Characterization of Grape Cultivars from China Using Microsatellite Markers

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Abstract

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A total of 32 different grape cultivars including representatives of local Chinese cultivars, some important and widely grown Chinese cultivars and international reference cultivars were genotyped at nine microsatellite loci in order to characterize their genetic diversities. The numbers of alleles detected per locus ranged from 9 to 18 with a total of 105 alleles and an average of 11.7 alleles per locus, while the number of microsatellite genotypes varied between 10 and 23, indicating that there are abundant allele diversities in Chinese grape cultivars. The expected heterozygosity varied between 0.740 and 0.915 and the polymorphism information content ranged from 0.716 to 0.908. According to the results of clustering and Principal Coordinates Analysis, three groups were identified among all these cultivars. The clusters of cultivars showed a clear separation of table grape, wine grape of *Vitis vinifera* and hybrids between European and American species. This study generated a microsatellite profile database for the cultivars from Chinese local and newly bred grapes.

Keywords: Chinese grape; genetic diversity; SSR; *Vitis vinifera*

Grape cultivation has been pursued in China for more than 2000 years (Kong 2004) and ranks the fifth in fruit production following the apple, citrus, pear and banana. More than 1500 grape cultivars are distributed in most regions of the country (Wan *et al*. 2008). The areas under grapevine are larger than 552 km^2 and over 8.43 million tons of grapes were produced in China in 2012.

Cultivars of *Vitis vinifera* are eco-geographically classified into 3 groups (convar.): *pontica*, *orientalis*, and *occidentalis* (Negrul 1938). Oriental cultivars commonly include Chinese and Japanese native cultivars, which were probably propagated along the Silk Road (Goto-Yamamoto *et al*. 2009). Many old indigenous cultivars existed in China and some of them are still cultivated widely (Kong 2004). The autochthonous cultivars have a high potential breeding value, so it is important to characterize the genetic diversity of the ancient grape cultivars for their further utilization. Initial efforts to evaluate the genetic diversity of Chinese grape cultivars were based on ampelographic traits (Yin *et al*. 1991). Preliminary surveys have shown that various local cultivars exhibited significant morphological variability. However, little is known about genetic diversity and relationships among them.

Microsatellite markers have been very useful in genetic diversity analysis due to their reproducibility, codominance and polymorphism (Powell *et al*. 1996). They have been extensively used in grape for variety identification in collections, pedigree analysis, or genetic mapping (Karatas *et al*. 2007; Vezzulli *et al*. 2008; Laucou *et al*. 2011; Moreno-Sanz *et al*. 2011) and are very useful for distinguishing grape genotypes and determining genetic relationships among *Vitis* cultivars and

species (Riahi *et al*. 2012). Only a limited number of microsatellite data on oriental cultivars, particularly on Chinese cultivars, has been reported (Goto-Yamamoto *et al*. 2009; Guo *et al*. 2012a, b).

In this study, a set of 9 microsatellite markers (This *et al*. 2004; Laucou *et al*. 2011) was used to investigate genetic polymorphism and relationships among the main local Chinese grape cultivars and some newly bred Chinese cultivars. International cultivars such as Pinot Gris and Cabernet Sauvignon were chosen as a base for comparison.

MATERIAL AND METHODS

Plant material and DNA extraction. Thirty-two cultivars including representative local cultivars, some widely grown Chinese cultivars and international reference cultivars were collected from the national grape germplasm repository of Zhengzhou Fruit Research Institute of the Chinese Academy of Agricul-tural Sciences (Table 1). Fresh young leaves were collected, frozen and preserved at –80°C. DNA was extracted from leaves as described by Marsal *et al.* (2011).

Table 1. List of grape cultivars used in this study

	Cultivars name	Pedigree	Species	Origin
1	Manai	ancient variety of China, unknown	*V. vinifera* L.	China
2	Munage	ancient variety of China, unknown	*V. vinifera* L.	China
3	Zijixin	ancient variety of China, unknown	*V. vinifera* L.	China
4	Baijixin	ancient variety of China, unknown	*V. vinifera* L.	China
5	Thompson Seedless	unknown	*V. vinifera* L.	West Asia
6	87-1	unknown	*V. vinifera* L.	China
7	Fenghuang 51	uncertain	*V. vinifera* L.	China
8	Guibao	Ispissar × Muscat Вира	*V. vinifera* L.	China
9	Jingxiu	Pannoniariiiacse × (Muscat Hamburg × Monukka)	*V. vinifera* L.	China
10	Shenyangmeigui	Sport of Muscat Hamburg	*V. vinifera* L.	China
11	Xiabai	unknown	*V. vinifera* L.	China
12	Zaomanao	Muscat Hamburg × Jingzaojing	*V. vinifera* L.	China
13	Zexiang	Muscat Hamburg × Longyan	*V. vinifera* L.	China
14	Jingzaojin	Queen of Vineyard × Thompson Seedless	*V. vinifera* L.	China
15	Zhengguodawuhe	unknown	*V. vinifera* L.	China
16	Zizhengxiang	Shenyangmeigui × Sport of Zixiangshui	*V. vinifera* L. × *V. labrusca* L.	China
17	Zhengzhouzaoyu	Queen of Vineyard × Italia	*V. vinifera* L.	China
18	Queen of Vineyard	Elisabeth × Pearl of Csaba	*V. vinifera* L.	Hungary
19	Muscat Hamburg	Schiava Grossa × Muscat of Alexandria	*V. vinifera* L.	England
20	Red Globe	C12~80 × S45~48	*V. vinifera* L.	USA
21	Kyoho	Ishihara Wase × Centenial	*V. vinifera* L. × *V. labrusca* L.	Japan
22	Zaojuxuan	chance seedling of Kyoho	*V. vinifera* L. × *V. labrusca* L.	China
23	Jingya	chance seedling of Black Olympia	*V. vinifera* L. × *V. labrusca* L.	China
24	Hongshuangwei	Queen of Vineyard × (Muscat Hamburg × TriumpH)	*V. vinifera* L. × *V. labrusca* L.	China
25	Pinot Gris	Sport of Piont Noir	*V. vinifera* L.	France
26	Cabernet Sauvignon	Cabernet Fran × Sauvignon Blan	*V. vinifera* L.	France
27	Beichun	Muscat Hamburg × *V. amurensis*	*V. vinifera* L. × *V. amurensis* Rupr.	China
28	Beiquan	Beichun × Dakeman	*V. vinifera* L. × *V. amurensis* Rupr.	China
29	Beihong	Muscat Hamburg × *V. amurensis*	*V. vinifera* L. × *V. amurensis* Rupr.	China
30	Beimei	Muscat Hamburg × *V. amurensis*	*V. vinifera* L. × *V. amurensis* Rupr.	China
31	Gongnian 1	Muscat Hamburg × *V. amurensis*	*V. vinifera* L. × *V. amurensis* Rupr.	China
32	Xiongyuebai	(Muscat Hamburg × *V. amurensis*) × Longyan	*V. vinifera* L. × *V. amurensis* Rupr.	China

Microsatellite amplification. A set of 9 microsatellite primers was selected: VVS2, VVMD5, VVMD6, VVMD7, VVMD27, VRZAG29, VRZAG62, VRZAG79 and SCU06. Six of these markers (VVMD5, VVMD7, VVMD27, VVS2, VRZAG62, and VRZAG79) have been recommended as a core set for the screening of grape cultivars (This *et al*. 2004). A multiplex PCR with these nine markers was employed. One primer of each pair was fluorescently labelled with FAM, HEX, or NED. The fragments were separated by capillary electrophoresis and genotyped with an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, USA) at Sangon Biotech (Shanghai) Co. Ltd., China.

Data analysis. PowerMarker (Version 3.25) (Liu & Muse 2005) was used to determine genetic diversity parameters of 9 microsatellite markers. These diversity measures consisted of: major allele frequencies (MAF); number of alleles (N_A); heterozygosity (Ho); expected heterozygosity (He) and polymorphism information content (PIC). Genetic similarity coefficients were calculated using the Jaccard coefficient. Cluster analysis was performed by the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method using the NTSYS package version 2.1 (Rohlf 2005). Principal Coordinates Analyses (PCoA) were conducted with GenAlEx 6 (Peakall & Smouse 2006).

RESULTS AND DISCUSSION

Microsatellite polymorphism

Genetic profiles of 9 nuclear microsatellites for grape cultivars are presented in Table 2. As a whole, high levels of heterozygosity were observed within the cultivars. The number of alleles ranged from 9 (VRZAG29) to 18 (VRZAG79) with a total of 105 alleles and an average of 11.7 alleles per locus. The average number of alleles per locus was higher than that from Spain (10.9) (Moreno-Sanz *et al*. 2011) and South America (9.67) (Martinez *et al*. 2006) with similar microsatellite markers. Some alleles common in European cultivars were also observed in Chinese grape cultivars, but several new alleles were also found, such as alleles 235, 237 and 241 of primer VRZAG79 (This *et al*. 2004) (Table 3). If we take into account only the oriental cultivars, more alleles were detected in this study than in the study of Goto-Yamamoto *et al*. (2009).

The observed heterozygosity at the marker sites across all cultivars varied from 0 (VVMD 7) to 0.844 (VVS2) with an average of 0.597. The expected heterozygosity over all cultivars ranged from 0.740 (VRZAG29) to 0.915 (VRZAG29) with a mean value of 0.827 for all loci (Table 2). These values are higher than those observed by Sefc *et al*. (2000) in European cultivars (0.677–0.819), but similar to those found in Spanish cultivars and in hybrids between French cultivars and American species (Martín *et al*. 2003). The observed heterozygosity of VVMD 7 is zero, which means the alleles detected by it are all homogeneous. It may be due to the existence of a large number of null alleles.

The PIC value provides information on the effectiveness of a marker. Accordingly, the most informative marker in the present study was VRZAG79, with a PIC of 0.908; while the least informative marker was VRZAG29, with a PIC of 0.716. The high value of PIC confirmed the

Table 2. Major allele frequencies (MAF), number of observed genotypes per locus (OG), number of observed alleles (N_A), expected (He) and observed (Ho) heterozygosity, and polymorphic information content (PIC) of the nine SSR markers for the cultivars discriminated in this study (including allelic variations)

Marker	MAF	OG	N_A	He	Ho	PIC
Vrzag29	0.453	14	9	0.740	0.688	0.716
Vrzag62	0.188	23	16	0.887	0.781	0.877
Vrzag79	0.141	23	18	0.915	0.563	0.908
VVMD5	0.359	19	11	0.805	0.750	0.784
VVMD6	0.297	13	7	0.771	0.594	0.734
VVMD7	0.156	10	10	0.875	0.000	0.862
VVMD27	0.266	14	6	0.813	0.688	0.787
SCU06	0.391	17	14	0.805	0.469	0.790
VVS2	0.313	22	14	0.833	0.844	0.816
Mean	0.285	17.2	11.7	0.827	0.597	0.808

Table 3. Genetic profiles (allele sizes in bp) of 32 grape cultivars (listed in Table 1) obtained for the nine SSR loci analysed in this study

	VRZAG29	VRZAG62	VRZAG79	VVMD5	VVMD6	VVMD7	VVMD27	SCU06	VVS2
1	–/108	186/192	–/238	230/237	206/209	246/246	178/184	–/163	132/148
2	108/110	188/196	–/248	221/232	189/206	–/–	–/193	189/206	145/154
3	–/108	191/203	250/252	230/234	189/206	232/232	179/193	162/163	141/148
4	108/110	–/204	–/249	234/236	189/209	242/242	184/193	171/–	130/132
5	109/111	186/188	–/249	232/234	207/209	248/248	184/193	171/–	130/139
6	108/112	202/–	–/245	236/287	–/209	–/–	182/184	–/172	–/122
7	108/112	186/204	–/245	224/226	207/209	246/246	178/193	171/175	130/148
8	–/108	–/186	250/253	226/234	207/209	246/246	178/184	170/171	132/148
9	108/112	194/204	247/257	234/238	189/209	–/–	180/193	164/–	132/154
10	–/108	186/204	253/257	234/–	189/209	247/247	178/180	164/171	130/–
11	108/113	188/204	–/241	–/226	–/207	242/242	184/193	–/171	132/150
12	108/111	192/204	253/255	230/236	206/209	242/242	179/193	–/171	130/148
13	–/109	193/201	235/237	–/236	199/209	240/240	178/184	–/171	120/132
14	107/109	189/192	–/246	236/–	199/206	246/246	–/179	170/171	132/–
15	109/111	–/187	–/245	–/232	207/209	238/238	180/180	166/167	143/150
16	108/112	186/206	235/238	229/234	207/209	234/234	178/184	163/171	132/148
17	–/109	186/204	–/238	230/234	189/209	246/246	–/–	171/–	132/154
18	–/108	186/–	–/238	234/236	209/–	248/248	178/184	164/–	130/132
19	108/110	188/192	250/255	230/234	186/207	237/237	184/193	161/–	138/141
20	–/108	188/196	–/238	226/238	–/206	232/232	178/193	171/206	141/148
21	108/112	187/203	235/241	–/230	–/207	234/234	178/184	170/171	120/130
22	112/108	188/204	241/249	229/234	207/–	234/234	182/184	–/171	120/132
23	108/112	189/204	235/249	230/234	207/–	234/234	178/184	162/171	130/132
24	106/108	–/–	237/253	229/234	200/207	248/248	178/184	163/–	122/132
25	102/108	188/196	–/241	–/234	–/207	242/242	180/184	–/207	124/132
26	–/108	–/193	241/249	224/238	189/199	238/238	–/180	162/163	130/141
27	109/110	185/187	253/257	224/234	–/209	238/238	179/180	167/–	–/132
28	109/113	187/191	237/251	230/234	–/206	240/240	–/182	171/–	132/141
29	111/113	185/204	250/254	–/234	–/189	240/240	–/182	171/175	126/132
30	–/102	175/192	256/262	230/234	–/206	236/236	–/–	162/163	130/132
31	109/113	188/192	253/262	234/236	–/206	240/240	–/182	162/163	126/148
32	108/112	188/192	–/253	234/236	206/209	240/240	182/–	–/179	148/–

– indicates that the cultivar is either homozygous or heterozygous with a null allele

finding of Martinez *et al.* (2006), who also observed high values of PIC (from 0.70 to 0.88) in grape. Based on the numbers of observed alleles, expected heterozygosity and PIC values in this study, the results indicated VRZAG 79 as the most informative marker (Table 2). Martinez *et al.* (2006) also regarded VRZAG62 as the most informative marker in their studies, not VVMD5. Clearly, these findings improved our knowledge of the genetic diversity of grape in China.

Genetic relatedness

The UPGMA analysis (Figure 1) confirmed the genetic diversity mentioned above. In fact, the constructed dendrogram exhibited 3 distinct groups (Figure 1), indicating complex genetic diversity of the Chinese grape germplasm.

The cultivars of C1 in the dendrogram are mainly wine grape. Two internationally important wine grape cultivars (Cabernet-Sauvignon, Pinot Gris)

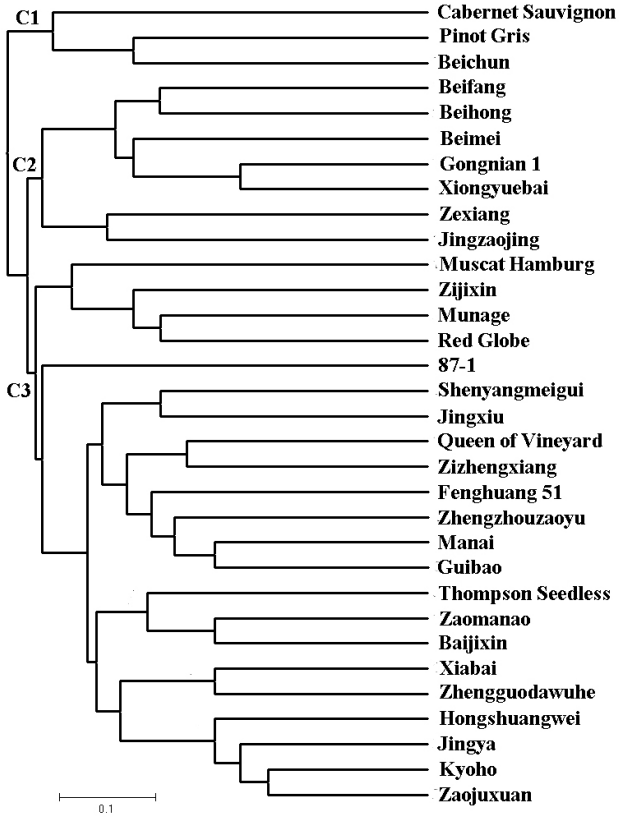

Figure 1. UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram obtained from microsatellite data for 32 grape cultivars

and one Chinese cultivar, Beichun, were in C1. The first subcluster of C2 comprised other wine grapes, Beifang, Beihong, Beimei, Gongnian 1 and Xiongyuebai, which are related with *V. amurensis*. It is in agreement with the results of SRAP (Sequence-Related Amplified Polymorphism) and SCoT (Start Codon Targeted) analysis in grape (Guo *et al*. 2012a, b) and previous microsatellite analysis (Aradhya *et al*. 2003). Zexiang and Jingzaojin are clustered into another subcluster of C2.

Muscat Hamburg, Red Globe, Munage and Zijixin grouped in the first subcluster of C3. Both Munage and Zijixin are the indigenous Chinese grape cultivars. 87-1 is an early ripening grape variety widely cultivated in China. However, nothing is known about its origin. In the dendrogram, it formed one single subcluster within C3. The second subcluster of C3 is the largest cluster, it included 8 cultivars, Shenyangmeigui, Fenghuang 51, Guibao, Jingxiu, Hongshuangwei, Zhengzhouzaoyu, Manai and Queen of Vineyard. The origin of Fenghuang 51 is also unknown. But, it was situated between Hongshuangwei and Zhengzhouzaoyu, it shares one allele at five loci with Queen of Vineyard, so it may have a certain close relationship with Queen of Vineyard. More proofs are needed to validate its parentage. Zaomanao, Baijixin and Thompson Seedless constituted the third subcluster of C3. Zaomanao is the offspring of Jingzaojin, while Jingzaojin is the descendant of Thompson Seedless. So, Zaomanao clustered with Thompson Seedless. The fifth subcluster of C3 consisted of Xiabai and Zhengguodawuhe. The cultivars in the last subcluster of C3 are all hybrids of European and American species.

In the dendrogram, not all parent cultivars were close to their offspring, such as Muscat Hamburg with Zaomanao and Zexiang, Thompson Seedless and Jingzaojin. This is not a surprise, since only one parent was available for pedigree validation in these cases. When reconstructing or validating a pedigree, the best results would be obtained when both parents were presented (Zoghlami *et al*. 2009). Microsatellite markers may succeed or fail at times for discriminating clones (Franks *et al*. 2002; Stenkamp *et al*. 2009). In summary, the dendrogram showed a clear separation among table grape, wine grape of *V. vinifera* and the hybrids of European and American cultivars.

The cultivars were also resolved based on PCoA analysis in order to obtain further cluster results. The first three coordinates of the PCoA analysis explained 23%, 19% and 16% of the total variance, respectively (Figure 2). The cultivars from the descent of *V. amurensis* (G3) are clearly differentiated from other accessions according to the first two factors, and the hybrid of European and American cultivars (G2) are also distinguished.

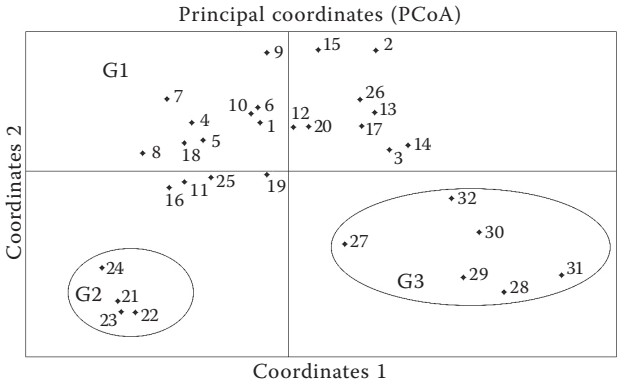

Figure 2. Principal coordinates analysis of 32 grape samples analysed with 9 SSR loci plotted on the first two coordinates; numbers are corresponding to the materials in Table 1

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