Molecular Characterization of Chinese Grape Landraces (*Vitis* L.) Using Microsatellite DNA Markers

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Abstract. In this study, we present the molecular characterization of 61 Chinese grape landraces and 33 foreign cultivars by using nine microsatellite DNA markers. A total of 115 distinct alleles were amplified, and the average allele number was 12.78. The average observed and expected heterozygosity values were 0.797 and 0.839, respectively. The effective allele numbers ranged from 5.011 to 8.575. The average polymorphism information content (PIC) was 0.816. Eighty distinct genotypes were detected, and new synonyms and homonyms were found. The clustering dendrogram indicated that 94 *Vitis* materials could be divided into five major groups, and the cluster analysis showed that part of the Chinese grape landraces had a close relationship with the foreign cultivars. Assessment of the true cultivar identity, and the identification of synonyms and homonyms will be a contribution to improve the grape germplasm management and protect breeders' intellectual rights.

The grape (*Vitis* L.), one of the most important fruit crops in the world, is grown in a wide range of environments (Liu et al., 2012). It is an important member of Vitaceae and has significant economic value. There are more than 70 species in the *Vitis* genus worldwide, and they are mainly found in the temperate regions of the Northern Hemisphere. The varieties mostly originated from three regions: Europe and western

Asia, North America, and eastern Asia (Alleweldt and Possingham, 1988; Kong, 2004; Mullins et al., 1992). Among these, *Vitis vinifera* L., which originates from Europe and west Asia, is the only species cultivated extensively around the world (This et al., 2006). About 6000–10,000 *V. vinifera* cultivars exist worldwide (Galet, 2000).

China has a long history of grape cultivation. Viticulture spread along the ancient Silk Road and it reached China in the second century AD (Nunez and Walker, 1989). In the midlate 19th century, the Catholic Church replaced the Romans in expanding grape cultivation (Royer, 1988). After the introduction of the grape into different locations in China, the original cultivar names were lost and they are still unknown today. Through long-term cultivation, many cultivars with excellent phenotypic traits were retained and regarded as local cultivars, and new names were given to these cultivars by local farmers. Therefore, many local grape cultivars exist in China. Currently, over 1400

grape accessions, including local cultivars from different sources, have been collected from China and overseas. Furthermore, these local cultivars have played a major role in breeding in China because of their rich morphological variation (Guo et al., 2012). Many new grape cultivars were bred with these local cultivars (Chang and Liu, 1993; Guo et al., 2012; Liu et al., 2002). Several local cultivars have also played key roles in promoting the development of the local economy. Cultivars such as the Guankouputao, one of the Geographical Indication Products in Jianshi County, Hubei Province, is highly accepted by the consumer (Giovannucci et al., 2009; Li et al., 2014). In recent years, with the continuous development of modern agriculture, growers abandoned the landrace varieties and instead switched to modern cultivars or recently bred cultivars. Therefore, many germplasm resources that were not preserved were lost permanently.

DNA molecular marker technique showing polymorphism at the DNA level is a powerful tool for the characterization of germplasms (Queiroz et al., 2015), the identification of cultivars (Gross et al., 2012; Semagn et al., 2006), and the estimation of genetic diversity (Mashilo et al., 2016; Parveen et al., 2016; Urrestarazu et al., 2015). Among the various DNA-based molecular markers, simple sequence repeats (SSRs), with the advantages of codominance inheritance, a high degree of polymorphism, reproducibility, and simple operation (Sefc et al., 2001), have been widely used in genetic diversity analyses of grape cultivars and the identification of germplasms (Emanuelli et al., 2013; Guo et al., 2010; Marinoni et al., 2009; This et al., 2004; Villano et al., 2014). Some countries have already used SSR markers to characterize grape collections, such as the United States (Adhikari et al., 2014), Hungary (Harangozó et al., 2013), Croatia (Maletić et al., 2015), Brazil (Leão et al., 2009), Slovenia (Stajner et al., 2011), Italy (Crespan et al., 2008), and France (Lauco et al., 2011).

However, only limited work has been published concerning Chinese grape landraces analyzed using SSR markers (Goto-Yamamoto et al., 2009; Guo et al., 2010). Most studies merely characterized several cultivars, and the genetic relationships among the landraces and introduced cultivars in China are not well known. Therefore, considerable effort should be exerted to characterize the genetic diversity among landraces and the introduced cultivars. In this study, we used a set of nine microsatellite markers recommended as the standard set for the screening of grapevine collections to characterize 61 Chinese grape landraces and 33 foreign cultivars (Mihaljevic et al., 2013). The aim of our study is to differentiate cultivars with similar morphological traits, and to better assess the genetic diversity and genetic relatedness among these landraces and introduced cultivars. The information obtained from our studies will be useful in the future

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Table 1. List of grape cultivars used in this study.

	Cultivar name	Pedigree	Species	Utility	Origin
1	Aierweiyin	Unknown	Unknown	Table/juice grape	Hebei, China
2	Alphonse Lavallée	Kharistvala Kolkhuri × Muscat of Hamburg	<i>Vitis vinifera</i>	Table grape	France
3	Atebage	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
4	Baiburuike	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
5	Baidalayi	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
6	Baiputao	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
7	Baiyouliang	Unknown	<i>V. vinifera</i>	Table grape	Unknown
8	Beijiagan	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
9	Cabernet Franc	Unknown	<i>V. vinifera</i>	Wine grape	France
10	Cabernet Gernischt	Moural × Cabernet Franc	<i>V. vinifera</i>	Wine grape	France
11	Cabernet Sauvignon1	Cabernet Franc × Sauvignon blanc	<i>V. vinifera</i>	Wine grape	France
12	Cabernet Sauvignon2	Cabernet Franc × Sauvignon blanc	<i>V. vinifera</i>	Wine grape	France
13	Campbell	Unknownm	Unknown	Wine grape	United States
14	Campbell Early	Moore Early × (Belvidere × Muscat of Hamburg)	<i>V. vinifera</i> × <i>Vitis labrusca</i>	Table/juice grape	United States
15	Champion	Unknown	<i>V. labrusca</i>	Juice grape	United States
16	Chardonnay	Pinot × Gouais Blanc	<i>V. vinifera</i>	Wine grape	France
17	Cizhongjiaotang	Unknown	<i>V. vinifera</i>	Wine grape	Yunnan, China
18	Concord	Chance seedling, uncertain	<i>V. labrusca</i>	Juice grape	United States
19	Cuiputao	Unknown	<i>V. vinifera</i>	Table grape	Shanxi, China
20	Dabaiputao	Unknown	<i>V. vinifera</i>	Table grape	Hebei, China
21	Guankouputao	Unknown	Unknown	Table/juice grape	Hubei, China
22	Guizhoushuijing	Unknown	Unknown	Table/juice grape	Guizhou, China
23	HashiHaerf	Unknown	<i>V. vinifera</i>	Table/wine grape	Xinjiang, China
24	Heijixin	Unknown	<i>V. vinifera</i>	Table grape	Shanxi, China
25	Heipohuang	Unknown	<i>V. vinifera</i>	Table grape	Shanxi, China
26	Heiputao1	Unknown	<i>V. vinifera</i>	Table grape	Shanxi, China
27	Heiputao2	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
28	Hetianhong	Unknown	<i>V. vinifera</i>	Table/wine grape	Xinjiang, China
29	Hetianlvputao	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
30	Hongdalayi	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
31	Honglianzi	Muscat Hamburg × Queen of Vineyard	<i>V. vinifera</i>	Table grape	China
32	Hongmanai	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
33	Hongmunage	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
34	Hongpinger	Unknown	<i>V. vinifera</i>	Table grape	Shanxi, China
35	Hongpohuang	Unknown	<i>V. vinifera</i>	Table grape	Shanxi, China
36	Hongputao1	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
37	Hongputao2	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
38	Hongputao3	Unknown	<i>V. vinifera</i>	Table/wine grape	Shanxi, China
39	Hongwuzilu	Unknown	<i>V. vinifera</i>	Table/raisin grape	Iran
40	Huabai	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
41	Huangmanji	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
42	Italia	Bicane × Muscat Hamburg	<i>V. vinifera</i>	Table grape	Italy
43	Jiaka	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
44	Kala	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
45	Kusikaqi	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
46	Lingdanputao	Unknown	<i>V. vinifera</i>	Table grape	Shanxi, China
47	Lizixiang	Unknown	<i>V. vinifera</i>	Table grape	Hebei, China
48	Longyan	Unknown	<i>V. vinifera</i>	Table/wine grape	Shanxi, China
49	Lvmunage	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
50	Lvputao	Unknown	<i>V. vinifera</i>	Table/wine grape	Xinjiang, China
51	Lvnai	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
52	Manai	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
53	Marezi	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
54	Meiguimi	Unknown	Unknown	Table/wine grape	Yunnan, China
55	Merlot1	Magdeleine Noire Des Charentes × Cabernet Franc	<i>V. vinifera</i>	Wine grape	France
56	Merlot2	Magdeleine Noire Des Charentes × Cabernet Franc	<i>V. vinifera</i>	Wine grape	France
57	Moyuputao	Unknown	<i>V. vinifera</i>	Table/wine grape	Xinjiang, China
58	Mudanhong	Unknown	<i>V. vinifera</i>	Table grape	Unknown
59	Munage	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
60	Muscat of Alexandria	Muscat Blanc a Petit Grain × Axina de Tres Bias	<i>V. vinifera</i>	Table grape	Egypt
61	Muscat Blanc a Petit Grain	Unknown	<i>V. vinifera</i>	Table/wine grape	Greece
62	Muscat Hamburg	Schiava Grossa × Muscat of Alexandria	<i>V. vinifera</i>	Table grape	England
63	Myckat Kpachbrh	Unknown	<i>V. vinifera</i>	Table/wine grape	Bulgaria
64	Nabuguzhu	Unknown	<i>V. vinifera</i>	Wine grape	Xizang, China
65	Niagara	Concord × Cassady	<i>V. vinifera</i> × <i>V. labrusca</i>	Table/juice grape	United States
66	Niunai	Unknown	<i>V. vinifera</i>	Table grape	Hebei, China
67	Niuxin	Unknown	<i>V. vinifera</i>	Table grape	Hebei, China

(Continued on next page)

Table 1. (Continued) List of grape cultivars used in this study.

	Cultivar name	Pedigree	Species	Utility	Origin
68	Pannoniavinesa	Ivan × Logan	<i>V. vinifera</i>	Table grape	Hungary
69	Pearl of Csaba	Muskatellier d'	<i>V. vinifera</i>	Table grape	Hungary
		Hongrie × Muscat Ottonel			
70	Pingdinghei	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
71	Pinger	Unknown	<i>V. vinifera</i>	Table grape	Shanxi, China
72	Pinot Noir	Unknown	<i>V. vinifera</i>	Wine grape	France
73	Qiaowushi	Unknown	<i>V. vinifera</i>	Table grape	Turkey
74	Qiligan	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
75	Riesling	Unknown	<i>V. vinifera</i>	Wine grape	Germany
76	Roter Traminer	Offspring of Uva Aminea	<i>V. vinifera</i>	Wine grape	Greece
77	Suosuoputao	Unknown	<i>V. vinifera</i>	Raisin grape	Xinjiang, China
78	Syrah	Unknown	<i>V. vinifera</i>	Wine grape	France
79	Taokekeluke	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
80	Thompson Seedless1	Unknown	<i>V. vinifera</i>	Table/raisin grape	Gongyi, China
81	Thompson Seedless2	Unknown	<i>V. vinifera</i>	Table/raisin grape	Ningxia, China
82	Thompson Seedless3	Unknown	<i>V. vinifera</i>	Table/raisin grape	Afghanistan
83	Thompson Seedless4	Unknown	<i>V. vinifera</i>	Table/raisin grape	West Asia
84	Triumph	Concord × Chasselas Musqué	<i>V. vinifera</i> × <i>V. labrusca</i>	Table grape	United States
85	Tuoxianputao	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
86	Urbana	Unknown	Unknown	Table/juice grape	United States
87	Xiabai	Unknown	<i>V. vinifera</i>	Table grape	Shanxi, China
88	Xiaoheputao	Unknown	Unknown	Wine grape	Jiangsu, China
89	Xiekelange	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
90	Xiying	Unknown	<i>V. vinifera</i>	Table grape	Shanxi, China
91	Yeliake	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China
92	Yilixiangputao	Unknown	<i>V. vinifera</i>	Table/wine grape	Xinjiang, China
93	Yunnanshuijing	Unknown	Unknown	Table/raisin grape	Yunnan, China
94	Zihongxingputao	Unknown	<i>V. vinifera</i>	Table grape	Xinjiang, China

The “origin” of the foreign cultivar refers to the geographical origin of that accession. The “origin” of the Chinese grape landrace refers to the region where it was collected.

breeding and management of these germplasm resources in ex situ conservation.

Materials and Methods

Plant materials and DNA extraction. Sixty-one local cultivars and 33 introduced cultivars were analyzed in this study (Table 1). These samples were collected from the National Grape Germplasm Repository of the Zhengzhou Fruit Research Institute of the Chinese Academy of Agricultural Sciences (34°42'47.52"N, 113°42'3.19"E).

The genomic DNA was isolated from young leaves using a Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. The DNA quality was checked by agarose gel electrophoresis analysis, and the DNA concentration and purity were determined using a NanoDrop 1000 spectrophotometer (NanoDrop, Wilmington, DE). Genomic DNA was normalized to 30–50 ng/μL and was stored at –20 °C.

Polymerase chain reaction amplification and fragment sizing. For the characterization of grape landraces, the following nine SSR loci distributed on six chromosomes in the grape were used: VVMD5 and VVMD7 (Bowers et al., 1996); VVS2 (Thomas and Scott, 1993); VVMD32, VVMD25, VVMD27, and VVMD28 (Bowers et al., 1999a); VrZAG62 and VrZAG79 (Sefc et al., 1999). These markers, each with high polymorphisms, were used by the European GrapeGen06 consortium (<http://www1.montpellier.inra.fr/grapegen06/accueil.php>) and have been frequently used to identify cultivars (Mihaljevic et al., 2013; This et al., 2004).

The polymerase chain reaction (PCR) was performed in a 20-μL volume containing 30–50 ng of template DNA, 1 × PCR buffer (Mg²⁺ plus) (TaKaRa, Japan), 0.25 mM dNTPs, 0.5 μM of each primer, and 1 U of Taq DNA polymerase (TaKaRa, Japan). The forward primers were labeled with FAM or HEX fluorescent dye at the 5'-end. The PCR amplification was conducted in a Biometra TGradient Thermocycler (Biometra, Göttingen, Germany) and was performed according to the following thermal cycles: 4 min at 94 °C for initial denaturation, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at optimum Ta for 30 s, 72 °C for 45 s and a final extension at 72 °C for 10 min, finally, the reaction was held at 4 °C. PCR amplification products were separated by capillary electrophoresis in an ABI 3730xl DNA Analyser (Applied Biosystems, Carlsbad, CA). Raw data obtained by capillary electrophoresis were converted into allelic sizes using Gene Mapper v3.2 software (Applied Biosystems, Foster City, CA), and fragment calibration was performed with an internal size standard (GeneScan-500ROX; PE Applied Biosystems).

Data scoring and analysis. The microsatellite data were analyzed with the statistical software POPGENE version 1.32 (<https://sites.ualberta.ca/~fyeh/popgene.html>), and the number of alleles per locus (N_a), number of effective alleles (N_e), observed heterozygosity (H_o), and expected heterozygosity (H_e) were calculated. The PIC of each SSR was calculated using the following formula: $PIC = 1 - \sum_{j=1}^n P_{ij}^2$ (Botstein et al., 1980), where P_{ij} is the frequency of the j th allele for the i th SSR marker.

For statistical analysis, the amplified fragments were scored as either 1 (present) or

0 (absent) for each of the SSR markers. Then, the data were converted into a binary matrix and analyzed using the software package NTSYSpc 2.1 (Rohlf, 2000). The genetic similarity matrix based on the Dice coefficient of similarity was used to construct a dendrogram showing the approximate relationships among a set of accessions based on the unweighted pair group with arithmetic mean (UPGMA) method using the Mega-5.0 software (Tamura et al., 2011).

Results and Discussion

Microsatellite analysis. DNA from all of the samples was successfully amplified using each of the 9 SSRs, and 80 distinct genotypes were detected by analyzing 94 accessions (Table 2). A total of 115 alleles, with sizes between 121 and 289 base pairs, were obtained from 9 SSR primers (Table 2). The number of alleles per locus ranged from 10 alleles (VrZAG62) to 17 alleles (VVMD28), with an average of 12.78 alleles per locus (Table 3), which is higher than the findings obtained from some other national collections (Alba et al., 2015; Maletić et al., 2015; Stajner et al., 2011; Tomić et al., 2012) but is in accordance with the results of the southern Italy collections (Villano et al., 2014). This difference is due to the increased existence of low-frequency alleles in large-scale sample sets (Laiadi et al., 2009).

In this study, the percentage of heterozygotes of the nine loci was high, indicating the high variability present in the samples. The expected heterozygosity values ranged from 0.805 at locus VVMD32 to 0.888 at locus VVS2, the average value of the expected

Table 2. Genetic profiles of 64 grape landraces and 30 introduced cultivars at 9 microsatellite loci.

	Cultivar name	Microsatellite loci								
		VrZAG79	VrZAG62	VVMD5	VVS2	VVMD7	VVMD28	VVMD25	VVMD27	VVMD32
14	Campbell Early	234/244	202/204	233/233	121/131	233/245	223/227	238/238	175/181	236/268
18	Concord	244/256	200/204	225/231	123/131	233/239	227/241	238/238	181/181	268/268
13	Campbell	244/256	200/204	233/233	123/131	233/239	227/241	238/238	181/181	268/268
54	Meiguimi	234/254	200/202	235/235	121/149	233/247	223/233	238/246	175/179	244/268
1	Aierweiyin	244/252	184/200	225/289	123/131	233/247	223/241	246/246	175/181	260/260
15	Champion	244/244	200/200	235/289	123/149	233/233	223/231	246/246	179/181	268/268
86	Urbana	236/244	200/204	235/235	123/133	233/239	223/241	240/252	181/181	242/268
58	Mudan hong	244/244	192/202	225/229	123/133	245/255	227/245	246/252	179/181	236/268
63	Myckat Kpachbrh	244/256	200/202	225/229	123/131	233/245	215/227	238/238	183/185	236/242
30	Hongdalayi	244/246	192/202	225/233	131/141	237/245	215/265	238/252	181/185	236/236
84	Triumph	236/252	184/184	235/235	123/147	233/247	227/241	246/246	181/181	268/268
62	Muscat Hamburg	236/252	184/190	229/235	133/147	245/247	233/241	246/252	175/181	268/268
2	Alphonse Lavallee	236/248	184/202	223/235	131/133	247/253	241/241	236/252	181/181	248/268
31	Honglianzi	236/248	184/190	223/235	131/133	245/247	241/241	238/252	181/181	268/268
42	Italia	252/254	190/202	229/235	131/147	241/245	231/241	236/246	175/191	248/268
60	Muscat of Alexandria	244/252	184/202	225/229	131/147	241/247	241/265	246/246	175/191	260/268
69	Pearl of Csaba	252/256	184/202	233/233	131/153	245/247	215/265	238/238	175/177	268/268
92	Yilixiangputao	248/252	184/202	225/233	131/131	245/247	243/265	238/246	175/181	236/268
61	Muscat Blanc a Petit Grain	248/252	184/194	225/233	131/131	231/247	243/265	238/246	175/191	260/268
81	Thompson Seedless2	244/254	186/186	231/237	139/149	251/251	215/241	246/252	177/191	246/252
82	Thompson Seedless3	254/256	186/186	229/231	141/149	237/237	215/241	244/246	191/191	246/270
39	Hongwuzulu	236/242	186/186	231/231	143/149	237/251	215/241	236/246	177/191	246/246
90	Xiying	236/248	186/186	231/231	143/149	237/251	215/241	236/246	177/191	246/246
37	Hongputao2	244/248	184/194	225/237	141/149	241/245	241/241	246/252	175/177	246/268
35	Hongpohuang	252/254	202/202	231/233	141/165	231/245	241/241	250/252	189/189	254/268
32	Hongmanai	244/254	186/194	225/237	149/153	231/251	241/241	242/246	175/191	252/254
40	Huabai	244/254	186/202	225/231	133/141	243/251	237/237	252/260	175/191	246/252
33	Hongmunage	244/254	186/200	225/231	131/131	231/251	235/241	246/260	175/191	246/268
45	Kusikaqi	244/254	186/202	225/233	133/149	237/247	215/255	236/246	191/191	246/248
89	Xiekelange	240/254	186/202	225/225	133/149	241/247	241/241	246/252	181/191	240/248
4	Baiburuike	248/248	186/194	221/233	137/153	237/241	241/241	242/252	177/191	246/254
51	Lvnai	248/248	194/194	221/233	133/153	237/241	241/255	242/260	175/191	246/268
85	Tuoxianputao	248/252	194/202	221/231	141/153	231/241	241/241	242/250	175/191	246/254
79	Taokekeluke	244/246	186/194	221/231	141/145	241/251	241/255	252/252	175/191	246/254
67	Niuxin	246/254	194/202	221/231	141/145	241/245	241/241	252/252	191/191	268/268
5	Baidalayi	248/254	186/194	223/233	149/153	241/245	241/241	242/246	181/191	254/254
71	Pinger	248/254	194/202	233/233	121/131	237/245	241/255	242/252	175/191	268/268
34	Hongpinger	248/254	194/202	233/233	141/153	237/245	241/255	242/252	175/191	268/268
7	Baiyouliang	244/248	186/202	231/233	133/147	231/241	233/241	246/252	175/181	268/268
47	Lizixiang	248/254	186/202	221/231	133/165	241/245	233/241	246/250	175/191	236/268
8	Beijiagan	240/242	192/192	223/225	141/149	245/255	241/241	242/250	175/191	246/254
20	Dabaiputao	248/252	192/192	221/231	141/149	231/241	231/241	246/252	177/177	248/248
28	Hetianhong	244/248	194/200	225/237	141/149	231/245	241/241	246/252	175/177	246/268
29	Hetianlvputao	244/244	186/202	223/225	133/139	231/241	241/245	238/252	175/175	254/254
25	Heipohuang	248/248	186/194	233/235	133/133	237/241	255/275	260/260	175/175	252/268
41	Huangmanji	246/248	186/192	231/233	133/153	241/247	233/255	236/242	175/191	254/268
43	Jiaka	244/248	194/200	225/233	123/133	231/243	241/241	238/252	175/181	254/254
53	Marezi	244/254	194/202	231/233	123/141	231/245	241/241	238/252	175/191	254/254
57	Moyuputao	244/244	200/202	225/231	149/153	231/245	241/241	260/260	175/177	248/268
70	Pingdinghei	246/254	186/192	231/233	135/139	247/251	233/235	236/254	177/191	246/254
74	Qiligan	244/244	192/202	225/231	133/145	245/247	233/241	252/262	177/181	248/268
73	Qiaowushi	246/248	186/194	235/243	133/141	237/247	233/255	246/252	175/175	260/268
68	Pannoniavinesa	248/254	184/186	225/233	131/141	241/247	231/265	238/252	181/191	236/268
75	Riesling	240/242	192/202	223/231	141/149	247/255	225/231	246/252	177/185	248/268
87	Xiabai	244/254	202/202	229/231	133/165	231/241	241/275	236/250	191/191	252/254
91	Yeliake	244/252	186/202	231/233	123/131	231/241	241/255	238/252	175/191	256/268
77	Suosuoputao	238/244	184/186	231/233	131/139	247/251	215/265	238/252	181/191	252/268
44	Kala	252/254	192/200	225/233	149/153	245/247	241/257	238/252	181/181	248/268
46	Lingdanputao	246/246	186/202	233/235	133/133	241/247	241/275	252/260	175/181	252/254
64	Nabuguzhu	244/244	186/202	229/237	121/131	237/245	231/233	236/246	175/181	236/236
16	Chardonnay	240/242	186/194	231/235	135/141	237/241	215/225	236/252	177/185	236/268
72	Pinot Noir	236/242	186/192	225/235	135/149	237/241	215/233	236/246	181/185	236/268
9	Cabernet Franc	244/256	192/202	223/237	137/149	237/261	225/233	236/252	177/185	236/254
10	Cabernet Gernischt	236/244	186/202	223/235	137/145	237/251	233/245	236/252	171/185	236/236
76	Roter Traminer	242/248	186/192	229/235	149/249	241/255	231/233	246/246	185/185	236/268
50	Lvputao	244/248	186/192	233/235	131/131	237/255	231/241	238/246	171/181	236/268
78	Syrah	242/248	186/192	223/229	131/131	237/237	217/227	238/238	185/187	236/268
88	Xiaoheiputao	254/256	168/192	233/247	133/135	227/241	215/247	238/252	187/191	222/238
17	Cizhongjiaotang	236/256	194/198	231/263	131/143	237/263	241/255	236/236	177/203	268/268
55	Merlot1 ^a	256/256	192/192	223/233	137/149	237/245	225/231	236/246	185/187	236/236
56	Merlot2 ^a	256/256	192/192	223/233	137/149	237/245	225/231	236/246	185/187	236/236
11	Cabernet Sauvignon1 ^b	244/244	186/192	229/237	137/149	237/237	231/233	236/246	171/185	236/236
12	Cabernet Sauvignon2 ^b	244/244	186/192	229/237	137/149	237/237	231/233	236/246	171/185	236/236

(Continued on next page)

Table 2. (Continued) Genetic profiles of 64 grape landraces and 30 introduced cultivars at 9 microsatellite loci.

	Cultivar name	Microsatellite loci								
		VrZAG79	VrZAG62	VVMD5	VVS2	VVMD7	VVMD28	VVMD25	VVMD27	VVMD32
80	ThompsonSeedless1 ^c	244/256	186/186	231/231	143/149	237/251	215/241	236/246	177/191	246/246
83	ThompsonSeedless4 ^c	244/256	186/186	231/231	143/149	237/251	215/241	236/246	177/191	246/246
65	Niagara ^d	234/256	200/202	233/233	121/131	233/239	227/233	238/238	175/181	244/268
21	Guankouputao ^d	234/256	200/202	233/233	121/131	233/239	227/233	238/238	175/181	244/268
22	Guizhoushuijing ^d	234/256	200/202	233/233	121/131	233/239	227/233	238/238	175/181	244/268
93	Yunnanshuijing ^d	234/256	200/202	233/233	121/131	233/239	227/233	238/238	175/181	244/268
3	Atebage ^e	244/254	186/186	237/237	133/149	241/251	233/233	246/252	175/191	246/254
36	Hongputao1 ^e	244/254	186/186	237/237	133/149	241/251	233/233	246/252	175/191	246/254
48	Longyan ^f	252/252	202/202	231/233	141/165	231/245	241/241	250/252	189/189	254/268
38	Hongputao3 ^f	252/252	202/202	231/233	141/165	231/245	241/241	250/252	189/189	254/268
24	Heijixin ^g	244/248	202/202	229/233	133/133	241/247	245/275	236/260	175/191	252/254
26	Heiputao1 ^g	244/248	202/202	229/233	133/133	241/247	245/275	236/260	175/191	252/254
23	Hashihaer ^h	244/254	186/202	225/231	133/141	243/251	235/235	252/260	175/191	246/252
6	Baiputao ^h	244/254	186/202	225/231	133/141	243/251	235/235	252/260	175/191	246/252
49	Lvmunage ⁱ	244/254	186/200	225/231	133/149	231/251	235/241	246/260	175/191	246/268
59	Munage ⁱ	244/254	186/200	225/231	133/149	231/251	235/241	246/260	175/191	246/268
52	Manai ^j	246/246	186/194	221/231	145/153	241/241	241/255	242/252	191/191	246/268
19	Cuiputao ^j	246/246	186/194	221/231	145/153	241/241	241/255	242/252	191/191	246/268
66	Niunai ^j	246/246	186/194	221/231	145/153	241/241	241/255	242/252	191/191	246/268
27	Heiputao2 ^k	248/254	186/192	225/243	133/151	241/247	231/231	252/252	181/191	252/268
94	Zihongxingputao ^k	248/254	186/192	225/243	133/151	241/247	231/231	252/252	181/191	252/268

Clones with identical profiles are represented by the same letters, i.e., a, b, c, d, e, f, g, h, i, j, k.

Table 3. Number of alleles (Na), effective number of alleles (Ne), observed (H_o) and expected (H_e) heterozygosity, and polymorphism information content (PIC) of the nine simple sequence repeat loci analyzed.

Locus	Na	Ne	H_o	H_e	PIC
VrZAG79	11	6.449	0.811	0.849	0.828
VrZAG62	10	5.250	0.768	0.814	0.784
VVS2	15	8.575	0.884	0.888	0.873
VVMD5	12	6.631	0.811	0.854	0.832
VVMD7	14	7.920	0.905	0.878	0.861
VVMD28	17	5.635	0.747	0.827	0.810
VVMD25	11	5.486	0.768	0.822	0.793
VVMD32	14	5.011	0.695	0.805	0.778
VVMD27	11	5.176	0.779	0.811	0.780
Mean	12.78	6.237	0.797	0.839	0.815

heterozygosity is 0.839, which was higher than that reported by Carimi et al. (2010) (0.791) among Sicilian grapevine cultivars. The values of observed heterozygosity for individual loci ranged from 0.695 at locus VVMD32 to 0.905 at locus VVMD7, with a mean of 0.797 (Table 3). The mean expected heterozygosity was slightly higher than the observed heterozygosity. This difference could be explained by the lack of both natural and human selection, which reduced homozygosity in grape plants (Sefc et al., 2001).

The values of PIC varied from 0.778 to 0.873, with a mean of 0.815. The most informative locus was VVS2 (0.873) and the least informative locus was VVMD32 (0.778) (Table 3). The mean PIC value was higher than that reported by Ramezani et al. (2009) (0.77) in grape, the high PIC value suggesting a high discriminating ability of the SSR markers for the grape landraces.

Duplicates and homonyms. Of the 94 cultivars analyzed in this study, the identities of several duplicates from different provenances were confirmed (Table 2), namely for Thompson seedless (Thompson Seedless1 and

Thompson Seedless4), Cabernet Sauvignon (Cabernet Sauvignon1 and Cabernet Sauvignon2), and Merlot (Merlot1 and Merlot2). Further research will be needed to establish the trueness-to-type of these cultivars.

Several homonyms, particularly Thompson Seedless (Thompson Seedless1, Thompson Seedless2, and Thompson Seedless3), Hongputao (Hongputao1, Hongputao2, and Hongputao3), Campbell and Campbell Early, and Heiputao (Heiputao1 and Heiputao2) (Table 2), were identified. The three clones of Thompson Seedless were grown in different locations (Table 1). Thompson Seedless1 and Thompson Seedless4 are the true Thompson Seedless (www.vivc.de). Thompson Seedless2 and Thompson Seedless3 shared identical genotypes at three loci (VrZAG62, VVMD27, and VVMD28) with Thompson Seedless1, but they had at least one allele at each locus in common, which could mean a parent–offspring relationship between them. Besides, the maturity of Thompson Seedless2 and Thompson Seedless3 is earlier than Thompson Seedless1, and the fecundity of Thompson Seedless2 and Thompson Seedless3 is better than that of Thompson Seedless1. Additionally, the clones of Hongputao and Heiputao all showed different microsatellite profiles in at least eight SSR loci analyzed. Among these, Hongputao1 and Hongputao2 both came from Xinjiang, and Hongputao3 came from Shanxi, whereas Heiputao1 came from Shanxi, and Heiputao2 came from Xinjiang. However, according to the record, only Hongputao1 and Heiputao2 were the real local cultivars of Xinjiang (Kong, 2004), whereas the other cultivars, named Hongputao and Heiputao, were likely misnamed by local farmers in the 19th century according to the berries' skin color. It is reported that Campbell and Campbell Early are the same cultivar (www.vivc.de), whereas in our study, they did not share the same genetic profile. It might

be that there was an error in the progress of germplasm transmission.

Synonyms. There were also cases of synonyms among samples with different names but identical SSR profiles at all nine analyzed loci, such as Guizhoushuijing, Yunnanshuijing, Guankouputao, and Niagara; Longyan and Hongputao3; Heijixin and Heiputao1; Lvmunage and Munage; Atebage and Hongputao1; Manai, Niunai, and Cuiputao; Hashihaer and Baiputao; and Heiputao2 and Zihongxingputao.

Niagara, obtained by cross-breeding in 1868, is native to the United States. It was imported into Japan in 1883 and was cultivated in the Hokkaido and Nagano areas. When and how Niagara was introduced into China is still unclear. Niagara was sporadically cultivated in the church of Hohhot, Inner Mongolia of China, in the 1940s (Kong, 2004). Meanwhile, Guizhoushuijing, Yunnanshuijing, and Guankouputao have been collected from Guizhou, Yunnan, and Hubei, respectively. All three have similar morphological characteristics to Niagara: the young shoot is green–yellow in color, the tender leaf is grey–green, the mature leaf is dark green, the berry skin color is chartreuse, the berry shape is near round, and the flesh has an intense strawberry fragrance with a soft texture and high juice content. Our results showed that the four cultivars have the same genotype. Guankouputao, Yunnanshuijing, and Guizhoushuijing could be local names for Niagara. The results were similar to those reported by Li et al. (2014), who analyzed the genetic relationship of Guankouputao grapes using SSR and IRAP markers. For Longyan and Hongputao3, Heijixin and Heiputao1, the four cultivars were all collected from Qingxu, Shanxi. Longyan and Heijixin belong to a class of Oriental cultivars and are important ancient Chinese cultivars (Guo et al., 2010). According to the skin color of Longyan and

Fig. 1. Dendrogram of genetic similarities based on nine simple sequence repeat loci of 94 genotypes by means of the Dice coefficient. The cultivars labeled with the same symbol are a group.

Heijixin, Heijixin also had another name, Heiputao; Longyan was also called Hongputao by the local people in Qingxu, Shanxi Province (Jin and Yang, 1982). In this study, Heijixin and Heiputao1, Longyan and Hongputao3 are actually the same variety. Similar cases also exist between Lvmunage and Munage and between Atebage and Hongputao1; all four accessions were from Xinjiang. The Uyghur names of Lvmunage and Hongputao1 are Munage and Atebage, respectively. In addition, Niunai, Manai, and

Cuiputao are also the same variety grown in different locations. Niunai was called Manai in Xinjiang and was also called Cuiputao in Shanxi (Kong, 2004). Hashihaer was collected from Xinjiang and was called Baiputao; they belonged to the same variety as well (Kong, 2004). Heiputao2 and Zihongxingputao were from Xinjiang and share the same genetic profile. Therefore, Zihongxingputao could be a local name for Heiputao. Similar synonyms often appear in apple (Gross et al., 2012) and pear (Cheng and Huang, 2009).

Furthermore, we also compared the Chinese accessions' genotype with the *Vitis* International Variety Catalogue database (www.vivc.de). We found that five Chinese accessions shared the same genetic profile with foreign cultivars: Hetianhong and Kuldzhinskii; Kusikaqi and Emerald seedless; Lvmunage and Gissary; Campbell and Eaton; Cizhongjiaotang and Baco noir; Yilixiangputao and Muscat fleur d'oranger. So they might be the same cultivar. Especially for Hetianhong and Kuldzhinskii, it

has been proved that they are synonyms (<http://www.vivc.de/index.php?r=passport%2Fview&id=6548>). In addition, we also found that Kala and Foster's White Seedling only mismatch at one locus (VrZAG79). We guess that variations might have occurred in Kala after Foster's White Seedling's arrival and settlement in China. As Milla-Tapia et al. (2013) reported that to adapt to particular environments, genetic variations might occur in grapevines. Further research is still needed. Finally, the identification of these homonyms and synonyms will help us to clearly identify germplasm sources and will contribute to the use of germplasms.

Genetic relatedness based on the cluster analysis. A dendrogram was constructed based on the matrix of the genetic distances using UPGMA clustering analysis. The grape accessions studied were distinctly classified into five major clusters (Fig. 1). Xiaohuiputao and Cizhongjiaotang separated from the other cultivars, forming two individual groups, namely, groups IV and V. Group I included all of the cultivars with foxy or muscat flavor. Group II consisted of most of the ancient Chinese accessions related to Oriental cultivars. Group III included most of the introduced cultivars.

Group I consisted of 20 cultivars and included all of the cultivars with foxy or muscat flavor (Fig. 1). In this group, samples related to Muscat Hamburg were grouped together, and samples with Concord in their pedigrees, such as Campbell Early, Niagara, and Campbell, were closely clustered. Some accessions with parent relationships were clustered together, such as Honglianzi and Alphonse Lavallee (both are offspring of Muscat Hamburg). Muscat of Alexandria clustered closely with one of its parents, Muscat Blanc a Petits grains (Cipriani et al., 2010).

In this group, Campbell shares the same genotype with Eaton which originated from United States (www.vivc.de). So we guess that they may be the same cultivar. Campbell and Concord mismatched at one locus (VVMD5) and clustered together, suggesting that Campbell may be a mutation of Concord (Einset and Lamb, 1951). Yilixiangputao from Xinjiang shares a common set of alleles with Muscat Blanc a Petit Grain, suggesting that these cultivars could have a familial connection. Meanwhile, we speculate that Yilixiangputao and Muscat fleur d'oranger may be the same cultivar with same genetic profile, and Muscat Blanc a Petit Grain is one of the parents of Muscat fleur d'oranger (Schneider et al., 2008). So Muscat Blanc a Petit Grain also may be one of the parents of Yilixiangputao.

Group II consisted of 57 cultivars and was the largest and most complex group. Most ancient Chinese accessions are in this cluster. For example, Longyan, Hongputao, Niunai, Pinger, Lizixiang, Niuxin, and Heijixin belonged to Oriental cultivars (Fig. 1). In this group, six seedless cultivars were grouped together, including Thompson Seedless4, Thompson Seedless1, Hongwuzilu, Xiying, Thompson Seedless2, and Thompson Seedless3.

The cluster analysis results were in agreement with Guo et al. (2010), who studied the genetic diversity and relationships among Chinese and European/American grape cultivars using SSRs.

Xiying came from Shanxi Qingxu, and Hongwuzilu originated from Iran. These two accessions are near synonyms, only mismatched at one locus (VrZAG79), revealing their close genetic relatedness and indicating a possible familial connection. Liu et al. (2006) investigated the morphological characteristics of 37 seedless grape cultivars in the National Zhengzhou Grape Germplasm Resources Repository and reported similar results. Longyan and Hongpohuang also mismatched at one locus (VrZAG79) and clustered together, reflecting the close genetic relationship between them. We guess that Longyan and Hongpohuang is probably the case of a near synonym. A similar case also exists between Hongputao2 and Hetianhong, a widely cultivar well known in China since the 19th century. It is reported that the groups of near synonyms are probably to be a mix of clones (Cipriani et al., 2010; Lefort and Roubelakis-Angelakis, 2001).

The foreign cultivars Syrah, Mycat Kpachbrh, Mudanhong, Cabernet Sauvignon1, Cabernet Sauvignon2, Chardonnay, Roter Traminer, Pinot Noir, Cabernet Gernischt, Merlot1, Merlot2, and Cabernet Franc were clustered with three Chinese local grapevines, Lvputao, Hongdalayi, and Nabuguzhu, to form group III (Fig. 1). This result indicates that they likely have a close genetic relationship with each other. In addition, in this group, some accessions with close genetic relationships are grouped closely; e.g., the cultivars Pinot Noir and Chardonnay cluster closely (Bowers et al., 1999b). Additionally, progenies are also grouped close to one of their parents, such as Cabernet Sauvignon and Cabernet Franc (Bowers and Meredith, 1996).

In group III, Hongdalayi collected from Xinjiang and Mycat Kpachbrh originated from Bulgaria share 50% of alleles, exactly one allele at each analyzed locus, and they could have a parent-offspring relationship (Tomic et al., 2012). Nabuguzhu was collected from Xizang, and its SSR profiles were compared with those of Cabernet Sauvignon from France. Despite the two primers (VVS2, VVMD27), they have at least one allele at each analyzed locus in common, suggesting a close relationship between them. Further research will be needed to explore its true identity.

Xiaohuiputao, namely Suxiaohong, is an old local variety from Suqian City of Jiangsu Province. Huang and Sheng (1993) reported that Suxiaohong had a close relationship with *V. pseudoreticulata*, a unique Chinese wild grape. Cizhongjiaotang from Yunnan Province is an ancient grape cultivar that has been cultivated in churches built by French missionaries in Yunnan. Furthermore, Cizhongjiaotang shares the same genotype with Baco noir which originated from France, so we speculated that Cizhongjiaotang could originate from France.

The results of clustering revealed that the origins of some Chinese local cultivars might be related to foreign cultivars. While most Chinese local cultivars are genetically separated from foreign cultivars to form a group, this separation might be the result of long-term domestication or might be the result of a lack of reference varieties. Further research is still needed.

Conclusions

The analysis of 94 grape accessions from China and abroad revealed 80 distinct genotypes. Some homonyms and synonyms were found, such as Hongputao1 and Hongputao2; Lvmunage and Munage; Hashihaer and Baiputao; Hetianhong and Kuldzhinskii; Kusikaqi and Emerald seedless, and so on. The results of the present work suggest that the Chinese grape landraces have a close relationship with the foreign cultivars. Our work will contribute to the effective management of germplasm repositories and the protection of breeders' rights. Meanwhile, we advise that the management of local accessions should be standardized.

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