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Genetic diversity of grape germplasm as revealed by microsatellite (SSR) markers

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In this work, cluster analysis and principal component analysis (PCA) were used to study the genetic diversity and relationships among 49 grape germplasm accessions analyzed with 19 simple sequence repeat (SSR) primer pairs. In total, 139 polymorphic loci were detected among these accessions with an average of 7.32 polymorphic loci per SSR primer pair. The average values for the effective number of alleles, Nei's gene diversity, and Shannon's information index were 1.5605, 0.3352 and 0.5064, respectively. The cluster analysis showed that the 49 accessions could be divided into five groups and an outgroup. The results of the PCA were nearly consistent with those of unweighted pair-group method with arithmetic averages (UPGMA) clustering analysis. These results will be useful for the exploitation of grape germplasm in basic and applied research.

Key words: *Vitis vinifera* L., simple sequence repeat (SSR), genetic diversity, principal component analysis.

INTRODUCTION

Vitis vinifera L. is a precious horticultural crop worldwide and is profoundly connected with the development of human culture (This et al., 2006). The genus *Vitis* L., with approximately 60 species, contains a large number of the Vitaceae and is primarily found in Europe, North America, and East Asia (Emanuelli et al., 2013). Due to the rising demand for higher-quality grape products, including fruits, raisins, juice, wine, etc., the economic value of excellent grape varieties is consistently increasing. Over the past few decades, the planting of single species with high quality and yield has resulted in the drastic reduction of genetic diversity in both cultivated and wild grapevines (Santana et al., 2008). The narrow genetic base of cultivated varieties makes them susceptible to diseases, pests, and environmental conditions. Likewise, the genetic variation of wild *V. vinifera* species has slowly diminished due to the loss of natural habitat (Emanuelli et al., 2013). To avoid further losses of valuable genes and genotypes, it is of significant importance to take effective protection measures, which requires research into genetic relationships and the reconstruction of pedigrees (Bowers et al., 1999; Benjak et al., 2005; Santana et al.,

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Abbreviations: PCA, principal component analysis; **SSR,** simple sequence repeat; **UPGMA,** unweighted pair-group method with arithmetic averages.

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2008). Cultivars with desirable traits have high potential breeding value, and those with genes of enological or organoleptic interest could be important resources to plant breeders and geneticists (Santana et al., 2008). Another crucial factor in breeding success is the phylogenetic relationships between parents. Information on the amount and distribution of genetic variation in grape germplasm collections is therefore essential for the development of conservation strategies and efficient use of *Vitis* germplasm resources (De Andrés et al., 2012).

The development of DNA-based markers has provided widely used methods for quantifying variation within germplasm, including that of grapes (Emanuelli et al., 2013). Simple sequence repeats (SSRs), also known as microsatellite makers, have been widely applied to investigate genetic diversity, distinguish populations, and determine reproductive characteristics in various organisms due to their high degree of polymorphism, reproducibility, and codominant nature (Doulati-Baneh et al., 2013). Recently, several studies have been conducted to decipher the origin, construct genetic maps, and determine the genetic structure of cultivated grapes using nuclear microsatellite analysis (Bowers et al., 1996; Scott et al., 2000; Santana et al., 2010; Doulati-Baneh et al., 2013). Santana et al. (2010) reported on the origins, genetic structure, and relationships of 421 cultivated and four (allegedly) wild grapevine samples from the Castilian Plateau of Spain based on six nuclear microsatellite loci (SSRs). Doulati-Baneh et al. (2013) examined 67 grape cultivars from Iran using SSR markers and analyzed the genetic distances and population structure in the studied germplasm.

Most previous studies have focused on *V. vinifera* L. cultivars from a single location (Agar et al., 2012), which limits the utilization of the species to some extent. In this work, we selected 49 grape germplasm accessions originating from several different countries and investigated their genetic diversity and evolutionary relationships using 19 SSR markers.

MATERIALS AND METHODS

Plant materials

A total of 49 accessions were collected and analyzed in this study. Accession names and their geographic origins are listed in Table 1. The accessions were all kindly provided by the grape germplasm repository of Yantai Changyu Pioneer Wine Company Limited. Young leaves were randomly sampled from adult trees and frozen in liquid nitrogen.

DNA extraction and SSR analysis

Total genomic DNA was extracted using the Ezup Column Plant Genomic DNA Purification Kit (Sangon, Shanghai, China) following the manufacturer's protocol. DNA concentration and purity were determined by UV-spectrophotometry at 260/280 nm, and its integrity was confirmed using 1% agarose gel electrophoresis. PCR was performed in a 25 µL total volume containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM of Mg^{2+}, 0.2 mM of each dNTP, 0.25 µM of each primer, and 1 unit of DNA Taq polymerase (Takara Biotech Co. Ltd., Japan) with 30 ng of DNA as templates. PCR was conducted as follows: 94°C for 5 min; 36 cycles consisting of denaturation at 94°C for 30 s, annealing at 48 to 63°C (depending on primer pair) for 30 s, and synthesis at 72°C for 1 min; and a final elongation at 72°C for 10 min. Twenty grapevine SSRs were used, and a set of 19 highly polymorphic markers were considered suitable for assessing variation among the studied samples (Table 2). The PCR products were separated on 6% (w/v) polyacrylamide gels and visualized with silver staining.

Genetic diversity analysis

The data were used for the following statistical analyses. The number of alleles per locus (N), effective number of alleles (Ne), Nei's gene diversity (H), and gene diversity (Shannon's information index = I) were calculated to estimate the genetic variation level. All of the above calculations were performed using POPGENE version 1.32 (Yeh et al., 1997). Cluster analysis was performed with the Numerical Taxonomy Multivariate Analysis System (NTSYS-PC) version 2.1 (Rohlf, 2002). A dendrogram was constructed via the unweighted pair-group method with arithmetic averages (UPGMA), and similarity coefficients were employed to reveal the relationships among the 49 accessions. Principal component analysis (PCA) was performed by NTSYS 2.1.

RESULTS

Polymorphism of SSR markers

The genetic variation statistics for the 19 SSR markers are summarized in Table 2. A total of 139 polymorphic alleles were amplified using the 19 SSR markers, ranging from 3 (scu16vv) to 17 (VrZAG62) alleles per locus. Ne among the studied markers ranged from 1.2376 (scu16vv) to 1.8449 (VrZAG64), with an average of 1.5605. The H of the 19 SSR markers ranged from 0.1834 (scu16vv) to 0.4543 (VrZAG64), with an average of 0.3352. The values of I ranged from 0.3183 (VVMD6) to 0.6458 (VrZAG64), with an average of 0.5064.

Genetic relatedness

To analyze the genetic relationships among the tested cultivars, the similarity coefficients were calculated with NTSYS-PC 2.1 using UPGMA. 'Cabernet Gernischet' 1–8 represent eight 'Cabernet Gernischet' cultivars from eight different areas in Yantai. The similarity coefficient between 'Cabernet Gernischet 6' and the other seven 'Cabernet Gernischet' cultivars, which were shown to be the same cultivar based on their similarity coefficients (1.0000), was 0.9712. The similarity coefficients of the tested grape accessions ranged from 0.4029 to 0.9856. The SSR UPGMA dendrogram partitioned the 49 tested cultivars into five main groups and an outgroup by clustering varieties with more than 60% similarity (Figure 1). Groups A, B, C, D, and E consisted of 11, 3, 8, 13, and 12 accessions, respectively. Group A was composed

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

Cultivar	Pedigree	Species	The introduction year	Source of collection
Chaush	Unknown	*V. vinifera* L.	1980s	Russia
Cabernet Franc	Ancient variety of France	*V. vinifera* L.	1890s	France
Malvasia Istriana	Ancient variety of Greece	*V. vinifera* L.	2000s	Italy
ВиНТА	Unknown	*V. vinifera* L.	1980s	Bulgaria
Yan Tai No: 73	Muscat hamburg × alicante bouschet	*V. vinifera* L.	----	China
Beta	Unknown	*V. vinifera* L.	1960s	America
Volga-Don	Unknown	*V. vinifera* L.	1960s	Uzbekistan
Xiongyuebai	(Muscat Hamburg× *V. Amurensis*) × Longyan	*V. vinifera* L. × *V. amurensis* Rupr.	----	China
Bacco Noir	Unknown	*V. vinifera* L. × *V .vulpina* L.	1950s	France
Gongniang No: 2	Muscat Hamburg × *V. Amurensis*	*V. vinifera* L. × *V. amurensis* Rupr.	----	China
Cabernet Gernischet 1	Ancient variety of France	*V. vinifera* L.	1890s	France
Cabernet Gernischet 2	Ancient variety of France	*V. vinifera* L.	1890s	France
Cabernet Gernischet 3	Ancient variety of France	*V. vinifera* L.	1890s	France
Cabernet Gernischet 4	Ancient variety of France	*V. vinifera* L.	1890s	France
Cabernet Gernischet 5	Ancient variety of France	*V. vinifera* L.	1890s	France
Cabernet Gernischet 6	Ancient variety of France	*V. vinifera* L.	1890s	France
Cabernet Gernischet 7	Ancient variety of France	*V. vinifera* L.	1890s	France
Cabernet Gernischet 8	Ancient variety of France	*V. vinifera* L.	1890s	France
Cabernet Sauvignon	Cabernet franc × sauvignon blanc	*V. vinifera* L.	1890s	France
Muscat Hamburg	Schiava Grossa × Muscat of Alexandria	*V. vinifera* L.	1890s	England
V.amurensis Rupr.	Ancient variety of China	*V. amurensis*	----	China
Ampelopsis brevipedunculata	Ancient variety of China	*A. brevipedunculata*	----	China
Kyoho	Campbell early × centenial	*V. vinifera* L. × *V. labrusca* L.	1960s	Japan
Ruby Seedless	Emperor × pirovan075	*V. vinifera* L.	1980s	Eurasian
Jiubai	Unknown	*V. vinifera* L.	----	China
Gamay	Pinot noir × Gouais	*V. vinifera* L.	1950s	France
Dragon Oeil	Unknown	*V. vinifera* L.	1980s	Eurasian
Muscat Ottonel	Chasselas × Muscat de Saumur	*V. vinifera* L.	2000s	France
Superior Seedless	Unknown	*V. vinifera* L.	1990s	America
Rizamat	Uncertain	*V. vinifera* L.	1960s	Russia
Saperavi	Unknown	*V. vinifera* L.	1980s	Georgia
Phoenix	Uncertain	*V. vinifera* L.	1980s	West Germany
Autumn Royal	Autumn black × g74-1	*V. vinifera* L.	1998	America

Table 1. Contd.

Purple Queen	Unknown	*V. vinifera* L.	1980s	America
Black Queen	Unknown	*V. vinifera* L.	1980s	Japan
Christmas Rose	(Hunisa × emperor × nocera) × (hunisa × emperor × italia)	*V. vinifera* L.	1980s	America
Magumi	Ancient variety of Japan	*V. vinifera* L.	1990s	Japan
Honey Juice	Unknown	*V. vinifera* L.	1980s	Euro-american hybrids
Amelia	Unknown	*V. vinifera* L.	1990s	Chile
Pinot Blanc	Mutation of Pinot noir	*V. vinifera* L.	1950s	France
Galbena Veral	Unknown	*V. vinifera* L.	1970s	Romania
Grasade Cotnali	Unknown	*V. vinifera* L.	1980s	France
Kadarka 1	Ancient variety of Hungary	*V. vinifera* L.	1980s	Bulgaria
Boulgal	Unknown	*V. vinifera* L.	1970s	Turkey
Kadarka 2	Ancient variety of Hungary	*V. vinifera* L.	1980s	Hungary
Unknown	Unknown	*V. vinifera* L.	2000s	Chile
Stary goru	Ancient variety of Japan	*V. vinifera* L.	1980s	Japan
Medoc Noir	Unknown	*V. vinifera* L.	1980s	France
Vidal Blanc	Ugni blanc × seyval blanc	*V. vinifera* L.	1940s	France

Table 2. Summary of genetic variation statistics for the 19 simple sequence repeat markers.

Primer name	5’ to 3’	T/°C	N	Ne	H	I
VMC4F3	F: AAAGCACTATGGTGGGTGTAAA R: TAACCAATACATGCATCAAGGA	52	5	1.5804	0.3491	0.5277
VVS2	F: CAGCCCGTAAATGTATCCATC R: AAATTCAAAATTCTAATTCAACTGG	50	5	1.3298	0.2367	0.3899
VVIv37	F: TTTTCTCCCTACTCTTAACTTC R: GGTAGACCTTGAAATGAAGTAA	52	5	1.3561	0.2471	0.4050
VVIv67	F: TATAACTTCTCATAGGGTTTCC R: TTGGAGTCCATCAAATTCATCT	52	5	1.8060	0.4402	0.6305
VVMD5	F: CTAGAGCTACGCCAATCCAA R: TATACCAAAAATCATATTCCTAAA	50	5	1.4599	0.2790	0.4335
VVMD6	F: ATCTCTAACCCTAAAACCAT R: CTGTGCTAAGACGAAGAAGA	50	11	1.2784	0.1902	0.3183
VVMD7	F: AGAGTTGCGGAGAACAGGAT R: CGAACCTTCACACGCTTGAT	55	8	1.6948	0.3940	0.5777
VVMD8	F: TAACAAACAAGAAGAGGAAT R: AGCACATCCACAACATAATG	48	9	1.5821	0.3573	0.5394

Table 2. Contd.

VVMD31	F: CAGTGGTTTTTCTTAAAGTTTCAAGG R: CTCTGTGAAAGAGGAAGAGACGC	55	6	1.4575	0.2804	0.4328
VVMD32	F: TATGATTTTTTAGGGGGGTGAGG R: GGAAAGATGGGATGACTCGC	56	13	1.6487	0.3852	0.5708
VrZAG21	F: TCATTCACTCACTGCATTCATCGGC R: GGGGCTACTCCAAAGTCAGTTCTTG	61	6	1.5808	0.3493	0.5279
VrZAG25	F: CTCCACTTCACATCACATGGCATGC R: CGGCCAACATTTACTCATCTCTCCC	62	5	1.6406	0.3802	0.5654
VrZAG62	F: GGTGAAATGGGCACCGAACACACGC R: CCATGTCTCTCCTCAGCTTCTCAGC	62	17	1.6851	0.3886	0.5710
VrZAG64	F: GAAAGAAACCCAACGCGGCACG R: TGCAATGTGGTCAGCCTTTGATGGG	62	8	1.8449	0.4543	0.6458
VrZAG67	F: ACCTGGCCCGACTCCTCTTGTATGC R: TCCTGCCGGCGATAACCAAGCTATG	63	7	1.8025	0.4392	0.6294
VrZAG79	F: AGATTGTGGAGGAGGGAACAAACCG R: TGCCCCCATTTTCAAACTCCCTTCC	62	7	1.7306	0.4198	0.6101
scu07vv	F: CCGAAGAGGAATATGGGTTTGAG R: CCTAACTTGAAACGAAAGGACTGC	58	4	1.3265	0.2306	0.3796
scu15vv	F: GCCTATGTGCCAGACCAAAAAC R: TTGGAAGTAGCCAGCCCAACCTTC	58	10	1.6065	0.3641	0.5463
scu16vv	F: CAAAGACAAAGAAGCCACCGAC R: ACCCTCTAAAGCACACACAGGAAC	58	3	1.2376	0.1834	0.3196

T, annealing temperature; N, number of alleles; Ne, effective number of alleles; H, Nei's gene diversity; I, Shannon's information index.

by 'Muscat Hamburg,' 'Kyoho,' 'Ruby Seedless,' 'Amilia,' 'Kadarka 2,' 'Boulgal,' 'Volga-Don,' 'Magumi,' 'Galbena Veral,' 'Grasade Cotnali,' and 'Honey Juice.' Group B contained only 3 accessions: 'Beta,' 'Purple Queen,' and 'Gongniang No. 2.' Group C contained 'Chaush,' 'Pinot Blanc,' 'Saperavi,' 'Kadarka 1,' 'Xiongyuebai,' 'Jiubai,' 'Dragon Oeil,' and 'Rizamat,' while the 'Cabernet Gernischet' cultivars from Yantai were predominantly grouped in group D, together with 'Cabernet Franc,' 'Cabernet Sauvignon,' 'Gamay,' 'Muscat Ottonel,' and 'Bacco Noir.' Group E contained the other 12 accessions, except for '*Vitis amurensis* Rupr.' and '*Ampelopsis brevipedunculata*,' which composed the outgroup.

The similarity coefficient between 'Kyoho' and 'Ruby Seedless' was the highest among all accessions. Additionally, in the UPGMA dendrogram, 'Kyoho' was very close to 'Ruby Seedless,' and both accessions were clustered with 'Muscat Hamburg.' 'Gongniang No. 2' is the offspring of 'Muscat Hamburg' and '*V. amurensis* Rupr.' However, these accessions were not in the same cluster, as can be seen in Figure 1. The similarity coefficient between 'Gongniang No. 2' and 'Muscat Hamburg' was 0.7122, while that between 'Gongniang No. 2' and '*V. amurensis* Rupr.' was only 0.6619. In group D, 'Cabernet Gernischet 6' was clustered with the other seven 'Cabernet Gernischet' cultivars. 'Cabernet Sauvignon' was close to 'Gamay,' and the two accessions were grouped together with 'Cabernet

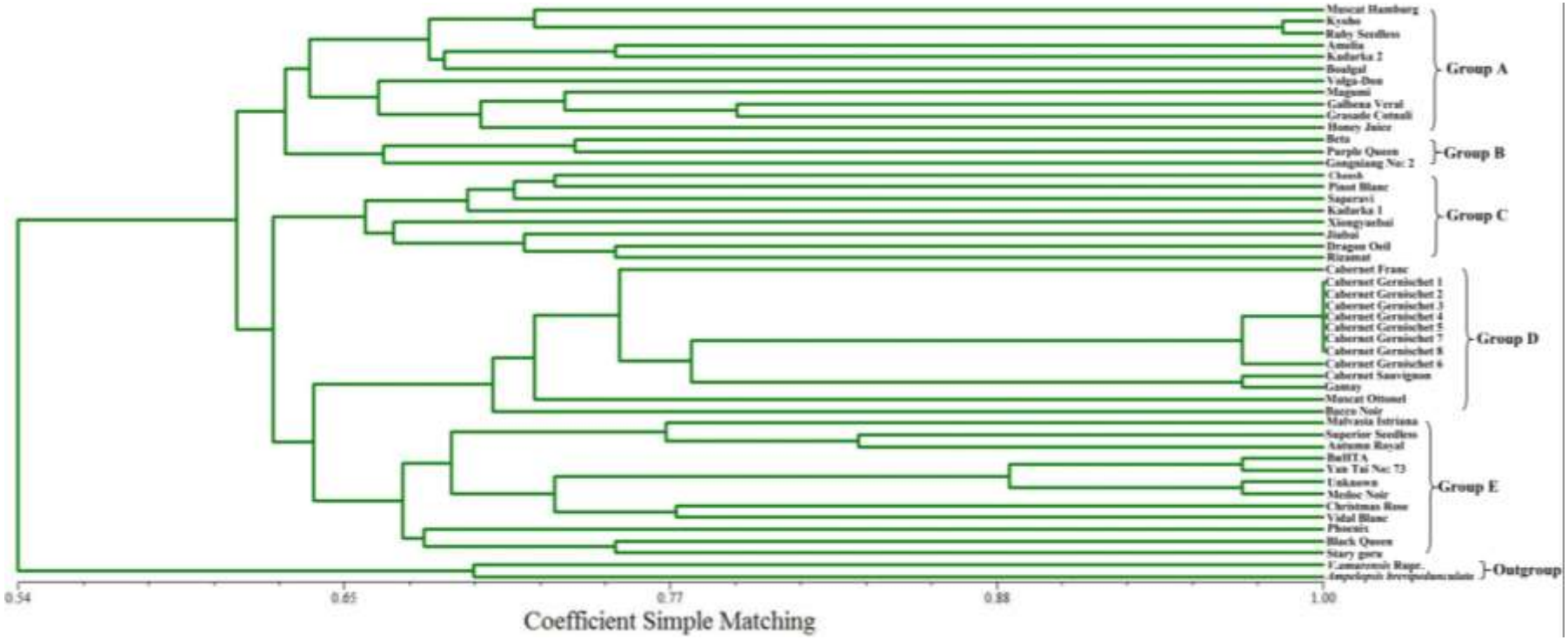

Figure 1. Unweighted pair-group method with arithmetic averages dendrogram of 49 grape germplasm accessions based on simple sequence repeat marker data

Franc.' 'ВиНТА' and 'Yan Tai No. 73' had a particularly close genetic relationship, as indicated by their similarity coefficient of 0.9712 and grouping into the same cluster. The similarity coefficient between the unknown Chilean accession and 'Medoc Noir' was also 0.9712, and a similar result can be seen in group E.

Principal component analysis

Conversely, the principal component analysis (PCA) based on the genotypic data from the SSR markers demonstrated the genetic divergence between the groups (Figures 2 and 3). Dim-1, dim-2, and dim-3 accounted for 17.33, 9.62, and 7.42% of the overall variation, respectively. The PCA results were nearly consistent with those of the UPGMA analysis, which had no difference among the 'Cabernet Gernischet' cultivars except for 'Cabernet Gernischet 6.' 'Kyoho' and 'Ruby Seedless,' the close genetic relationship which is shown in Figure 1, clearly overlapped in the PCA. However, the PCA results separated 'Saperavi' from group C. This result may have been due to dimensionality reduction.

DISCUSSION

In the present study, we selected SSR markers from these previous experiments to assess the phylogenetic relationships among 49 cultivated grapevines originating from different countries. Our results show that VrZAG64 had the highest level of genetic diversity (H = 0.4543; I = 0.6458) among all of the studied SSR markers, which suggested that VrZAG64 should have priority to be considered when estimating the genetic variation of grape cultivars. In the SSR UPGMA dendrogram, the unknown cultivar from Chile and

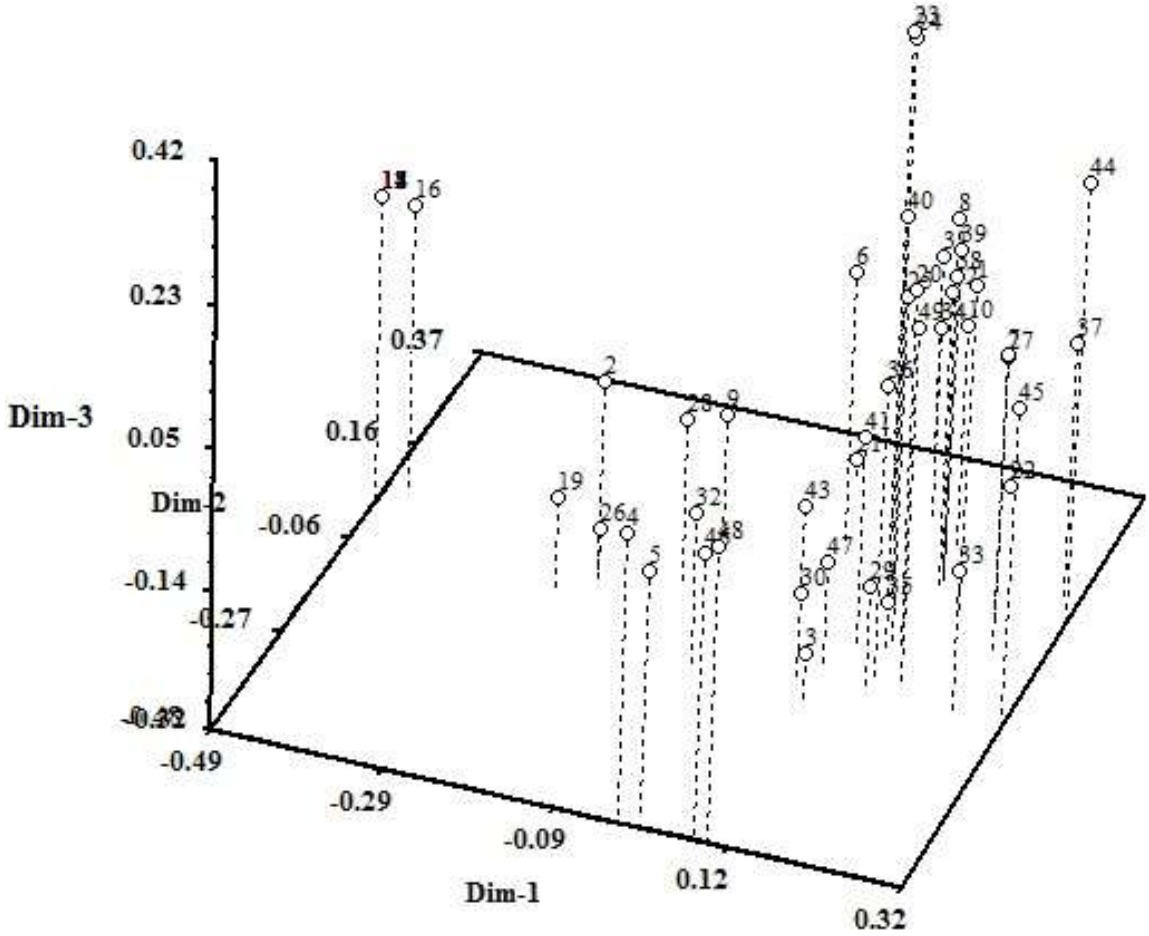

Figure 2. Principal component analysis of the simple sequence repeat markers associated with the grape germplasm accessions. The serial numbers of the accessions are shown in Table 1.

'Medoc Noir' from France (similarity coefficient = 0.9712, Figure 1) were clustered into a clade in Group E. This result implied that the unknown cultivar from Chile likely shares a common ancestry with 'Medoc Noir.' The dendrogram and similarity coefficients indicated that 'Cabernet Gernischet 6' was different from the other seven 'Cabernet Gernischet' cultivars, and the different geography and climate may be the reason why they have differences. This result also serves as a reminder that the protection of germplasm resources should be conducted to the greatest possible extent at the origins of the germplasm, as protection via relocation may potentially damage the germplasm resources. The similarity coefficient between 'Cabernet Franc' and 'Cabernet Sauvignon' was 0.7122 (Figure 1), close to the previous value found by D'Onofrio et al. (2010) using AFLP markers (0.688). These low values did not reflect the fact that 'Cabernet Sauvignon' is a cross of 'Cabernet Franc' and 'Sauvignon Blanc.' Therefore, to clarify the genetic relationship between 'Cabernet Sauvignon' and 'Cabernet Franc,' more information from the nuclear and chloroplast genomes should be considered. 'Kyoho' and 'Ruby Seedless' had a very close genetic relationship according to the cluster results and their similarity coefficient. This result is consistent with the knowledge that both accessions are offspring of the 'Emperor' cultivar. In contrast, the genetic distance between 'Muscat Hamburg' and 'Yan Tai No. 73' (Muscat Hamburg × Aicante Bouschet) is comparatively large despite their parent-offspring relationship; these cultivars were even assigned to two different groups. This result was consistent with that obtained by a previous SRAP marker study (Guo et al., 2012). A similar result also occurred between 'Muscat Hamburg,' '*V. amurensis*,' and 'Xiongyuebai' ((Muscat Hamburg × *V. amurensis*) × Longyan) (Figure 1). These observations indicated that some offspring displayed obvious heterosis, inheriting different superior qualities from their parents to obtain more desirable biological characteristics.

We also found that the similarity coefficients between cultivars from different countries were generally small, with the exception of that between 'ВиНТА' from Bulgaria

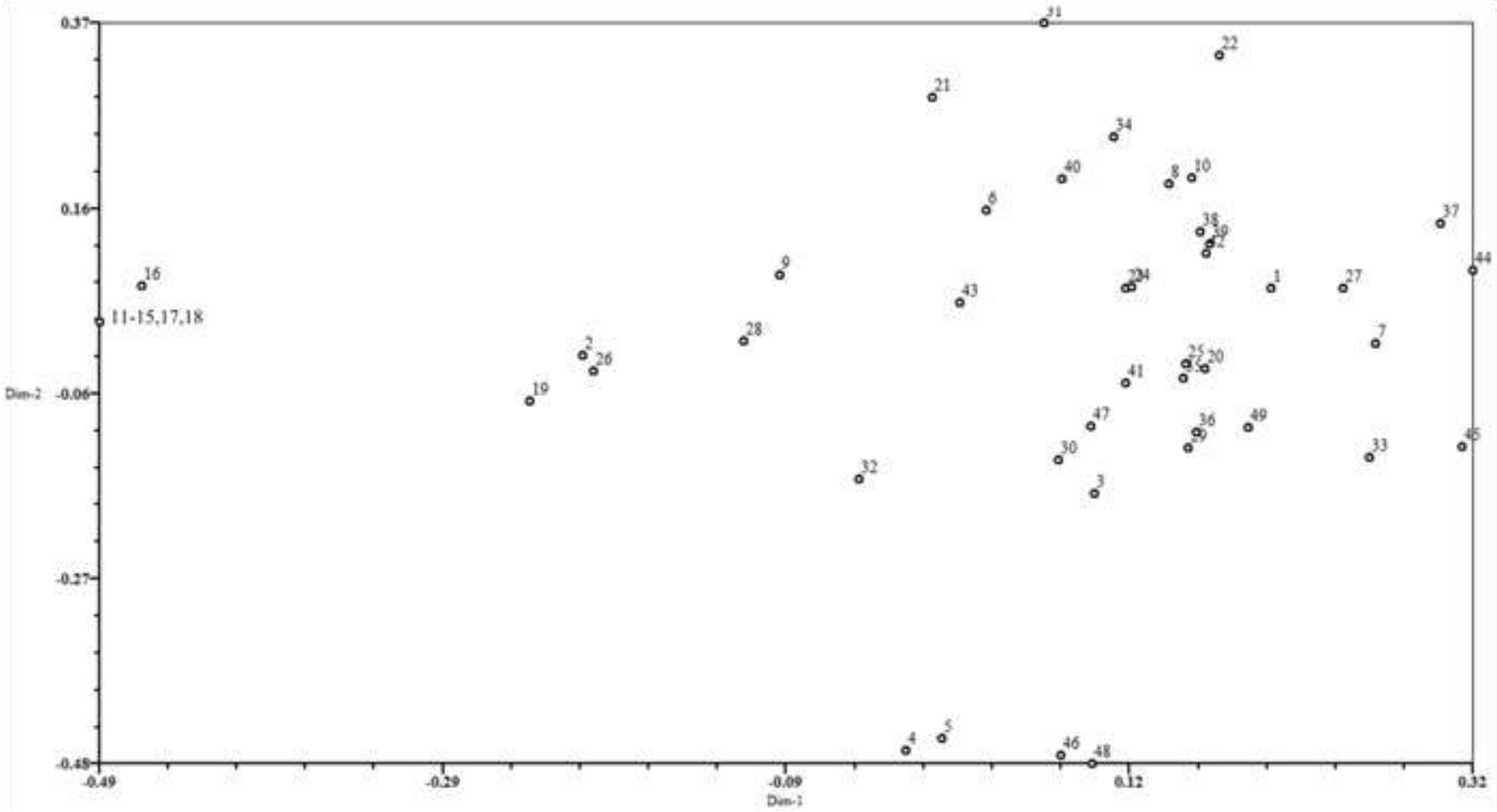

Figure 3. Simple sequence repeat markers associated with grape germplasm accessions based on principal components 1 and 2. The serial numbers of the accessions are shown in Table 1.

and 'Yan Tai No: 73' from China (0.9712, Figure 1). Given that the parents of 'ВиНТА' are not clear, 'ВиНТА' and 'Yan Tai No: 73' likely have a similar origin. In the UPGMA dendrogram, the groupings were not obviously related with the geographic origins of the cultivars (Figure 1). Cultivated populations from different countries may tend towards uniformity due to long-term adaptation to climate and human activities during the long history of cultivation for these accessions. Due to the high economic value of *V. vinifera* L., we strongly advise that core germplasm accessions of this species should be cultivated for conservation in their original regions instead of a single grape germplasm repository with a uniform

growth environment.

In conclusion, our work shows that the polymorphism of SSR molecular markers can provide important information on the inheritance and phylogenetics of grape germplasm. We identified the unknown Chilean accession using SSR markers, although we could not definitively determine its parentage. To better preserve genetic diversity, we suggest that new natural protection habitats should be established at the origins of germplasm accessions, and we recommend that the conservation and management of grape species prioritize populations with high allelic richness and heterosis (Lu et al., 2013). This work shows that assessing the genetic diversity of grape germplasm collections using SSRs is very efficient for basic and applied research. Further experiments should be performed to study grape genetic diversity. Based on the relationships among and characteristics of accessions, scientists can better protect germplasm resources and conduct breeding programs.

Conflict of interests

The authors did not declare any conflict of interest.

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REFERENCES

Agar G, Yildirim N, Ercisli S, Ergul A, Yuksel C (2012). Determination of genetic diversity of *Vitis vinifera* cv. Kabarcik populations from the Coruh Valley using SSR markers. Biochem. Genet. 50(5): 476-483.

Benjak A, Ercisli S, Vokurka A, Maletic E, Pejic I (2005). Genetic relationships among grapevine cultivars native to Croatia, Greece and Turkey. Vitis. 44(2):73-77.

Bowers JE, Dangl GS, Meredith CP (1999). Development and characterization of additional microsatellite DNA markers for grape. Am. J. Enol. Viticult. 50(3):243-246.

Bowers JE, Dangl GS, Vignani R, Meredith CP (1996). Isolation and characterization of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). Genome 39(4):628-633.

D'Onofrio C, De Lorenzis G, Giordani T, Natali L, Cavallini A, Scalabrelli G (2010). Retrotransposon-based molecular markers for grapevine species and cultivars identification. Tree Genet. Genomes 6(3):451-466.

De Andrés MT, Benito A, Perez-Rivera G, Ocete R, Lopez MA, Gaforio L, MuÑOz G, Cabello F, MartÍNez Zapater JM, Arroyo-GarcÍA R (2012). Genetic diversity of wild grapevine populations in Spain and their genetic relationships with cultivated grapevines. Mol. Ecol. 21(4):800-816.

Doulati-Baneh H, Mohammadi SA, Labra M (2013). Genetic structure and diversity analysis in *Vitis vinifera* L. cultivars from Iran using SSR markers. Sci. Hortic. 160:29-36.

Emanuelli F, Lorenzi S, Grzeskowiak L, Catalano V, Stefanini M, Troggio M, Myles S, Martinez-Zapater J, Zyprian E, Moreira F, Grando M (2013). Genetic diversity and population structure assessed by SSR and SNP markers in a large germplasm collection of grape. BMC Plant. Biol. 13(1):1-17.

Guo D, Zhang J, Liu C, Zhang G, Li M, Zhang Q (2012). Genetic variability and relationships between and within grape cultivated varieties and wild species based on SRAP markers. Tree Genet. Genomes 8(4):789-800.

Lu X, Xu H, Li Z, Shang H, Adams RP, Mao K (2013). Genetic diversity and conservation implications of four Cupressus species in China as revealed by microsatellite markers. Biochem. Genet. 52: 1-22.

Rohlf F (2002). NTSYSpc: Numerical taxonomy system, ver. 2.1 Setauket, New York: Exeter Publishing, Ltd.

Santana JC, Heuertz M, Arranz C, Rubio JA, Martínez-Zapater JM, Hidalgo E (2010). Genetic structure, origins, and relationships of grapevine cultivars from the Castilian Plateau of Spain. Am. J. Enol. Viticult. 61(2):214-224.

Santana JC, Hidalgo E, De Lucas AI et al. (2008). Identification and relationships of accessions grown in the grapevine (*Vitis vinifera* L.) germplasm bank of Castillay Léon (Spain) and the varieties authorized in the VQPRD areas of the region by SSR-marker analysis. Genet. Resour. Crop Evol. 55: 573-583.

Scott KD, Eggler P, Seaton G, Rossetto M, Ablett EM, Lee LS, Henry RJ (2000). Analysis of SSRs derived from grape ESTs. Theor. Appl. Genet. 100(5):723-726.

This P, Lacombe T, Thomas MR (2006). Historical origins and genetic diversity of wine grapes. Trends Genet. 22(9): 511-519.

Yeh F, Yang R, Boyle T (1997). POPGENE: a microsoft windows-based freeware for population genetic analysis: version 1.32, 32 bit University of Alberta, Edmonton, Canada