversity in this perennial herbaceous species, reveal the partitioning of genetic diversity within and among populations and discuss the possible implications for its conservation.

MATERIALS AND METHODS

Plant material

The fresh leaves used in this study were collected from each of 22 *P. Paniceum* (Lam.) Hack. populations, which were sampled in Zhuang et al. (2009) (Figure 1 and Table 1). Each individual was randomly sampled in populations in each location according to the probability sampling method (Luan et al., 2006). Fresh leaves were collected and preserved in plastic zip-lock bags with silica and stored at -80℃ prior to DNA extraction.

DNA extraction and ISSR-PCR amplification

DNA was extracted using the modified CTAB (cetyltrimethyl ammonium bromide) protocol (Doyle, 1991) and then dissolved in 0.1 × TE buffer (10 mM pH 8.0 Tris-HCl; 1 mM pH 8.0 EDTA) for subsequent use.

ISSR-PCR amplifications were performed in a PTC^{100TM} (MJ Research Inc., USA) and commenced with 5 min at 94℃, followed by 45 cycles of 30 s at 94℃, 45 s at 48℃ and 2 min at 72℃ and ended with 7 min at 72℃. One hundred primers (UBC primer set No. 9, Biotechnology Laboratory, University of British Columbia) were initially screened for PCR amplification. Fourteen ISSR primers that generated clear, reproducible banding patters were chosen for final analysis (Table 2). The 20 µl reaction mixture contained 25 ng genomic DNA templates, 2 µl 10 × PCR buffer (TaKaRa), 0.8 U Taq DNA polymerase, 0.25 mM dNTPs and 0.3 µmol/L primers. The amplification products were electrophoresed on a 2.0% agarose gels buffer with 0.5 × TBE at 120 V for 4 h along with a 100 bp DNA ladder, stained with ethidium bromide and photographed with a Gene Genius Bioimaging System. Molecular weights were estimated based on a 100 bp DNA ladder.

Statistical analysis

Since ISSR markers were dominantly inherited, each band was assumed to represent the phenotype at a single bialleliclocus (Williams et al., 1990). Only bright and discernible fragments across all the population samples were included in the statistical analysis. ISSR bands were scored as presence (1) or absence (0) characters, to construct the binary data matrix.

Assumption Hardy-Weinberg equilibrium, POPGENE1.32 (Yeh et al., 1997) was used to calculate various genetic diversity parameters which include the percentage of polymorphic bands (PPB), observed number of alleles (Na), effective number of alleles (Ne), Nei gene diversity (H) and Shannon's Information index (I). Nei's unbiased genetic identity (I) and genetic distance (D) between

Table 1. Characteristics of *P. paniceum* (Lam.) Hack. samples used in for ISSR analysis.

Origins	Pop. code	Altitude (m)	Latitude (N)	Longitude (E)	Soil pH
Wuzhou, Guangxi	WZ	50	23°28′59.8″	111°18′09.0″	6.00
Bishan, Chongqing	QG	283	29°49′45.4″	106°24′04.1″	5.13
Jinyun mountain, Chongqing	JYS	309	29°26′25.0″	106°12′12.7″	4.97
Qljiang, Chongqing	QJ	320	29°01′52.9″	106°38′49.3″	6.46
Ziyang, Sichuan	XY	387	30°00′26.1″	104°52′42.2″	8.43
Ziyang, Sichuan	BH	380	30°12′05.1″	104°45′06.8″	8.43
Pengzhou, Sichuan	PZ	450	30°59′13.0″	103°56′29.9″	5.02
Yibin, Sichuan	YB	600	28°46′13.6″	104°37′12.2″	6.68
Shuangliu, Sichuan	HY	475	30°30′32.5″	104°03′15.3″	8.23
Jintang, Sichuan	JT	412	30°38′09.9″	104°29′23.1″	8.01
Emei, Sichuan	DL	456	29°36′57.3″	103°27′49.8″	8.12
Emei, Sichuan	QYG	582	29°35′19.6″	103°24′08″	8.12
Renshou ,Sichuan	SX	390	30°02′14.2″	104°11′57.3″	8.40
Renshou ,Sichuan	ZJ	393	30°02′17.2″	104°11′58.3″	8.43
Renshou ,Sichuan	HH	620	30°13′08.6″	104°07′02.8″	8.65
Renshou ,Sichuan	CJ	719	30°12′19.8″	104°06′22.2″	8.36
Renshou ,Sichuan	YD	723	30°12′21.0″	104°06′23.4″	8.54
Yanbian, Sichuan	PYB	1200	26°53′21.0″	101°30′19.6″	6.38
Luliang, Yunnan	YTZG	1912	25°03′27.4″	103°34′16.9″	5.72
Luliang, Yunnan	DCB	1920	25°03′21.1″	103°34′16.9″	4.42
Luliang, Yunnan	HZG	1928	25°03′25.3″	103°34′17.6″	5.29
Luliang, Yunnan	YTG	1936	25°03′27.4″	103°34′15.9″	4.59

Table 2. Primers used for ISSR amplification.

Primer	Sequence of Primer 5′to 3′	No. of bands scored	No. of polymorphic bands
801	$(AG)_8T$	18	18
808	$(AG)_8C$	14	14
809	$(AG)_8G$	18	17
810	$(GA)_8T$	19	19
816	$(CA)_8T$	11	11
817	$(CA)_8A$	18	17
835	$(AG)_8YC$	21	20
856	$(AC)_8YA$	17	16
860	$(TG)_8RA$	16	15
861	$(ACC)_6$	16	13
887	$DVD(TC)_7$	19	17
888	$BDB(CA)_7$	24	23
891	$HVH(TG)_7$	13	12
900	ACT TCC CCA CAG GTT AAC ACA	15	15
Average		17.07	16.21
Total		239	227

B = C/G/T; D = A/G/T; H = A/C/T; R = A/G; V = A/C/G; Y = C/T.

populations were computed using the program (Nei, 1972). To examine the genetic relationship among populations, a dendrogram was also constructed by UPGMA (unweighted pair group method with arithmetic mean) method of cluster analysis using arithmetic averages of NTSYSpc version 2.02c (Rohlf, 1997).

The Mantel test was performed to reveal the correlation between the genetic (D) and geographic distances matrices using TFPGA (tools for population genetic analysis) software package (Miller, 1997). The range standardization was used to deal with the geographic distances (Pfeifer and Jetschke, 2006). Statistical significance

Table 3. Nei's Genetic Identity (above diagonal) and Genetic distance (below diagonal) of *Pogonatherum paniceum* in ISSR analysis.

	WZ	QG	JYS	QJ	XY	BH	PZ	YB	HY	JT	DL	QYG	SX	ZJ	HH	CJ	YD	PYB	YTZG	DCB	HZG	YTG
WZ	***	0.598	0.644	0.586	0.586	0.632	0.619	0.590	0.653	0.699	0.674	0.678	0.598	0.615	0.582	0.598	0.611	0.598	0.582	0.636	0.623	0.640
QG	0.514	***	0.736	0.720	0.678	0.649	0.695	0.649	0.703	0.640	0.674	0.670	0.674	0.682	0.690	0.640	0.611	0.590	0.707	0.678	0.649	0.623
JYS	0.440	0.306	***	0.724	0.741	0.661	0.699	0.611	0.674	0.653	0.653	0.682	0.670	0.619	0.594	0.653	0.623	0.603	0.628	0.682	0.611	0.644
QJ	0.535	0.329	0.323	***	0.707	0.695	0.699	0.695	0.757	0.670	0.644	0.657	0.678	0.670	0.619	0.653	0.548	0.619	0.661	0.649	0.653	0.552
XY	0.535	0.389	0.300	0.347	***	0.711	0.749	0.703	0.749	0.686	0.711	0.699	0.695	0.678	0.653	0.678	0.640	0.661	0.678	0.665	0.661	0.636
BH	0.459	0.433	0.414	0.365	0.341	***	0.695	0.716	0.770	0.699	0.690	0.653	0.716	0.674	0.690	0.699	0.619	0.623	0.632	0.670	0.640	0.665
PZ	0.479	0.365	0.359	0.359	0.289	0.365	***	0.703	0.749	0.736	0.728	0.665	0.686	0.711	0.644	0.619	0.615	0.603	0.670	0.674	0.653	0.619
YB	0.528	0.433	0.493	0.365	0.353	0.335	0.353	***	0.736	0.699	0.707	0.661	0.716	0.682	0.690	0.640	0.619	0.598	0.665	0.670	0.665	0.615
HY	0.427	0.353	0.395	0.278	0.289	0.262	0.289	0.306	***	0.745	0.803	0.724	0.720	0.728	0.678	0.695	0.649	0.644	0.686	0.682	0.695	0.661
JT	0.359	0.446	0.427	0.401	0.377	0.359	0.306	0.359	0.295	***	0.749	0.711	0.716	0.690	0.665	0.623	0.603	0.615	0.665	0.711	0.674	0.649
DL	0.395	0.395	0.427	0.440	0.341	0.371	0.317	0.347	0.219	0.289	***	0.787	0.682	0.690	0.690	0.690	0.703	0.674	0.649	0.661	0.665	0.707
QYG	0.389	0.401	0.383	0.420	0.359	0.427	0.408	0.414	0.323	0.341	0.240	***	0.703	0.745	0.703	0.720	0.699	0.661	0.670	0.665	0.653	0.653
SX	0.514	0.395	0.401	0.389	0.365	0.335	0.377	0.335	0.329	0.335	0.383	0.353	***	0.782	0.741	0.707	0.678	0.640	0.699	0.720	0.707	0.707
ZJ	0.486	0.383	0.479	0.401	0.389	0.395	0.341	0.383	0.317	0.371	0.371	0.295	0.245	***	0.741	0.665	0.653	0.649	0.690	0.703	0.716	0.649
HH	0.542	0.371	0.521	0.479	0.427	0.371	0.440	0.371	0.389	0.408	0.371	0.353	0.300	0.300	* * *	0.757	0.678	0.665	0.690	0.670	0.690	0.690
CJ	0.514	0.446	0.427	0.427	0.389	0.359	0.479	0.446	0.365	0.473	0.371	0.329	0.347	0.408	0.278	* **	0.745	0.640	0.657	0.670	0.632	0.682
YD	0.493	0.493	0.473	0.601	0.446	0.479	0.486	0.479	0.433	0.507	0.353	0.359	0.389	0.427	0.389	0.295	***	0.636	0.636	0.682	0.628	0.678
PYB	0.514	0.528	0.507	0.479	0.414	0.473	0.507	0.514	0.440	0.486	0.395	0.414	0.446	0.433	0.408	0.446	0.453	***	0.724	0.686	0.674	0.674
YTZG	0.542	0.347	0.466	0.414	0.389	0.459	0.401	0.408	0.377	0.408	0.433	0.401	0.359	0.371	0.371	0.420	0.453	0.323	***	0.787	0.766	0.690
DCB	0.453	0.389	0.383	0.433	0.408	0.401	0.395	0.401	0.383	0.341	0.414	0.408	0.329	0.353	0.401	0.401	0.383	0.377	0.240	****	0.820	0.787
HZG	0.473	0.433	0.493	0.427	0.414	0.446	0.427	0.408	0.365	0.395	0.408	0.427	0.347	0.335	0.371	0.459	0.466	0.395	0.267	0.198	***	0.766
YTG	0.446	0.473	0.440	0.594	0.453	0.408	0.479	0.486	0.414	0.433	0.347	0.427	0.347	0.433	0.371	0.383	0.389	0.395	0.371	0.240	0.267	***

of the Mantel test (Mantel, 1967) was determined by random per-mutation, with the number of permutation set to 1000. Meanwhile, the Mantel test was also applied to assess the correlation between the genetic and altitude distance.

RESULTS

ISSR polymorphism

The fourteen selected primers amplified with 239 different ISSR bands among 22 populations, was less than half of the AFLP bands (Zhuang et al., 2009). The bands per primer produced ranged from 14 to 24 and average band loci was 17.07 (Table 2). Their molecular weights ranged from approximately 450 to 2000 bp. Of these bands, 227 were polymorphic and 12 were monomorphic, that is, PPB for *P. paniceum* was 94.98%. This was beyond the PPB of AFLP which was 4.03% (Zhuang et al., 2009)

Genetic variation among populations

In the present study, observed number of alleles was 1.950 ± 0.1304, effective number of alleles were 1.530 ± 0.2739, both were similar as that of AFLP (Zhuang et al., 2009). Assuming Hardy–Weinberg equilibrium, Nei's gene diversity and Shannon's information index were 0.312 ± 0.1308 and 0.471 ± 0.1435, respectively, which were a few higher than that of AFLP (Zhuang et al., 2009). The values of genetic identity (I) ranged from 0.548 to 0.820 with a mean of 0.673 (Table 3), but when compared with the values of genetic identity by AFLP, both the range and mean of ISSR were less than that. Estimates of genetic distance (Nei's measure) between pairs of popu-

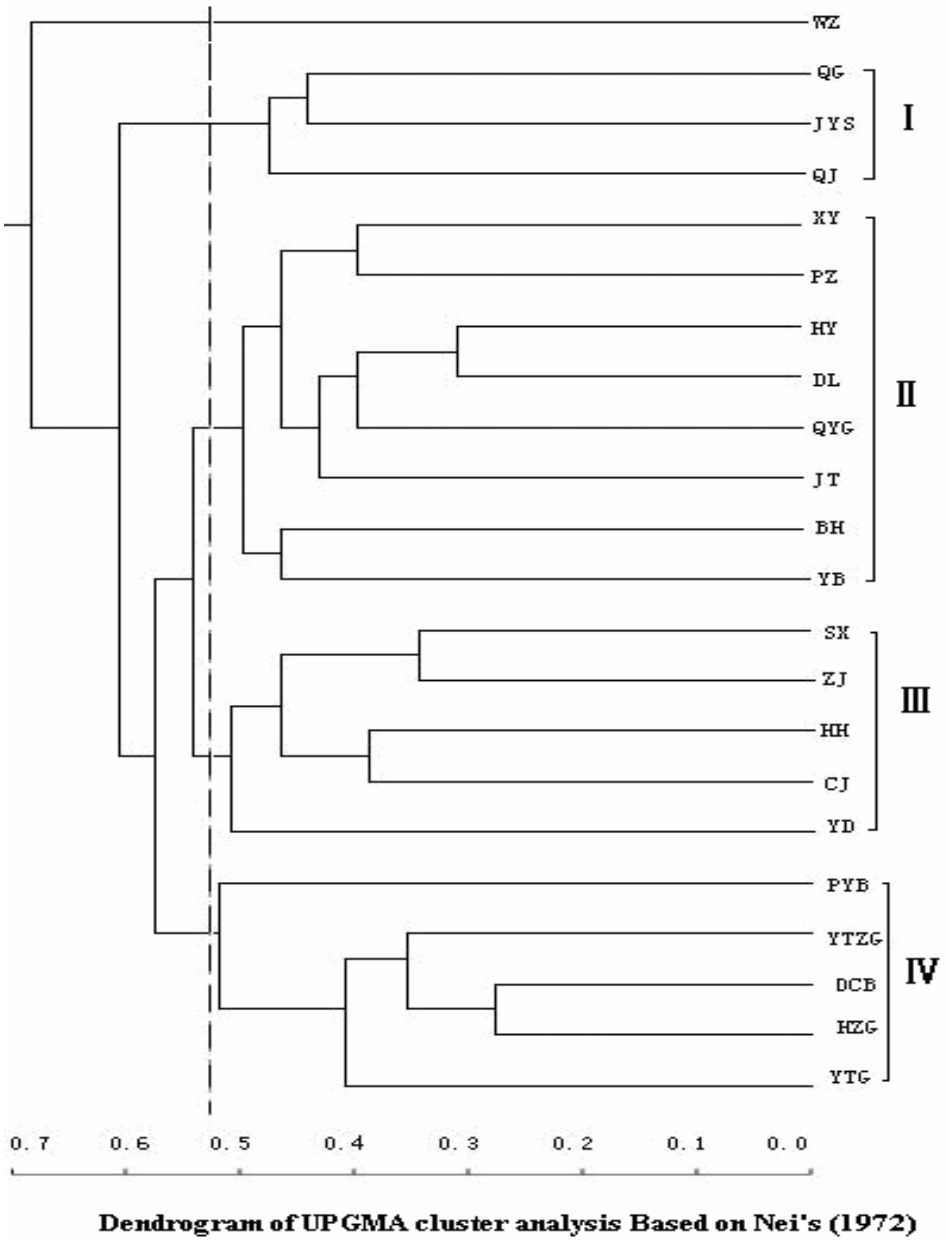

Figure 2. Genetic distance among populations of *P. panium* (Lam.) Hack.

lations were calculated based on 239 markers scored. Nei's genetic distance between populations ranged from 0.198 (between populations DCB and HZG) to 0.601 (between populations QJ and YD) (Table 3) which was more widely ranged by AFLP. This indicated a closer relationship between populations DCB and HZG. In general, population WZ is the most distant from the other populations (Table 3).

Genetic relationship

In order to represent the relationships among populations, cluster analysis (UPGMA) was used to generate a dendrogram based on Nei's genetic distances between populations (Figure 2). In this dendrogram, all populations form a distinct cluster. Four main groups were identified, less than that in AFLP analysis (seven groups) (Zhuang et al., 2009), when transect line was placed at approximately 0.52 on the distance scale (Figure 2). In both analyses of ISSR and AFLP, population WZ fell outside the main clusters and group I comprised populations QG, JYS and QJ from Chongqing. In ISSR analyses, Group II, III and IV were different with that of AFLP. In ISSR analyses, Group II included eight populations from Sichuan. All populations (five) of Group III came

Table 4. The genetic variation among groups of *Pogonatherum paniceum* by ISSR analysis.

Group	Population code	PPB	H	I
I	QG, JYS, QJ	41.00	0.150	0.224
II	XY, BH, PZ, YB, HY, JT, DL, QYG	68.20	0.225	0.341
III	SX, ZJ, HH, CJ, YD	59.83	0.201	0.304
IV	PYB, YTZG, DCB, HZG, YTG	53.97	0.167	0.259

PPB = the percentage of polymorphic bands; H = Nei (1973) gene diversity; I = Shannon's Information index.

from Renshou County, Sichuan. Group IV possessed five populations which came from Luliang County, Yunnan except PYB population (Table 4).

Genetic differentiation

The dendrogram generally showed the clear correlation among genetic and geographic and altitude distances. To assess overall relationship between genetic and geographic distances, the Mantel test analyses were completed. The correlation coefficient (r) among genetic and geographic and altitude distances using Mantel test for all populations were 0.5155 (p = 0.001) and 0.3188 (p = 0.005), respectively. The correlation coefficient (r) between genetic and geographic distances was more obvious than that of AFLP. The dendrogram of UPGMA cluster based on ISSR revealed a similar result that the genetic distances among populations showed a spatial pattern corresponding to their geographic locations (Figure 1). Similarly, the genetic correlated well with altitude distributions of populations studied.

Genetic variations between groups

Nei's genetic distance between groups I and II, III, IV was 0.147, 0.206 and 0.195, respectively. Between groups II and III, IV was 0.102 and 0.110, respectively, while between groups III and IV was 0.103. This showed a close relationship between groups II and III. The highest level of variation was detected within group II, within which 68.20% of detected bands were polymorphic. The PPB of Groups III and IV was 59.83 and 53.97%, respectively. Whereas, the least varied group I contained 41.00% polymorphic bands. The Nei's gene diversity of groups II was 0.225, very similar to that (H = 0.201) of group III and the values of Shannon's information index (I) also showed an identical trend (Table 4). Both distant form groups I and IV (Table 4). This suggested that genetic diversity among the different group areas were different and low.

Between the groups, the genetic differentiation was 0.4879 among populations. This indicated that 48.79% of the total genetic variation existed among the groups studied and the level of gene flow (Nm) was estimated to be 0.525.

DISCUSSION

On the basis of previous study (Zhuang et al., 2009), a further survey on the genetic diversity of 22 natural populations of *P. paniceum* (Lam.) Hack. from Southwest China by ISSR analysis was made. The result indicated that both AFLP and ISSR analyses could get a high PPB among 22 natural populations of *P. paniceum* (Lam.) Hack.. In ISSR analysis, the PPB was 94.98%, which was higher than that in AFLP analysis (Zhuang et al., 2009; Cao et al., 2006) and RAPD analysis (Bai et al., 2002; Yao et al., 2004; Ning et al., 2005). Similar interpretations of genetic diversity of populations of *P. paniceum* (Lam.) Hack. were revealed by analyses by ISSR and AFLP using different parameters (Nei's gene diversity, Shannon's information index and genetic distance). Though the PPB from *P. paniceum* (Lam.) Hack. in ISSR analysis was high, Nei's genetic distance between populations by AFLP analysis was wider than that in ISSR survey and in genetic cluster. Only four main groups were identified in ISSR analysis, which was less than that revealed by AFLP. The PPB was not an appropriate coefficient to assess the level of genetic variation because it depended on the size of the population and the number of DNA markers used. On the contrary, the present research showed both the average values of Nei's genetic diversity and Shannon index of diversity. Two coefficients independent of the size of population, were very low as revealed by AFLP, were 0.312 and 0.471, respectively. Under the assumption of Hardy-Weinberg equilibrium, the calculated value of effective number of alleles (1.530) in ISSR was also low, which suggested a contribution of low-frequent mutations.

Therefore, both the results revealed by ISSR and AFLP suggested that the level of genetic variation of *P. paniceum* (Lam.) Hack. in the Southwest China was low and the low-frequent polymorphism distributed in different populations might be the reason for the high score of PPB.

Specific characteristics and evolutionary history of plant would influence the maintenance of genetic diversity (Zhou et al., 2010). *P. paniceum* (Lam.) Hack. is a widespread long lived, perennial herbaceous species and occurs in a range of habitats. It could even appear in extreme growth conditions such as arid surface of bare rock. These biological and ecological traits may induce

the species to maintain a low-frequent polymorphism in different populations.

By AFLP and ISSR analyses, a significant correlation between genetic and geographic distances of populations was revealed based on Mantel test. 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 and Table 1) while their genetic distances (from 0.198 between populations DCB and HZG to 0.371 between populations YTZG and YTG) were close among four populations from Luliang County, Yunnan Province. These were the largest geographic distances (Figure 1 and Table 1) between population WZ (from Wuzhou City, Guangxi Zhuang Autonomous Region) and other populations. Accordingly, their genetic distance was larger (from 0.359 to 0.542). The rest of the populations also showed similar trend. In general, genetic variations were correlated with geographic distances. These results revealed by ISSR suggested again that geographic isolation might not be one of the reasons leading to low level genetic diversity of *P. paniceum* (Lam.) Hack. This result was the same as that revealed by AFLP. The reasons leading to low level of genetic diversity of *P. paniceum* (Lam.) Hack. however, needs further research.

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