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Analysis of genetic diversity among Chinese wild *Vitis* species revealed with SSR and SRAP markers

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ABSTRACT. The genetic diversity among 80 *Vitis* materials including 62 indigenous accessions of 17 wild *Vitis* species in China and 7 interspecific hybrids, 10 *V. vinifera* L. cultivars, and 1 *V. riparia* Michaux were evaluated by simple sequence repeat and sequence-related amplified polymorphism markers. A total of 10 simple sequence repeat primers and 11 sequence-related amplified polymorphism primer combinations were amplified, and 260 bands were generated, of which 252 were polymorphic with an average polymorphism rate of 97.02%. Genetic relationships among the different *Vitis* species indicated that *V. ficifolia* and *V. yeshanensis* could be considered a separate species. As for the 4 major ecogeographic regions of Chinese wild *Vitis* species, the genetic diversities of Chinese wild *Vitis* species from the Qinling Mountain region (H = 0.1947, I = 0.3067) and the mid-downstream

Yangtze River region ($H = 0.1834$, $I = 0.2925$) were higher, with results suggesting that these regions may be one of the major centers of *Vitis* origin. An understanding of the genetic diversity of these Chinese wild *Vitis* species could provide the theoretical foundation for further protection and reasonable utilization in grape breeding.

Key words: Chinese wild grape; *Vitis*; Genetic diversity; SSR; SRAP

INTRODUCTION

Grape (*Vitis vinifera* L.) is one of the most important fruit crop in the world, and grows in South Europe, Asia Minor, East Asia, and North and Central America (Wan et al., 2008). There are more than 70 *Vitis* species, mainly distributed in the Northern Hemisphere (Ercisli et al., 2008).

China is one of the major germplasm gene centers of *Vitis* species origination. More than 35 *Vitis* species have their origin in China (Wan et al., 2008). Chinese wild *Vitis* species have many excellent characteristics, including 1) high-quality wine-making attributes, such as high sugar content and moderate acidity (Li and He, 2000); 2) a high level of resistance to fungal diseases, including powdery mildew [*Uncinula necator* (Schw.) Burr.], anthracnose [*Elsinoë ampelina* (de Barry) Shear], ripe rot [*Glomerella cingulata* (Ston.) Spauld and Schrenk], and crown gall [*Agrobacterium tumefaciens* Smith and Townsend] (He et al., 1991; Wang et al., 1995, 2007); 3) highly efficient photosynthesis in *V. quinquangularis* (Zhu et al., 1994); and 4) the facts that Chinese wild *Vitis* can be easily crossed with American *Vitis* species, and the undesirable "foxy" flavor compounds in the berries of American *Vitis* species are nonexistent in Chinese wild *Vitis* species (He, 1999b; Wan et al., 2008).

Up to now, selection for the desirable characteristics of Chinese wild grape has enlarged the scope of applications for grape breeding. This has resulted in many intermediate and transitional types of *Vitis*, which cause difficulties in germplasm research of the wild Chinese *Vitis*. Moreover, the classification of a few Chinese *Vitis* species adopted by Chinese taxonomists has led to some controversy and confusion, and further studies are necessary to classify and elucidate the evolutionary relationships between the various *Vitis* species. In previous studies, the taxonomy and germplasm identification of some wild *Vitis* species natives to China have been completed based on morphological and isoenzyme approaches (He et al., 1996; Niu and He, 1996; Ma and He, 1998). However, the morphological characteristics and isoenzymes of grapes are easily affected by the developmental period and the environment (Luo et al., 2001). DNA molecular marker technologies are available for the analysis of genetic diversity at the DNA level. A few studies applying random amplified polymorphic DNA (RAPD), sequence-related amplified polymorphism (SRAP), and start codon targeted techniques have reported the use of DNA markers to classify and analyze the genetic relationships between and within Chinese wild grape, and foreign cultivars (Luo et al., 2001; Guo et al., 2012a,b). So far, there are no reports to assess the genetic diversity and relationships for more than 18 Chinese wild *Vitis* species by combination of simple sequence repeat (SSR) and SRAP markers. Two technologies, SSR and SRAP, are recognized as being the most used molecular markers because they are simple, reliable, and effective (Guo et al., 2012b).

In this study, we combined SSR and SRAP markers to analyze the genetic diversity and relationships among wild *Vitis* species native to China, America, and European cultivars from different ecogeographic regions in China. Our studies will facilitate the efficient evalua-

tion, conservation, management, and utilization of Chinese wild grape germplasm resources.

MATERIAL AND METHODS

Plant materials

A total of 80 *Vitis* materials including 62 clones of 17 wild *Vitis* species native to China, and 7 interspecific hybrids, 10 European cultivars, and 1 *V. riparia* Michaux were collected from the orchard of grape germplasm resources, Northwest A&F University, Yangling, Shaanxi, China. The 62 wild grapes were from 4 ecogeographic regions in China. Information for the 80 *Vitis* materials is listed in Table 1. Young healthy leaves from plants of each accession were collected and stored under -80°C for later DNA extraction.

Table 1. *Vitis* materials used in this study.

Species	Pop. code	Code No.	Clones	Origin	Species	Pop. code	Code No.	Clones	Origin
V. pseudoreticulata W.T. Wang	A	1	Baihe-13-1	R4			41	Liu-9	
		2	Guangxi-2				42	Baishui-40	
V. amurensis Rupr.	B	3	Zuoshan-75079	R1	*V. davidii* (Roman) Foex	I	43	Tangwei	R3
		4	Taishan-11				44	Fujian-4	
		5	Zuoshan-2				45	Xuefeng	
		6	Zuoshan-1				46	Lueyang-4	
		7	Heilongjiang♂				47	Ningqiang-6	
		8	Tonghua-3		*V. adstricta* Hance	J	48	Taishan-1 ♂	R3
		9	Shuangyou				49	Taishan-2	
		10	Zuoshan-76097				50	Taishan-1	
		11	Zuoshan-12				51	Anlin-3	
V. baihensis L.X. Niu	C	12	Baihe-40	R3			52	Anlin-2 ♂	
V. bashanica P.C. He	D	13	Baihe-41	R2	*V. hancockii* Hance	K	53	Jiangxi-3	R3
V. liubaensis L.X. Niu	E	14	Langao-2	R3			54	Jiangxi-3 ♂	
		15	Liu-10		*V. qinlingensis* P.C. He	L	55	Pingli-5	R2
V. quinquangularis Rehd.	F	16	Weinan-3	R2			56	Lueyang-4	
		17	83-4-94 ♀		*V. yeshanensis* J.X. Chen	M	57	Yanshan-1	R2
		18	83-4-96 ♀		*V. ficifolia* Bunge.	N	58	Sangye	R2
		19	Shang-24		*V.* spp (Qiufuyie)	O	59	Meixian-6	R1
		20	83-4-49 ♀				60	Liu-7	
		21	83-4-49 ♂		*V. riparia* Michaux	P	61	Hean-3	US
		22	Dan-2		Interspecific hybrid (*V. binifera* x *V. labrusca*)	Q	62	Tengnian	
		23	Huaxian-11				63	Early-Takasumi	
V. romanetii Roman.	G	24	Jiangxi-2	R3			64	Jingya	
		25	Pingli-2				65	Kyoho	
		26	Pingli-2 ♂				66	Jingyou	
		27	Jiangxi-1(green)♂				67	Hutai-8	
		28	Jiangxi-1(green) ♀				68	Campbell Early	
		29	Jiangxi-2(red) ♂		*V. vinifera* L.	R	69	Merlot	
		30	Liuba-11				70	White Riesling	
		31	Jiangxi-1(red) ♀				71	Chardonnay	
		32	Jiangxi-2(red) ♀				72	Early-Rose	
		33	Pingli-7				73	Italian Riesling	
		34	Baihe-22				74	Jingxiu	
		35	Liuba-1				75	Christmas Rose	
V. piasekii Maxim	H	36	Nanzheng-2	R2			76	Carignane	
		37	Gansu-91 ♂				77	Pinot Noir	
		38	Gansu-91 ♀				78	Princes seedless	
		39	Liu-8		*Vitis* sp	S	79	Bianye	R4
		40	Liu-6		Unknown	T	80	Wanxian-15	R3

R1, R2, R3, R4 = 4 major ecogeographic regions of Chinese wild grape distribution. R1 = Changbaishan and Xiaoxing'anling Mountain regions; R2 = Qinling Mountain region; R3 = Yangtze River region; R4 = Guangxi region.

DNA extraction

Genomic DNA was extracted from fresh leaves using a modification of the hexadecyltrimethylammonium bromide method described by Qu et al. (1996), and the quality was tested by 0.8% (w/v) agarose gel electrophoresis. DNA concentrations were determined with a UV-VIS spectrophotometer, and samples were diluted to 40 ng/μL and then stored at -20°C for PCR amplification.

PCR amplification and electrophoresis

A total of 10 SSR primers and 11 SRAP primer combinations were synthesized by Beijing Aoke Biological Technology and Service Co. Ltd. The SSR and SRAP primer sequences are listed in Table 2. For SSR analysis, each 20-μL amplification reaction system consisted of 20 ng template DNA, 10X PCR buffer (100 mM Tris-HCl, pH 8.3, and 500 mM KCl), 0.20 mM each dNTP, 0.75 mM each primer, 2.0 mM $MgCl_2$, and 1 U *Taq* DNA polymerase. The PCR amplification was conducted under the following conditions: initial 5 min at 94°C; followed by 35 cycles of 1 min at 94°C, annealing at appropriate temperature for 1 min, 90 s extension at 72°C; and a final extension of 8 min at 72°C. The PCR amplification of SRAP markers was carried out in a 20-μL volume, containing 20 ng template DNA, 10X PCR buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 0.18 mM each dNTP, 0.75 mM each primer, 1.87 mM $MgCl_2$, and 1 U *Taq* DNA polymerase. The PCRs were performed as follows: 5 min of denaturing at 94°C followed by 5 cycles of 3 steps: 1 min of denaturing at 94°C, 1 min of annealing at 35°C, and 2 min of elongation at 72°C. In the following 30 cycles, the annealing temperature was increased to 50°C, with a final elongation step of 5 min at 72°C.

The PCR products were separated on 6% denatured polyacrylamide gels and detected by silver staining. Then, clearly and reproducibly distinguished bands were recorded and used in the following analysis. The DL 2000 DNA ladder (TaKaRa Biotechnology Dalian Co., Ltd., China) was used as DNA markers.

Data analyses

After silver staining, each SRAP and SSR fragment was scored as present (1) or absent (0) for each of the 80 DNA samples, excluding the weak and blurred bands, thus generating a binary data matrix. The binary data matrix was analyzed using the NTSYS-pc version 2.1e software package (Rohlf, 2000). The pairwise genetic distances among all accessions, according to Nei (1978), were calculated based on the Jaccard similarity coefficient. Cluster analyses (SSR + SRAP) were performed using the unweighted pair-group method with arithmetic average (UPGMA). The dendrogram was constructed using the NTSYS-pc version 2.1 software package and MEGA version 4.0 (Tamura et al., 2007). The principal coordinate analysis (PCoA) was performed using the DCENTER and EIGEN programs in the NTSYS-pc version 2.1 software package. Finally, the observed number of alleles (N_A), effective number of alleles (N_E), Nei's gene diversity index (H), and Shannon information index (I) were calculated using POPGENE version 1.32 (Yeh et al., 1999).

RESULTS

Polymorphism analysis

A total of 36 SSR primer pairs and 168 SRAP primer combinations were used to screen for polymorphism between 5 accessions from different species. Among them, 10 SSR primer pairs and 11 SRAP primer combinations amplified clear and abundant fragments, and were used to evaluate the genetic diversity of 80 *Vitis* accessions.

A total of 260 bands ranging from 200 to 2000 bp were scored with an average of 12.38 bands per primer, of which 252 (97.02%) were polymorphic. Each primer generated 5 (UDV-060) to 25 (Me12 + Em8) bands, and the percentage of polymorphic bands produced by each primer ranged from 85.71 to 100%. The results of the SSR and SRAP analysis are listed in Table 2.

Table 2. Characterization of SSR and SRAP primers used in this study and their polymorphism and diversity analysis.

Primer	*t*	*p*	P%	Primer sequence
VVMD15	15	15	100.00	F: CTGCAGTGCACTCAAAGTTGG; R: TGAAACACCAAGGGAAACCTC
VVMD19	11	10	90.91	F: TGAAATATCATCAATGCTCTCTCTCC; R: GGTTGATATTGCTTCCTTTTCCC
UDV-046	15	13	86.67	F: CGTCATGGCTTCTGCTCAT; R: TGATACCACAGTCTGCTGATTTTT
UDV-048	9	9	100.00	F: CACTTGTGTGTGCAGTCCT; R: CTTTCCTCACCGAACACTC
UDV-050	8	7	87.50	F: TAATGGCCCCTTACAACACC; R: AGCTTCACTGCCAAAGGATG
UDV-060	5	5	100.00	F: CCTGCCACACCACAATACAA; R: TGGGGTAAAACTGGGTGTTT
UDV-067	10	10	100.00	F: TCATGGACTCACATCCTCAAA; R: TGAGTGGATGAAGGACAGTTC
UDV-088	11	11	100.00	F: CCATGCACACACGCACAT; R: CCACCAAACAAGTGGAGGTT
VMC9a2.1	10	10	100.00	F: AGCTCGGCTAGCTGCAAAATC; R: ACCCTTCCCTCTTCAAAACCC
VMC7h3	11	11	100.00	F: TCAGATATTGAAGAACACCACA; R: ACTAGAAAATGCACAATCTCCC
Me1+Em3	13	13	100.00	F: TGA GTC CAA ACC GGATA; R: GAC TGC GTA CGA ATT GAC
Me2+Em5	9	9	100.00	F: TGA GTC CAA ACC GGAGC; R: GAC TGC GTA CGA ATT AAC
Me2+Em7	16	15	93.75	F: TGA GTC CAA ACC GGAGC; R: GAC TGC GTA CGA ATT CAA
Me2+Em3	22	22	100.00	F: TGA GTC CAA ACC GGAGC; R: GAC TGC GTA CGA ATT GAC
Me3+Em5	14	12	85.71	F: TGA GTC CAA ACC GGAAT; R: GAC TGC GTA CGA ATT AAC
Me3+Em3	10	10	100.00	F: TGA GTC CAA ACC GGAAT; R: GAC TGC GTA CGA ATT GAC
Me8+Em14	13	13	100.00	F: TGA GTC CAA ACC GGACT; R: GAC TGC GTA CGA ATT CTT
Me8+Em19	10	10	100.00	F: TGA GTC CAA ACC GGACT; R: GACTGCGTACG AATT TCA
Me12+Em8	25	25	100.00	F: TGA GTC CAA ACC GGAGA; R: GAC TGC GTA CGA ATT CAC
Me12+Em19	14	13	92.86	F: TGA GTC CAA ACC GGAGA; R: GACTGCGTACG AATT TCA
Me13+Em19	9	9	100.00	F: TGA GTC CAA ACC GGAAG; R: GACTGCGTACG AATT TCA
Total	260	252	2037.4	
Average	12.38	12	97.02	

t = number of total loci; *p* = number of polymorphic loci; P% = percentage of polymorphic loci.

Genetic diversity analysis

The N_A, N_E, *H*, and *I* values were estimated for different *Vitis* species (Table 3). For accessions numbers less than 1, the parameters were zero. For the other 13 species, N_A ranged from 1.1158 (O) to 1.5483 (B), with an average value of 1.3009. N_E varied from 1.1158 (O) to 1.2991 (B), with an average value of 1.2021. *H* ranged from 0.0579 (O) to 0.1804 (B), with an average value of 0.1144. *I* varied from 0.0803 (O) to 0.2752 (B), with an average value of 0.1686. The maximum *H* (0.1804) and *I* (0.2752) values among the different species in B indicated that it was genetically more diverse than the other species.

For the 4 major ecogeographic regions, the *H* and *I* values increased from R4 to R1, R3, and R2 (Table 4), this trend showing that the genetic diversity of the 4 regions varied.

Table 3. Parameter of genetic diversity among different *Vitis* species.

Pop. code	Acc. No.	N_A	N_E	*H*	*I*
A	2	1.1390	1.1390	0.0695	0.0963
B	9	1.5483	1.2991	0.1804	0.2752
C	1				
D	1				
E	2	1.1737	1.1737	0.0869	0.1204
F	8	1.4093	1.2495	0.1464	0.2193
G	12	1.3822	1.1684	0.1075	0.1698
H	7	1.3282	1.1992	0.1182	0.1773
I	5	1.3089	1.2013	0.1186	0.1759
J	5	1.3514	1.2400	0.1389	0.2044
K	2	1.2162	1.2162	0.1081	0.1499
L	2	1.1969	1.1969	0.0985	0.1365
M	1				
N	1				
O	2	1.1158	1.1158	0.0579	0.0803
P	1				
Q	7	1.3205	1.1953	0.1157	0.1734
R	10	1.4208	1.2330	0.1404	0.2135
S	1				
T	1				

N_A = observed number of alleles; N_E = effective number of alleles; *H* = Nei's gene diversity; *I* = Shannon information index. The parameters of accession numbers less than 1 were zero.

Table 4. Parameter of genetic diversity of 4 major ecogeographic regions for Chinese wild *Vitis* species.

Origin	N_A	N_E	*H*	*I*
R1	1.5714	1.2986	0.1805	0.2769
R2	1.7220	1.3119	0.1947	0.3067
R3	1.7490	1.2884	0.1834	0.2925
R4	1.1395	1.1310	0.1165	0.1559

N_A = observed number of alleles; N_E = effective number of alleles; *H* = Nei's gene diversity; *I* = Shannon information index.

According to the Nei analysis of gene diversity, genetic differentiation and gene flow existed for different *Vitis* species. The values of genetic differentiation and gene flow were estimated to be 0.6570 and 0.2610, respectively.

Cluster analysis

The UPGMA dendrogram obtained from the SSR and SRAP data is shown in Figure 1. UPGMA grouped the 80 *Vitis* accessions into 3 main clusters (Figure 2). Cluster 1 comprised a single species (*V. quinquangularis*). Cluster 2 comprised 55 *Vitis* accessions that were further divided into 6 subclusters. Within Cluster 2, the first subcluster consisted of a single species (*V. pseudoreticulata*). The second subcluster also contained a single species (*V. amurensis*). The third subcluster consisted of *V. yeshanensis*, *Vitis* spp (Qiufuyie), *V. piasekii*, *V. bashanica*, *V. liubaensis*, *V. romanetii*, and *V. baihensis*. The fourth subcluster contained *V. davidii* and *V. riparia*. The fifth subcluster comprised a single species (*V. adstricta*). The sixth subcluster contained *Vitia* sp, *V. qinlingensis*, *V. hancockii*, and *V. ficifolia*. Cluster 3 was composed of 18 accessions, and all American and European cultivars were in this cluster, along with Chinese wild *Vitis* accession Wanxian-15.

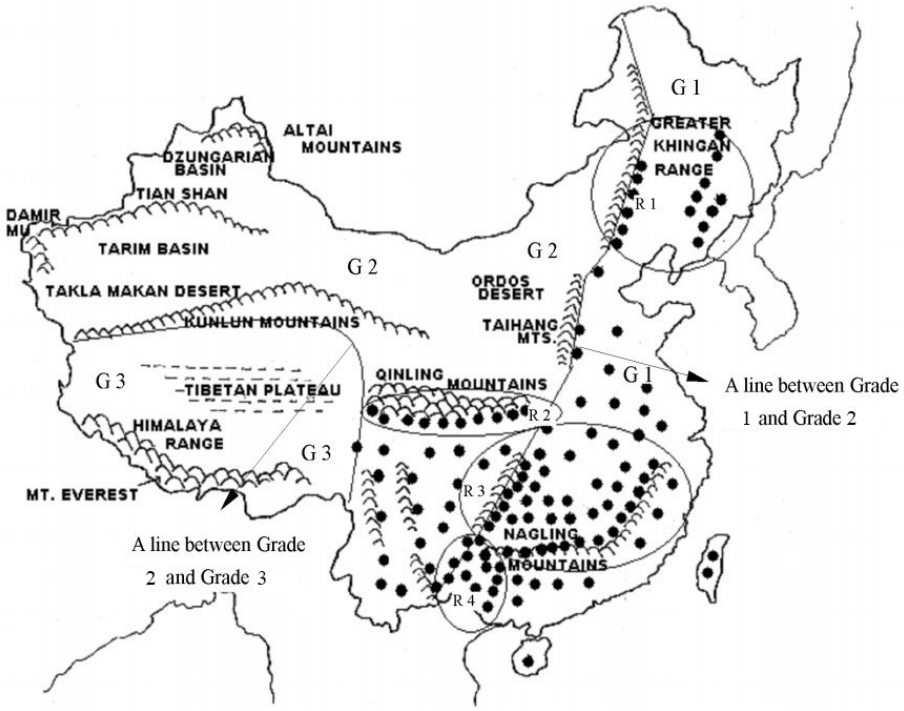

Figure 1. Map showing wild grapes distributed in China (from Wan et al., 2008). R1, R2, R3, R4: 4 major eco-geographic regions of Chinese wild grape distribution. R1 = Changbaishan and Xiaoxing'anling Mountain regions; R2 = Qinling Mountain region; R3 = Yangtze River region; R4 = Guangxi region. Circles or ellipses in the map representing the 4 major ecogeographic regions of Chinese wild grapes are mainly distributed.

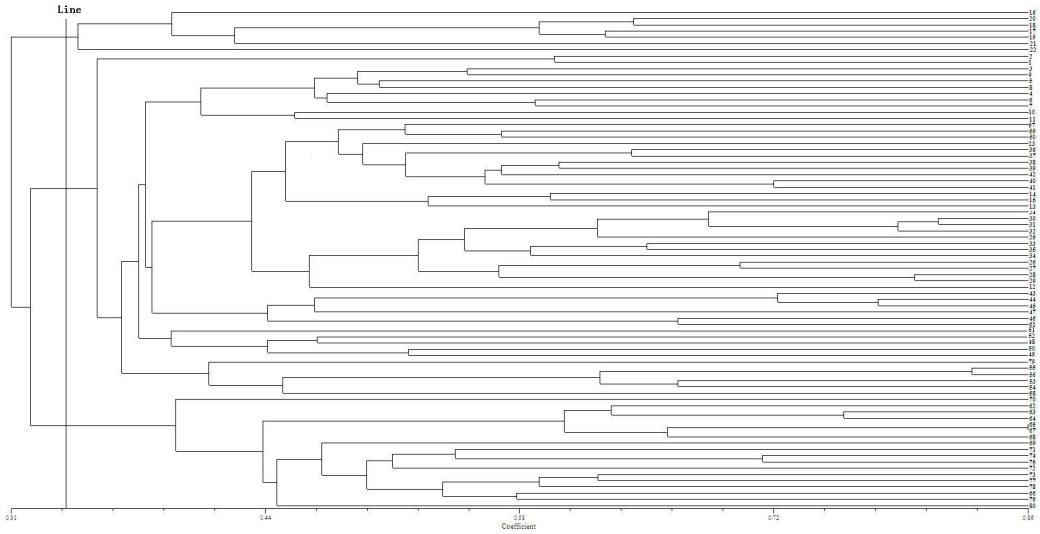

Figure 2. Unweighted pair-group method with arithmetic average dendrogram of the 80 *Vitis* constructed using the genetic similarity analysis based on SSR and SRAP analysis.

Principal coordinate analysis

PCoA further helped in describing the variability among these accessions in a 2-dimensional model. PCoA data based on the genetic similarity matrix are shown in Figure 3. The PCoA analysis indicated that the first and the second principal components accounted for 19.01 and 14.18% of the total variation (Figure 3), respectively. The classification of all accessions derived from PCoA was similar to the result of the UPGMA analysis.

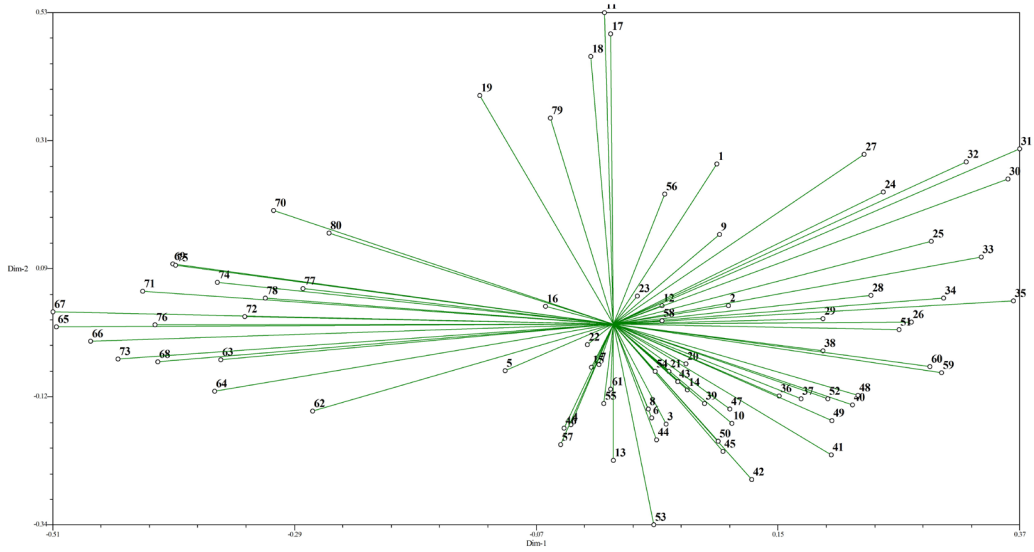

Figure 3. Relationships among the 80 *Vitis* by principal coordinate analysis based on SSR and SRAP data.

Analysis of Nei's genetic identity and genetic distance among different species

The Nei genetic identity and the genetic distance among different species were analyzed using the POPGENE software (data not shown). Based on the genetic distance, the dendrogram of 20 species was constructed using the MEGA software (Figure 4). The genetic identity ranged from 0.6988 to 0.9563. The highest genetic identity was between *V. davidii* and *V. qinlingensis*, and the lowest was between *V. bashanica* and *V. ficifolia*. The genetic distance ranged from 0.0447 to 0.3639. Among Chinese wild *Vitis* species, the largest genetic distance was found between *V. ficifolia* and the other wild species.

DISCUSSION

SSR and SRAP markers

DNA markers are powerful and reliable tools for evaluating the genetic diversity of plants. SSR and SRAP are efficient molecular marker systems that have been used to assess

genetic diversity in different plants (Brini et al., 2008; Erfani et al., 2012; Guo et al., 2012b). SSR and SRAP markers amplify the different parts of the genome. SSR marker amplification targets are the simple sequence repeats, which include microsatellite DNA. However, SRAP marker amplification targets are located in the open reading frame, which includes the intron(s) and promoter region. Therefore, SRAP markers could better reflect the diversity of the plants. SSR and SRAP markers have been successfully applied to some wild and cultivated plants; however, for more than 18 Chinese wild *Vitis* species, the application of the SSR and SRAP combined markers has been scarce. In this study, SSR and SRAP markers were used to evaluate the genetic diversity among 18 Chinese wild grape and varieties from America and Europe. The average polymorphic band amplified by each primer was 12.38, and the percentage of polymorphic loci reached 97.02%. This result is highly comparable to the one described by Luo et al. (2001), who reported a 68.7% polymorphism level in 83 similar wild Chinese *Vitis* clones or accessions using RAPD markers. Polymorphism levels also suggest that SSR and SRAP combined markers is an effective tool for identifying genetic diversities of wild *Vitis* species native to China, and to separate Chinese wild *Vitis* from American and European cultivars.

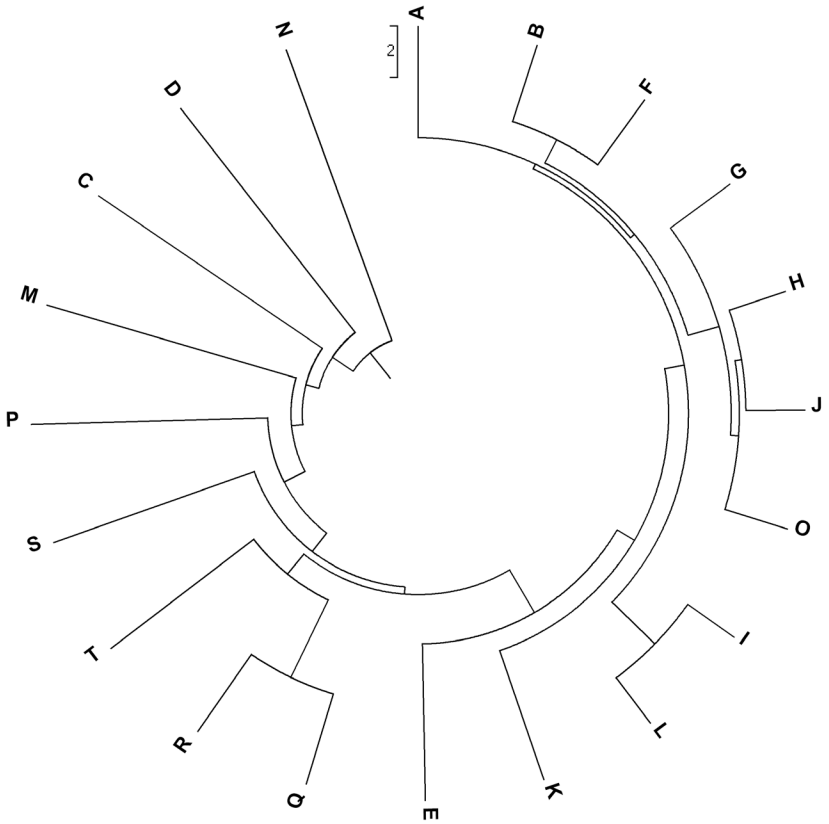

Figure 4. Dendrogram showing genetic relationships among grape species from 4 major ecogeographic regions by the MEGA software. The species numbers are the same as those listed in Table 1.

Genetic diversity and cluster analysis

The abundant genetic diversity of Chinese wild *Vitis* species in different ecogeographic regions has resulted from many causes, including climatic, ecologic, and geographic factors, and cultivation history. Chinese wild *Vitis* species are found predominantly in the Qinling Mountains and the Bashan Mountains, and the Jiangxi, Hubei, and Hunan Provinces have relatively high diversity, with over 30 species found in these areas (Wan et al., 2008). In this study, the genetic diversities of Chinese wild *Vitis* species from R2 (H = 0.1947, I = 0.3067) and R3 (H = 0.1834, I = 0.2769) were higher. These results are consistent with those of Wan et al. (2008). The ecogeographic regions R2 and R3 comprised the Shaanxi, Henan, Gansu, Hunan, Jiangxi, Zhejiang, Hubei, Anhui, Jiangsu, and some of Shanghai Provinces. The temperature, annual rainfall, and soil are suitable for grape growth, and thus these regions have a higher *Vitis* diversity (He, 1999b; Kong, 2004).

Genetic similarity values among all the 80 grape accessions ranged from 0.31 to 0.86, which showed a high genetic diversity among these *Vitis* accessions. The classification of a few Chinese *Vitis* species adopted by Chinese taxonomists has some controversy and confusion. Whether *V. ficifolia* is a subspecies of *V. quinquangularis* or a separate species is still being disputed (Kong, 2004). In this study, the largest genetic distance was found between *V. ficifolia* and the other wild species, and the relationship between *V. ficifolia* and *V. quinquangularis* was distant (Figure 4); therefore, our result agrees with that of Wang (1979) in that *V. ficifolia* could be considered a separate species. Through morphological trait analysis, He et al. (1996) found that a close relationship existed between *V. yeshanensis* and *V. amurensis*, and *V. yeshanensis* was considered to be a variety of *V. amurensis* (Lu and Liang, 1993). However, the leaf and shoot morphologies as well as drought tolerance and cold hardiness of *V. yeshanensis* are different from those of *V. amurensis*, suggesting that it may be a separate species (Niu and He, 1996; He, 1999a). In the present study, a large distance was found between *V. yeshanensis* and *V. amurensis* (Figure 4), supporting the view that *V. yeshanensis* is a separate species.

In this study, SSR and SRAP markers classified studied accessions into different species (Figure 2); for example, Huaxian-11 was clustered into *V. piasekii*. The reasons were that interspecific hybridization and gene introgression had probably been involved in the evolution and conservation of the various species (Bell and Hough, 1986). This was also confirmed by the gene flow (N_m = 0.2610) among different *Vitis* species.

Theoretical significance for Chinese wild *Vitis* germplasm and breeding

The evaluation and conservation of wild grape germplasms are important for grape breeding programs. Chinese wild *Vitis* species have been used in breeding program. For example, *V. amurensis*, *V. quinquangularis*, and *V. davidii* are the most widely used wild *Vitis* species for breeding in China, the United States, and Germany (He, 1999a). Large genetic variations could provide the opportunity to select and breed new varieties with adaptability to different environments (Hummer and Sugar, 1998). According to the previous and present results, there is a high degree of genetic diversity in Chinese wild *Vitis* species. Results indicated that parent materials of grape breeding could be selected from the Chinese wild grape species mentioned in this paper, and the genetic distance of parents should be considered first, whereas geographic distance is not important. Furthermore, it is necessary to reinforce the collection and protection

of wild grape germplasm resources from ecogeographic regions with higher diversity, such as the Qinling Mountains region and the mid-downstream Yangtze River region.

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