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Genetic Diversity and Population Structure Analysis of Chinese Wild Grape Using Simple Sequence Repeat Markers

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Abstract. **Chinese wild *Vitis* is a useful gene source for resistance to biotic and abiotic stresses, although there is little research on its genetic diversity and structure. In this study, nine simple sequence repeat (SSR) markers were used to assess the genetic diversity and genetic structure among 100 *Vitis* materials. These materials included 77 indigenous accessions representing 23 of 38 wild *Vitis* species/cultivars in China, 18 *V. vinifera* cultivars, and the five North American species *V. aestivalis*, *V. girdiana*, *V. monticola*, *V. acerifolia*, and *V. riparia*. The SSR loci used in this study for establishing an international database (*Vitis* International Variety Catalogue) revealed a total of 186 alleles in 100 *Vitis* accessions. The mean values for the gene diversity (GD) and polymorphism information content (PIC) per locus were 0.91 and 0.90, respectively, which indicates that the discriminatory power of the markers is high. Based on the genetic distance data, the 100 *Vitis* accessions were divided into five primary clusters by cluster analysis, and five populations by structure analysis; these results indicate these Chinese wild grapes were more genetically close to European grapes than to North American species. In addition, the clustering patterns of most accessions correlated with the geographic distribution. An analysis of molecular variance (AMOVA) revealed that 3.28%, 3.27%, and 93.46% of the variance occurred between populations, between individuals within populations, and between individuals within the entire population, respectively. In addition, we identified three previously undescribed accessions (Wuzhi-1, MZL-5, and MZL-6) by cluster analysis. Our results reveal a high level of genetic diversity and variability in *Vitis* from China, which will be helpful in the use of genetic resources in future breeding programs. In addition, our study demonstrates that SSR markers are highly suitable for further genetic diversity analyses of Chinese wild grapes.**

Grapes (*Vitis* sp.) are one of the most economically valuable horticultural crops in the world. They are widely used to produce wine, table grapes, raisins, juice, and health care products (Ren and Wen, 2007; Wan et al., 2013; Zhou et al., 2017). The genus, which includes more than 70 *Vitis* species, consists of two subgenera—*Vitis* and *Muscadinia*—in which more than 38 *Vitis* species originated from China (Liu et al., 2012; Wan et al., 2008). Currently, most cultivated grapes in China originated from Europe, and grape commercial breeding populations in China share a narrow genetic base as a result of their common origins from a number of popular cultivars, such as Muscat Hamburg and Kyoho. However, China, as one of the major gene centers of *Vitis* species, has abundant wild grape species that occur naturally in all the provinces except Xinjiang, and the number of wild grape species per province ranges from 1 to 29 (Jiang et al., 2015). Chinese wild grapes have strong abiotic stress tolerance and could be widely used for modern grape breeding (Liu et al., 2014; Wan et al., 2013). There is still a great interest among grape breeders in broadening the genetic base of cultivated grapes and also in tapping into the gene pool of the wild relatives to enhance stress resistance.

Traditionally, the methods used for assessing the genetic diversity and relationships among wild grape species have relied mostly on morphology, palynology, and isoenzymes (Chao and Niu, 1981; He and Chao, 1982; Wan et al., 2008). However, these methods are easily affected by environmental conditions and developmental stages (Luo et al., 2001). Fortunately, DNA molecular marker techniques are able to overcome these limitations and act as powerful tools for evaluating genetic diversity and relationships (Guan et al., 2019; Queiroz et al., 2015; Riaz et al., 2018). Therefore, a variety of molecular markers, such as random amplified polymorphic DNA (Luo et al., 2001)], intersimple sequence repeat (Jing and Wang, 2013; Zhang et al., 2011)], sequence-related amplified polymorphism (SRAP) (Liu et al., 2013), and SSR (Jing et al., 2013; Liu et al., 2012)], have been used recently to examine the genetic diversity and relationships among wild Chinese grape species. Klein et al. (2018) also used high-throughput sequencing to clarify evolutionary relationships among North American *Vitis* species. Because SSR markers show high levels of polymorphisms, reproducibility, and codominant inheritance (Aradhya et al., 2013; Li et al., 2017), they have been widely used to study

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the genetic diversity of grapes (Cao et al., 2020; Guo et al., 2010), sugarcane [*Saccharum officinarum* (Ali et al., 2019)], wheat [*Triticum aestivum* (Abbasov et al., 2018)], cabbage [*Brassica oleracea* (El-Esawi et al., 2016)], and hazelnuts [*Corylus heterophylla* (Zhao et al., 2020)].

Early research focused on the classification of Chinese wild grapes, but the taxonomic status of many of the indigenous Chinese species and cultivars remains obscure, such as *V. amurensis* var. *yanshanensis* (Zhang et al., 2018). Later, as a result of the importance of wild grapes in breeding, a number of reports focused on the analysis of genetic diversity among wild grapes native to China and cultivated grapes originating from North America and Europe (Jing and Wang, 2013; Jing et al., 2013; Péros et al., 2011). However, there is still a need to investigate further the genetic diversity of wild grape resources to enable breeders to broaden the genetic base of cultivated grapevines through the incorporation of wild relatives (Fan et al., 2015; Jing et al., 2013; Liu et al., 2012; Zhang et al., 2018).

Recently, Liu et al. (2012) used SSR and SRAP to analyze the genetic diversity of 15 Chinese wild grape species (Liu et al., 2012). To understand more fully the genetic background of Chinese wild grapes, we extend that work in this study to include 23 species/cultivars, in which most wild species are included. This study aimed to characterize the genetic diversity and population structure of 100 accessions belonging to Chinese wild *Vitis* species/cultivars, *V. vinifera*, *V. aestivalis*, *V. girdiana*, *V. monticola*, *V. acerifolia*, and *V. riparia*. The results may provide invaluable information for the better use of Chinese wild grape germplasms in breeding. Moreover, to connect our findings with the *Vitis* International Variety Catalogue database (Maul and Töpfer, 2015), we assessed the genetic diversity and relationships of 100 *Vitis* materials standardized by using the nine SSR markers used in the database to obtain useful information on these materials. The genotypes obtained in this study can be connected to the *Vitis* International Variety Catalogue database after standardization, which will facilitate the sharing of information among different laboratories.

Materials and Methods

Plant materials. Plant materials were collected from the China Grape Germplasm Repository in Zhengzhou Henan Province. A total of 100 *Vitis* accessions were used in this study. They consisted of 77 accessions representing 23 Chinese wild *Vitis* species/cultivars, 18 *V. vinifera* cultivars, and the North American *V. aestivalis*, *V. girdiana*, *V. monticola*, *V. acerifolia*, and *V. riparia*. Moreover, four cultivars of the 18 *V. vinifera* cultivars were used for SSR standardization for comparison within international databases (Table 1). The young leaves of each accession were harvested and stored at –80 °C for later DNA extraction.

Genomic DNA extraction and genotyping. Genomic DNA was extracted from the young leaves using a Plant Genomic DNA Kit (Aidlab, Beijing, China) according to the manufacturer's instructions. The DNA quality was checked by 1% (w/v) agarose gel electrophoresis. The genomic DNA was diluted to 30 to 50 ng·μL^{-1} and was stored at –20 °C.

Nine SSR loci distributed over six chromosomes in the grape were used, and they have shown a high discrimination capacity: VVMD5 and VVMD7 (Bowers et al., 1996); VVS2 (Thomas and Scott 1993); VVMD 32, VVMD25, VVMD27, and VVMD28 (Bowers et al., 1999); and VrZAG62 and VrZAG79 (Sefc et al., 1999). All the forward primers were labeled with FAM (Carboxyfluorescein) or HEX (Hexachloro-Fluorescein) fluorescent dye at the 5′-end.

The polymerase chain reaction (PCR) was performed in a 20-μL volume containing 30 to 50 ng of template DNA, 1× PCR buffer (Mg^{2+} plus) (TaKaRa, Beijing, China), 0.25 mM deoxynucleoside triphosphates, 0.5 μM of each primer set, and 1 U of Taq DNA polymerase (TaKaRa). The PCR amplification was conducted under the following thermal conditions: 4 min at 94 °C for initial denaturation, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at the optimum annealing temperature for 30 s, then at 72 °C for 45 s, with a final extension at 72 °C for 10 min. The final reaction was held at 4 °C.

SSR data acquisition. The PCR-amplified products were separated using capillary electrophoresis on an ABA-3730XL DNA Analyzer (Thermo Fisher Scientific, Waltham, MA). An internal size standard (GeneScan-500ROX, Thermo Fisher Scientific) was used to estimate the approximate molecular weights of the amplified products. Ultimately, the peaks were shown by size and height using generic description software (GeneMapper version 3.2, Thermo Fisher Scientific).

SSR data analysis. To assess the genetic diversity among the accessions, the following parameters were applied using the statistical software POPGEN version 1.32 (Krawczak et al., 2006): the number of alleles (N_a), number of effective alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), and Shannon's information index (I) for each locus. The PIC and GD of each primer pair was estimated using PowerMarker version 3.25 (Kejun and Muse, 2005). The genetic diversity index among different populations was calculated using GenALEx version 6.41 (standard model) (Peakall and Smouse, 2006).

Genetic distances were calculated in accordance with Nei (1973) and were used to construct a dendrogram based on the unweighted pair–group method of mathematical averages (UPGMA) using MEGA5 software (Tamura et al., 2011), and the dendrogram was visualized using EvolView version 2 (He et al., 2016). A principal coordinate analysis (PCA) and an AMOVA were also conducted with GenALEx version 6.41. The population structure was investigated with STRUCTURE software (Li et al., 2019), which is based on an admixture model with correlated allelic frequencies. The number of populations (K) was set from 2 to 10, and 10 independent runs for each K were conducted using a burn-in period of 10,000 steps, followed by 100,000 iterations. The best K was estimated based on the delta K method using STRUCTURE HARVESTER (Earl and Vonholdt, 2012).

Results

Allelic variance and genetic diversity. During this study, nine SSR primers were used to detect the molecular variance among the 100 grape samples; 100 unique genotypes were detected (Supplemental Table 1). Each of the nine loci analyzed was polymorphic, and resulted in 186 alleles, with an average of 20.667 alleles per locus (Table 2). N_a varied from 16 (VVMD7) to 29 (VVMD28). N_e ranged from 8.306 to 17.549, with a mean value of 11.472. GD was between 0.883 and 0.943, with an average of 0.910. The average value for I was 1.245, with a range from 0.966 to 1.582. PIC values varied from 0.873 for VVMD5 to 0.940 for VVMD28, with an average of 0.903

Table 1. Geographic characteristics and the group information of 100 *Vitis* materials examined in this study.

Species	Code no.	Clone	Origin	Cluster no.
V. riparia	83	DVIT1884 b43-15M44	United States	I
V. aestivalis	84	DVIT2382Xiaputao	United States	I
V. monticola	85	DVIT1847Tianshanputao	United States	I
V. acerifolia	87	DVIT1296Qiyeputao	United States	I
V. girdiana	86	DVIT1387Shanguputao	United States	I
V. davidii	88	ZLL	Hunan Province, R6^{y}	II
	90	HHBG	Huaihua, Hunan Province, R6^{y}	II
	89	BPT-1	Hunan Province, R6^{y}	II
	92	HT-1	Huaihua, Hunan Province, R6^{y}	II
	91	WH-ci	Wuhan Province, R6^{y}	II
	98	Gaoshan-1	Jiangxi Province, R2^{y}	II
	99	Ziqiu	Hunan Province, R2^{y}	II
	100	Gaoshan-2	Jiangxi Province, R2^{y}	II
	94	Black pearl	Hunan Province, R6^{y}	II
	93	ZF-1	Huaihua, Hunan Province, R6^{y}	II
	95	Xiangzhenzhu (Red)	Hunan Province, R6^{y}	II
	96	Xiangzhenzhu (Green)	Hunan Province, R6^{y}	II
	97	ES1424	Hubei Province, R6^{y}	II
V. amurensis	78	SPT-1	Northeastern China, R4^{y}	III
	79	SPT-2	Northeastern China, R4^{y}	III
	80	SPT0933	Northeastern China, R4^{y}	III
	81	DLS	Beijing Province, R3^{y}	III
V. amurensis var. *yanshanensis*	82	Beizhi1702	Beijing Province, R3^{y}	III
V. vinifera	72	Sauvignon Blanc^{z}	France	III
	74	Carignan^{z}	Spain	III
	75	Gamay^{z}	Spain	III
	76	Syrah^{z}	Iran	III
	67	Cardinal	United States	III
	62	Muscat Hamburg	England	III
	59	6-28	China	III
	60	Jinanzaohong	China	III
	65	Jingfeng	China	III
	64	Centennial Seedless	United States	III
	73	Jingkejing	China	III
	70	Jingyu	China	III
	66	Queen of Vineyard	Hungary	III
	63	Shenyangmeigui	China	III
	71	Augusta	Romania	III
	68	Miskat Plevenski	Bulgaria	III
	61	Pobeda	Uzbekistan	III
	69	Flaming Muscat	South Africa	III
Unknown	56	MZL-5	Songxian, Henan Province, R6^{y}	III
Unknown	55	MZL-6	Songxian, Henan Province, R6^{y}	III
V. betulifolia	58	BYS	Songxian, Henan Province, R6^{y}	III
	57	SX	Songxian, Henan Province, R6^{y}	III
V. ficifolia	45	QTH-6	Jiaozuo, Henan Province, R6^{y}	IV
	44	NWH1403	Xinyang, Henan Province, R6^{y}	IV
	41	QYS-8	Luoyang, Henan Province, R6^{y}	IV
	43	JLG-4	Luoyang, Henan Province, R6^{y}	IV
	42	JLG-1	Luoyang, Henan Province, R6^{y}	IV
	51	BTM-1	Nanyang, Henan Province, R6^{y}	IV
	53	FJG-3	Luoyang, Henan Province, R6^{y}	IV
	52	XY	Xinyang, Henan Province, R6^{y}	IV
	47	QL-3	Shaanxi Province, R5^{y}	IV
	46	HX	Xian, Shaanxi Province, R5^{y}	IV
	48	WDS-1	Henan Province, R6^{y}	IV
	50	SBY-5	Linzhou, Henan Province, R6^{y}	IV
	49	JLG1401	Luoyang, Henan Province, R6^{y}	IV

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Table 1. Continued.

Species	Code no.	Clone	Origin	Cluster no.
V. romaneti	26	BTM	Nanyang, Henan Province, R6[y]	IV
	27	LB-qiu	Henan Province, R6[y]	IV
	38	ZX1734	Hubei Province, R6[y]	IV
V. bellula	3	MLPT1104	Hubei Province, R6[y]	IV
V. pseudoreticulata	36	GX	Guangxi Province, R2[y]	V
	37	LY	Jiangsu Province, R2[y]	V
	4	WX1135	Chongqing, R6[y]	IV
	5	HDPT1057	Hubei Province, R6[y]	IV
V. piasezkii	7	LYW-1	Luoyang, Henan Province, R6[y]	IV
	10	LJS-1	Luoyang, Henan Province, R6[y]	IV
	77	SNJ	Hubei Province, R6[y]	III
V. shenxiensise	40	CHS1401	Xian, Shaanxi Province, R5[y]	IV
	15	LB-shanxi	Henan Province, R6[y]	IV
V. wuhanensis	12	DW1707	Hubei Province, R6[y]	V
	9	XN1710	Hubei Province, R6[y]	V
V. bryoniaefolia	39	WH-3	Wuhan Province, R6[y]	V
	6	WD1418	Wuhan Province, R6[y]	V
	21	QYS-1	Luoyang, Henan Province, R6[y]	V
V. balanseana	1	XGPT-2	Guangxi Province, R2[y]	V
	2	NN1606	Guangxi Province, R2[y]	V
V. heyneana	13	YC-1	Chongqing, R1[y]	V
	14	YMN-1	Yuanmou, Yunnan Province, R1[y]	V
	18	YMCB	Yuanmou, Yunnan Province, R1[y]	V
	54	NWH1402	Xinyang, Henan Province, R1[y]	IV
V. yunnanensis	25	YM-2	Yunnan Province, R1[y]	V
	28	YM-3	Yunnan Province, R1[y]	V
	29	YN-hn	Hunan Province, R2[y]	V
	30	YN-2	Yunnan Province, R1[y]	V
V. chungii	24	XS1614	Jiangxi Province, R2[y]	V
V. chunganensis	23	SQS	Jiangxi Province, R2[y]	V
	19	WYS1709	Fujian Province, R2[y]	V
V. hancockii	20	LYPT0945	Jiangxi Province, R2[y]	V
V. sinocinerea	8	CS	Changsha, Hunan Province, R6[y]	V
V. wilsonae	34	BTM-2	Nanyang, Henan Province, R6[y]	V
	35	BTM-1	Nanyang, Henan Province, R6[y]	V
	31	LS-2	Luoyang, Henan Province, R6[y]	V
	33	LS-1	Luoyang, Henan Province, R6[y]	V
V. adenoclada	16	SX-1	Hongjiang, Hunan Province, R6[y]	V
	17	GX4020101	Guangxi Province, R2[y]	V
V. hekouensis	22	HKPT	Yunnan Province, R1[y]	V
V. ruyuanensis	11	HZ199	Wuhan Province, R6[y]	V
Unknown	32	Wuzhi-1	Wuhan Province, R6[y]	V

[z]Sauvignon Blanc, Carignan, Gamay, and Syrah are used for simple sequence repeat standardization for comparison with international databases.
[y]R1 = southwestern China (i.e., Yunnan and Chongqing); R2 = southeastern China (i.e., Guangxi, Guangdong, Jiangxi, Jiangsu, and Fujian); R3 = north China (i.e., Beijing); R4 = northeastern China (i.e., Heilongjiang); R5 = northwestern China (i.e., Shanxi and Qinling); R6 = central China (i.e., Hubei, Hunan, Henan).

(Table 2). H_o ranged from 0.476 to 0.859, with a mean value of 0.644; H_e ranged from 0.503 to 0.726, with a mean value of 0.608 (Table 2). The fixation index (F) values were close to zero for most of the loci, indicating that the locus fit Hardy-Weinberg equilibrium. The gene flow values of all the SSR loci were less than one, revealing that the allelic variation at different SSR loci showed genetic differentiation among the different materials.

According to the cluster analysis, 100 grape samples could be divided into five groups. The genetic diversity of the different groups (I, II, III, IV, and V) evaluated using the SSR markers is shown in Table 3. According to the values for N_e and I, group V had the highest diversity, followed by groups IV, III, I, and II, whereas the number of accessions in groups III and II are more than group IV and I, respectively, which indicates a little relationship with the number of accessions in each group.

To understand the genetic variation in grape accessions from different origins, genetic diversity was also evaluated with the SSR data (Table 4). Central China (R6) had the highest values

Table 2. Genetic diversity analysis of the 100 *Vitis* accessions by the nine simple sequence repeat markers.

Locus	Parameter[z]								
	N_a	N_e	H_o	H_e	GD	PIC	I	F	Nm
VrZAG62	20	10.959	0.637	0.646	0.909	0.902	1.319	–0.023	0.608
VrZAG79	18	11.138	0.691	0.610	0.911	0.904	1.274	–0.142	0.489
VVS2	23	13.441	0.859	0.726	0.927	0.923	1.582	–0.216	0.937
VVMD5	19	8.306	0.582	0.544	0.883	0.873	1.102	–0.091	0.413
VVMD7	16	9.268	0.515	0.503	0.894	0.885	0.966	–0.057	0.309
VVMD28	29	17.549	0.622	0.626	0.943	0.940	1.358	0.003	0.480
VVMD32	22	12.107	0.476	0.543	0.918	0.912	1.051	0.099	0.369
VVMD25	21	10.455	0.751	0.667	0.906	0.899	1.358	–0.171	0.675
VVMD27	18	10.030	0.662	0.605	0.899	0.891	1.197	–0.101	0.539
Mean	20.667	11.472	0.644	0.608	0.910	0.903	1.245	–0.078	0.535

[z]N_a = observed number of alleles; N_e = effective number of alleles; H_o = observed heterozygosity; H_e = expected heterozygosity; GD = gene diversity; PIC = polymorphism information content; I = Shannon's information index; F = average fixation index; Nm = gene flow estimate according to Wright's equation.

Table 3. The parameters of genetic diversity among five *Vitis* groups estimated by nine simple sequence repeat markers.

Group no.	Parameter[z]					Total genotypes (no.)
	N_a	N_e	I	H_o	H_e	
I	5.556	4.433	1.473	0.733	0.700	5
II	5.667	3.455	1.345	0.744	0.650	13
III	11.556	5.764	2.000	0.798	0.816	28
IV	12.556	7.034	2.141	0.755	0.838	24
V	14.667	8.503	2.362	0.566	0.878	30

[z]N_a = observed number of alleles; N_e = effective number of alleles; I = Shannon's information index; H_o = observed heterozygosity; H_e = expected heterozygosity.

Table 4. The parameters of genetic diversity of *Vitis* from different regions estimated by nine simple sequence repeat markers.

Origin[z]	Parameter[y]						Total genotypes (no.)
	N_a	N_e	I	H_o	H_e	F	
R1	7.778	5.819	1.783	0.611	0.775	0.028**	8
R2	9.444	6.861	2.052	0.640	0.850	0.248**	13
R3	2.667	2.415	0.866	0.722	0.528	–0.378	2
R4	3.111	2.690	0.973	0.667	0.556	–0.187	3
R5	3.667	3.003	1.126	0.778	0.611	–0.272**	3
R6	16.778	10.002	2.469	0.692	0.891	0.225***	48

[z]R1 = southwestern China (i.e., Yunnan and Chongqing); R2 = southeastern China (i.e., Guangxi, Guangdong, Jiangxi, Jiangsu, and Fujian); R3 = North China (i.e., Beijing); R4 = northeastern China (i.e., Heilongjiang); R5 = northwestern China (i.e., Shanxi and Qinling); R6 = Central China (i.e., Hubei, Hunan, Henan).
[y]N_a = observed number of alleles; N_e = effective number of alleles; I = Shannon's information index; H_o = observed heterozygosity; H_e = expected heterozygosity; F = average fixation index.
**, ***Significant at $P \leq 0.10$ or 0.05, respectively, calculated over 1000 permutations.

of the major parameters N_e and I, followed by southeastern China (R2), southwestern China (R1), northwestern China (R5), northeastern China (R4), and north China (R3), and this trend showed that the genetic diversity of the six regions varied, with the accessions derived from central China exhibiting the greatest genetic diversity. This result may be related to the sample size of each region.

Population differentiation analysis. AMOVA was performed among the 100 accessions by considering the five populations generated by the structure analysis (Table 5). The AMOVA results in Chinese wild grape accessions indicated that 3.28% of the total variance was among populations, 3.27% was among individuals within populations, and 93.46% was within individuals. The inbreeding coefficients within subpopulations relative to the total, within individuals relative to the subpopulation, and within individuals relative to the total parameters were 0.033, 0.034, and 0.065, respectively (Table 5).

Principal coordinate analysis. PCA was chosen to complement the cluster analysis information and genetic relationships (Zhu et al., 2018). The PCA results for 100 accessions based on the genetic distance matrix data from the nine SSR markers is shown in Fig. 1. Generally speaking, 100 accessions could be divided into three primary groups (i.e., North American species, European species, and East Asian species), although there was little overlap between the European species and East Asian species, which is consistent with the geography [i.e., North America, East Asia, and Europe (Fig. 1)].

Cluster analysis. To assess the genetic diversity and relationships among 100 *Vitis* materials, a cluster analysis was conducted based on the genetic distance matrix data estimated by using SSR markers. The dendrogram constructed by UPGMA is shown in Fig. 2. In addition, the North American accessions separated from the other cultivars, forming one individual group—namely, group I—which showed there was a marked divergence between the North American accessions and the Eurasian species of the subgenus *Vitis*, whereas the cultivated grapes (European species) and Chinese wild grape species (East Asian species) were grouped together in group III. These results provide additional support for the sister relationship between the East Asian species and the European species.

All 100 grape accessions examined were divided into five groups based on the cluster analysis. Group I contained exclusively North American accessions; group II contained only *V. davidii*. Group III was subdivided into three subgroups. Group III-1 contained *V. amurensis* and *V. amurensis* var. *yanshanensis*, and one accession belonged to *V. piasezkii*. Group III-2 contained European cultivars. Group III-3 included *V. betulifolia*, and two accessions shared similar morphologies with *V. betulifolia*. Group IV consisted of two subgroups. Group IV-1 included *V. ficifolia*. Group IV-2 contained *V. heyneana*, *V.*

Table 5. Results of the analysis of molecular variance carried out among 100 *Vitis* accessions considering the five populations generated by the structure analysis.

Source of variation	df	Sum of squares	Variance components	Proportion of variation (%)	Coefficient[z]		
					F_{st}	F_{is}	F_{it}
Among populations	4	36.102	0.135	3.28			
Among individuals within populations	95	390.298	0.134	3.27			
Within individuals	100	384.000	3.840	93.46			
Total	199	810.400	4.109	100	0.033	0.034	0.065

[z]F_{st} = the inbreeding coefficient within subpopulations relative to the total; F_{is} = the inbreeding coefficient within individuals relative to the subpopulation; F_{it} = the inbreeding coefficient within individuals relative to the total.

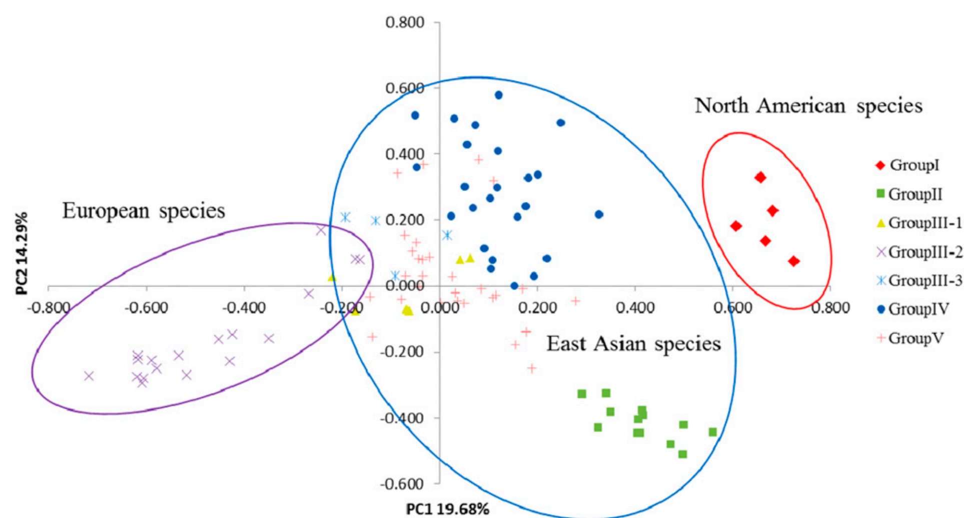

Fig. 1. Associations among the 100 *Vitis* accessions revealed by principal coordinate (PC) analysis as performed on Nei's genetic distance matrix data calculated from nine simple sequence repeat markers. The red circle represents the North American species. The green circle represents the East Asian species. The purple circle represents the European species.

shenxiensise, *V. piasezkii*, and *V. romaneti*. Group V was the largest, with 30 accessions, and included 15 wild species native to China. Group V was subdivided into 12 subgroups. Group V-1 included the single species *V. wuhanensis*. Group V-2 included *V. bryoniaefolia*. Group V-3 contained *V. balanseana*, *V. pseudoreticulata*, and *V. bellula*. Group V-4 included a single species, *V. yunnanensis*. Group V-5 contained *V. chungii*. Group V-6 consisted of *V. chunganensis*. Group V-7 contained a single species, *V. hancockii.* Group V-8 contained only *V. sinocinerea*. Group V-9 included two accessions of *V. pseudoreticulata*, and four accessions of *V. pseudoreticulata* did not group closely within one branch. Group V-10 contained *V. wilsonae* and one accession, Wuzhi-1. Group V-11 consisted of *V. adenoclada* and *V. hekouensis*. Group V-12 contained only *V. ruyuanensis*.

In addition, some important exceptions were discovered by cluster analysis. Three previously undescribed accessions [Wuzhi-1 (ID 32), MZL-5 (ID 56), and MZL-6 (ID 55)] were assigned to the *V. wilsonae* and *V. betulifolia* cluster, with mean genetic distance values of 0.39, 0.31, and 0.38, respectively.

Population structure analysis. The statistic ρK calculated with STRUCTURE was used to determine a suitable K value (population number). The maximum likelihood value and a sharp peak were revealed at K = 5. Thus, the 100 grape accessions were divided into five populations: POP I, POP II, POP III, POP IV, and POP V (Fig. 3). On the whole, the results of the genetic structure analysis were consistent with those revealed by the cluster analysis. POP I contained five accessions that originated only from group I, and the accessions of POP I were from the US. POP II included 13 accessions, all of which were from group II, and the accessions of this population were from Hunan, Hubei, Jiangxi, and Wuhan. The 24 accessions that originated from group III were assigned to POP III, which were mostly from Europe and Heilongjiang, among others. POP IV consisted of 18 accessions, which were mostly from Henan, including 14 accessions from group IV, and the other four accessions that originated from group III. POP V included 40 accessions containing all 30 accessions from POP V along with 10 accessions from group IV, which were from Yunnan, Hunan, Jiangxi, Guangxi, and Fujian, among others.

Discussion

SSR marker. The SSR marker is a powerful, reliable, and useful tool for molecular breeding and for assessing the genetic diversity of plants because of its codominance and ability to reveal a high number of alleles per locus (Bassil et al., 2018; El-Esawi et al., 2016; Liang et al., 2015; Liu et al., 2015; Rodolfi et al., 2018). This is the first study to assess the genetic diversity and relationships among 23 wild Chinese *Vitis* species/cultivars using this powerful microsatellite technique. The nine SSR markers selected in this study, which are high in polymorphisms, have been used by the European GrapeGen06 consortium (Maul et al., 2012) and have been used frequently to identify grape cultivars (Li et al., 2018; Mihaljevic et al., 2013; This et al., 2004). Internationally, France, Germany, Italy, and other countries have used the nine SSR markers to establish a molecular database of grape cultivars (Maul and Töpfer, 2015), providing an inquiry service for grape research workers. Furthermore, Li et al. (2017, 2018) showed that the nine SSR markers can be used to identify the Chinese grape landraces and Euro-American hybrids.

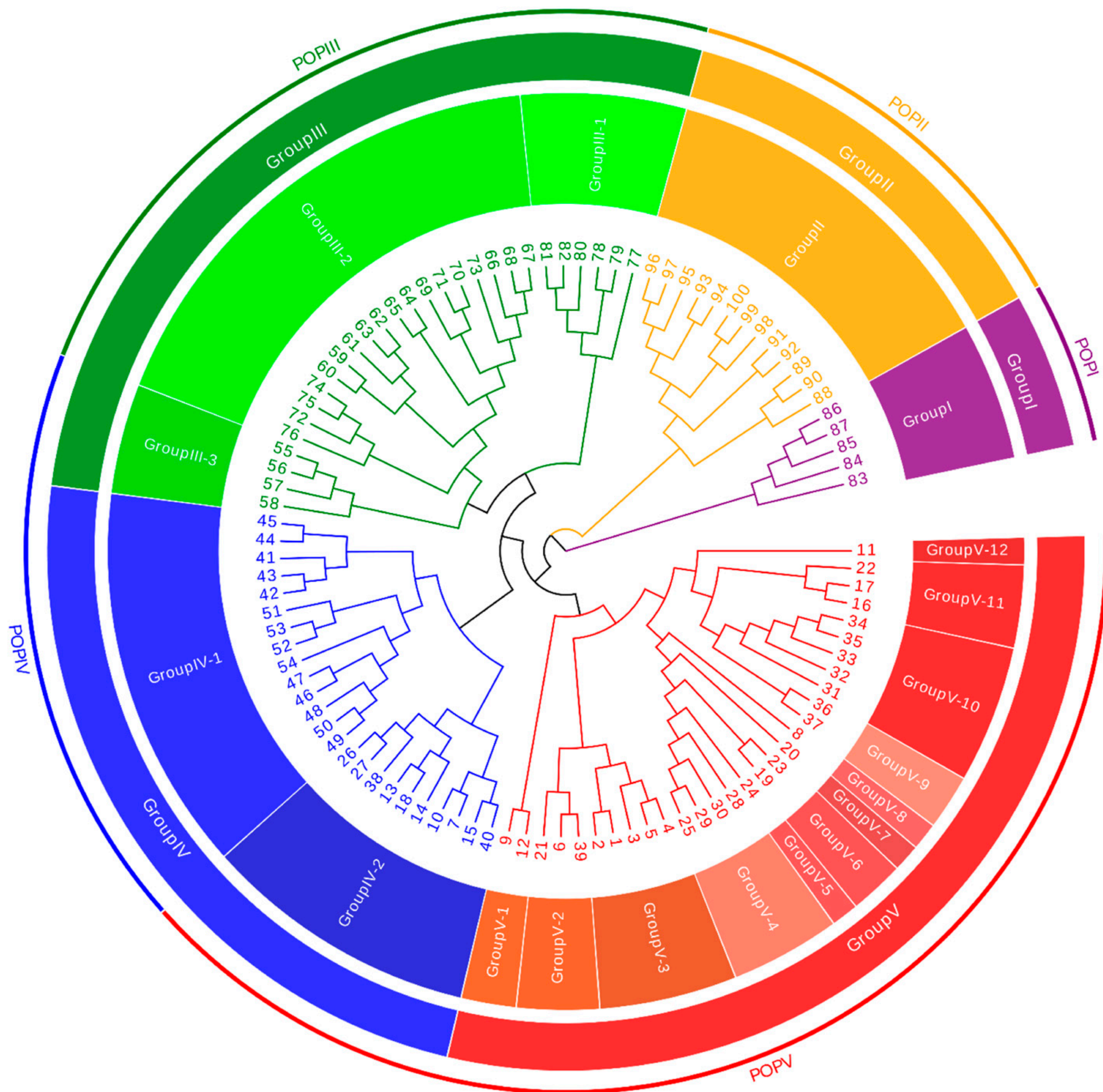

Fig. 2. Phylogenetic trees of 100 *Vitis* accessions based on simple sequence repeat data. A distance tree was constructed in MEGA5 (Tamura et al., 2011) using the unweighted pair-group method of mathematical averages method. Code number is the same reported in Table 1. POP = population.

The average allele per marker (20.667) is greater than that noted in the earlier report by Jing et al. (2013), who found an average of 10.5 alleles per marker in 62 Chinese wild grapes using 10 SSR markers. The PIC value (0.903) is greater than the values reported by Karataş et al. (2014) (SSR, 0.63) and Ramezani et al. (2009) (SSR, 0.89) in grapes. In particular, the PIC value in our study (0.903) is greater than the earlier report by Li et al. (2018), who found an average PIC value of 0.815 in 94 cultivated accessions using the nine SSR markers. The greater PIC values evidence a greater genetic diversity in the currently studied Chinese wild grape species than in the cultivated accessions that were studied before. In addition, we found greater H_o values than expected (0.644 and 0.608, respectively), which is in accordance with Riaz et al. (2018), who reported greater H_o values than expected among 1378 grape accessions (0.742 and 0.678, respectively). The high heterozygosity values found our study suggest high levels of genetic variation among the analyzed Chinese wild grape species. Currently, many wild grapes have been used in breeding programs, and some even were applied in production directly (Liu et al., 2014). Therefore, this set of SSR primers can be used for the identification of wild grape cultivars in the grape industry.

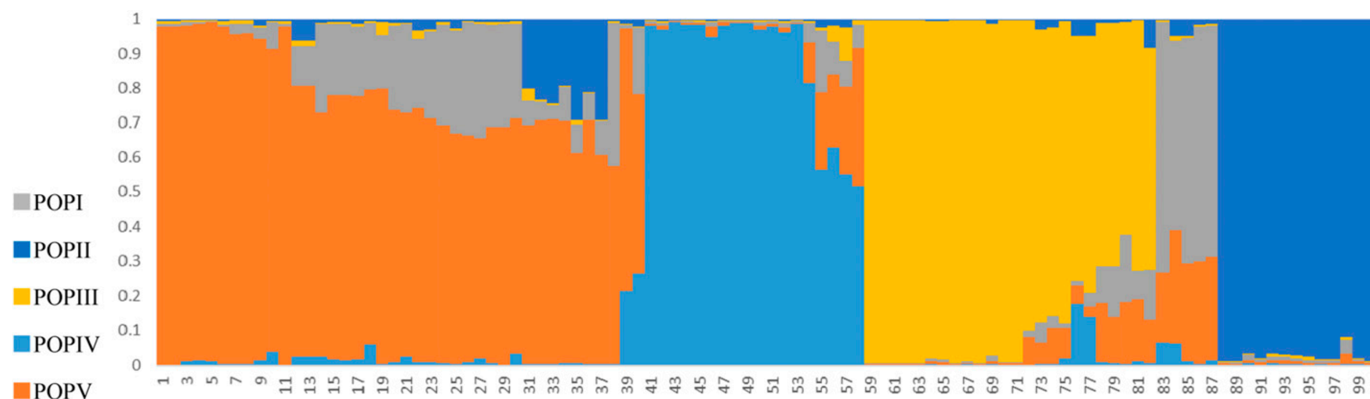

Fig. 3. Genetic structure of the 100 *Vitis* accessions inferred by a Bayesian mixed-model-based cluster analysis assuming correlations among allele frequencies across clusters. Each individual is represented by a thin line partitioned into K colored segments representing the membership fraction in K clusters. Each color represents a population. POP = population; K = number of populations.

SSR markers are also a powerful tool for characterizing the cultivars. In this study, cluster, principal coordinate, and structure analyses allowed us to identify three previously undescribed accessions (Wuzhi-1, MZL-5, and MZL-6). For instance, Wuzhi-1, MZL-5, and MZL-6 not only have a closer relationship with *V. betulifolia* at the molecular level, but also have similar morphological characteristics. The branchlets have arachnoid tomentum and fall off later; the shape of the mature leaf is oval and the number of mature leaf lobes is three; the lower side prostrate hairs between the veins of young leaves are sparse; and there are no glandular hairs on shoots. Bassil et al. (2018) also used SSRs to identify four accessions of previously undescribed hybrid origin during an analysis of the genetic diversity of wild *Vaccinium* species. Cao et al. (2020) reported that five previously unknown grape accessions were matched by SSRs.

Pattern of genetic diversity distribution among different populations. The genetic diversity within and among the different geographic groups in Chinese wild grape species, as demonstrated by I and N_e, indicates there is significant diversity (Table 4). The *Vitis* accessions from R6 (i.e., Hubei, Hunan, and Henan) had the highest number of effective alleles, and the I value suggests this region may be the center of diversity for wild grape species. These results may be a result of the large sample size for this region. The H_o value of the accessions from R1, R2, and R6 was slightly less than the H_e values, with a contrasting trend for the accessions from R3, R4, and R5. These differences are consistent with the positive F values, particularly in the populations from R2 and R6, which indicates a close genetic relationship among individuals from the same populations (Table 4). The negative F values for accessions from R2, R3, and R6 indicate an excess of heterozygotes, but only the last one (R6), was statistically significant (–0.272) (Table 4).

The AMOVA results showed that similar levels of genetic variation among and within populations were observed, and low levels of genetic diversity were present among populations, whereas high levels of genetic diversity were found within individuals (Table 5). These results are in accordance with the findings from Riaz et al. (2018), who analyzed the genetic diversity of cultivated and wild grapevine accessions around the Mediterranean Basin and Central Asia. Riaz et al. (2018) also reported that the level of genetic diversity within individuals was greater than that among populations.

Genetic structure and differentiation. A significant differentiation between wild North American *Vitis* species and Eurasian *Vitis* species was detected by cluster analysis and PCA (Figs. 1 and 2). This result is consistent with previous studies that support the division of the subgenus *Vitis* into two clades that mirror their geographic distribution—North America and Eurasia—based on resequencing technology, plastid and nuclear markers, and son on (Klein et al., 2018; Liu et al., 2016; Ma et al., 2018; Péros et al., 2011; Wan et al., 2013). Nevertheless, the relationship between European and East Asian species remains controversial.

In our study, the European species and the cultivated accessions native to China were grouped with two Chinese wild grape species, indicating these Chinese wild grape species are more genetically close to European grapes than North American species. On the other hand, these results were not consistent with the findings of Liang et al. (2019), who indicated that wild East Asian and wild North American accessions shared more similarity in genetic background than Eurasian accessions based on the whole-genome resequencing method. This finding is consistent with those of Liu et al. (2016) and Zhang et al. (2018), all of whom affirmed that the European species (*V. vinifera*) was grouped within the East Asian clade. Many explanations have been made regarding this phenomenon. For example, Péros et al. (2011) held the opinion that there might be an Asian origin for the subgenus *Vitis*, and their study suggested that the subgenus *Vitis* first diverged in Asia and later colonized Europe. Moreover, one hypothesis was suggested that hybridization could be conducive to the integration of the European grape genome into the Chinese germplasm (Zhang et al., 2018). This finding is based on the observation that viticulture has been common since ancient times in regions where wild species grew as well. This finding will increase the gene flow possibility between the cultivated grapes (*V. vinifera*) and the wild grape species.

Furthermore, 100 *Vitis* accessions were divided into five major groups by their SSRs (Fig. 2), and the PCA also showed similar groupings. The reason that group V exhibited greater genetic diversity might be related to the sample size. In addition, the American *Vitis* species from the US grouped together and formed group I. Group II was distributed primarily in the south–central and southeastern regions of China. Groups III and IV were distributed primarily throughout the central region, with some extending to the northern, northeastern, and

northwestern regions of China, whereas the accessions that originated from Europe grouped together and were also assigned to group III. Group V was distributed primarily throughout the southwestern and southeastern regions of China. Thus, the clustering patterns of most accessions correlated with geographic distribution (Fig. 2).

In addition, a population structure analysis showed that five populations were obtained from the 100 *Vitis* accessions. However, there were a few discrepancies between the cluster analysis and the structure analysis, which might be because each accession was assigned a fixed branch by cluster analysis, whereas the structural analysis assigned individuals to groups based on the percentage of population members (Zhu et al., 2018). The admixture of all the populations might indicate gene flow in these accessions (Aradhya et al., 2013).

In addition, the results of the cluster analysis revealed a close relationship among *V. piasezkii*, *V. romaneti*, *V. shenxiensise*, and *V. heyneana*. This finding is similar to the results of Ma et al. (2018). Our result supports the idea that some types of *V. piasezkii* have similar morphological characteristics relative to *V. shenxiensis* (Ren and Wen, 2007), which also indicates that a high level of genetic diversity is present within *V. piasezkii*. In addition, according to the review by Wan et al. (2008), *V. romaneti* and *V. shenxiensise* belong to section *Romanetianae*. *V. heyneana* did not cluster with *V. ficifolia*, unlike the results reported by Zhang et al. (2018) and Wang et al. (2008), but consistent with those of Liu et al. (2016). In addition, the results also revealed a closer relationship between *V. wilsonae* and two accessions of *V. pseudoreticulata*, and this finding is similar to the results of the phylogenetic studies on the grape genus using five plastid and two nuclear markers (Liu et al., 2016). We also found that some accessions belonging to the same species are in different clades (Fig. 2), such as *V. pseudoreticulata* and *V. piasezkii*. This result implies high genetic variation and genetic diversity within species, which was further supported by AMOVA.

Conclusion

Today, China holds more than 3000 accessions of *Vitis*, of which some are wild types. These accessions are either native to China or through foreign introduction. With the increase of grape germplasms, genetic information among accessions is becoming more critical for maintenance and utilization strategies in China's breeding programs. We conclude that the estimation of genetic diversity and the population structure of 100 accessions of *Vitis* using SSR markers may provide more accurate information to grape breeders than the classical pedigree method. In addition, the nine SSR markers used in our study are significantly polymorphic and can be used efficiently to distinguish wild Chinese grape species. Moreover, three previously undescribed accessions were identified to species (Wuzhi-1, MZL-5, and MZL-6). The nine SSR primer pairs in our study may also be of potential value for further research on genetic mapping, marker-assisted selections, and so on, in grapes.

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