Genetic relationships of 34 grapevine varieties and construction of molecular fingerprints by SSR markers

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ABSTRACT

In order to protect and promote the effective differentiation and rational utilization of grape germplasm resources, 34 grapevine varieties were selected from the National Germplasm Resources, Taigu Grape Nursery in China: 12 wine grapes (Group I), 14 table grapes (Group II) and 12 seedless grapes (Group III), 4 of which were in Group II, too. For the purpose of genetic characterization, 15 pairs of SSR (simple sequence repeat) primers were used. Sixty-six alleles were generated among Group I, including 64 polymorphic bands, with 1–8 alleles per locus. In Group II, a total of 54 alleles, from 1 to 8 (3.6 on average) alleles per locus, were identified. In Group III, 54 alleles with an average of 3.6 alleles per locus were identified and the percentage of polymorphic alleles was 94%. The 34 varieties clustered into two major clades in the dendrogram: *V. vinifera* with hybrid of *V. vinifera* and *V. labrusca* or hybrid of *V. amurensis* and *V. vinifera* were clearly differentiated. After processing and filtering the raw data, we produced the molecular fingerprint code of each variety. These results showed that the SSR markers are useful for discrimination and analysis of genetic diversity of grapevine varieties. The SSR markers could be used to examine and distinguish the genetic resources among closely related varieties. This is also an effective tool for construction of a grapevine molecular fingerprinting system. The obtained data will be useful in grape breeding in the future.

Introduction

Grapevine (*Vitis vinifera* L.) is economically important around the world, as grape can be consumed directly or processed into wine, juice and others. There are more than 70 species in the *Vitis* genus worldwide, mainly distributed in the temperate regions of the Northern Hemisphere. With the grape industry growing, authorized varieties increase rapidly; consequently, a lot of different grape varieties use the same name (homonyms) or varieties get more than one name (synonyms). The growing seedling production, the protection of varieties and intellectual property rights, management of germplasm resources and demand of grape breeding put forward the requirements for grape variety identification. The traditional field evaluation method had been considered an important approach for the grape variety identification, however, it is time-consuming, laborious and error-prone due to environmental factors, which poses great challenges [1]. As an alternative, SSR (simple sequence repeat) markers, also known as short tandem repeats, are now widely used and have become a rewarding tool in the construction of genetic maps, description of distinctive individuals and assessment of genetic relatedness of grapevine varieties, for their reproducibility, high polymorphism, openness and co-dominance [2–4]. Since 1993, when Thomas et al. [5] first used SSR markers to identify multiple Eurasian grape varieties and several other representatives of genus *Vitis*, SSR markers became more and more widely used in the research of different species [6–7], cultigens and rootstock varieties [8–9], and in distinguishing and identification of homonym and synonym varieties [3,10–13]. This et al. [14] demonstrated the usefulness of SSR markers in the identification of grape varieties, and Aradhya et al. [15] reported that SSR genetic analysis with 244 grape varieties matched the classical eco-geographic grouping of grape cultivars: occidentalis, pontica and orientalis. Sefc et al. [16] showed that 162 grape cultivars from seven European vine-growing regions had significantly different allele frequencies among the regions, speculating that these cultivars could possibly be assigned to their regions of origin according to their genotypes.
Compared with the phenotypic characterization methods, which may cause incorrect judgment, SSR markers technology could directly reflect the existing differences, producing more trustworthy results which are not affected by the environmental factors [17]. Therefore, this technology gradually became a very useful tool in the study of the origin of species, as well as in the investigation of the evolution and genetic diversity of grape varieties [18].

Some researchers began to build the molecular fingerprinting maps of grape varieties by SSR markers. In 2004, ten different laboratories from France, Germany, Italy and four other countries chose six high polymorphism SSR primers and analyzed 46 grape cultivars for the standardization of reference allele coding [14].

In this study, we selected 34 grape varieties: 12 wine grapes (Group I), 14 table grapes (Group II) and 12 seedless table grapes (Group III), four of which were in Group II, from the National Germplasm Resources, Taigu grape Nursery in China. Fifteen SSR markers were used to investigate the relationship and genetic diversity among three groups of different types grape varieties. The molecular fingerprint code of each variety was also produced. The obtained results would be a useful reference for the more efficient utilization of grape germplasm and would be helpful in the selection of breeding parents and research in the future.

Materials and methods

Plant materials

The leaf samples of 34 grapevine varieties used in this study were gathered from the national fruit germplasm resources, Taigu grape nursery of Pomology Institute, Shanxi Academy of Agricultural Sciences (37°23’N, 112°32’E). Fresh samples were frozen and kept at –80 °C for genomic DNA extraction. The accession names, species, genetic relationship and their geographic origins are listed in Table 1.

Genomic DNA extraction

Genomic DNA was extracted from young leaf tissues by the method of Thomas and Scott [4] with minor modifications. DNA integrity was confirmed using 1% (w/v) agarose gel electrophoresis, and DNA concentration and purity were determined by Bio-Photometer (Eppendorf

| Accession | Species | Pedigree | Origin |
|-----------|---------|----------|--------|
| Gongniang 1 | Hybrid of V. amurensisi and V. vinifera | Muscat hamburg x V. amurensisi | China |
| Gongniang 2 | Hybrid of V. amurensisi and V. vinifera | V. amurensisi x Muscat hamburg | China |
| Zuoshan | V. amurensisi | – | China |
| Muscat blanc | V. vinifera | – | France |
| Muscat Hamburg | V. vinifera | Frankenenthal x Muscat hamburg | England |
| Blue French | V. vinifera | Gouais Blanc | Austria |
| Cabernet Sauvignon | V. vinifera | Sauvignon Blanc x Cabernet Fanc | France |
| Merlot 181 | V. vinifera | – | France |
| Merlot 343 | V. vinifera | – | France |
| Petit Manseng | V. vinifera | – | France |
| Petit verdot | V. vinifera | – | France |
| Chenin blanc | V. vinifera | – | France |
| Zaoheibao | V. vinifera | Guibao x Zaomeigui | China |
| Muscat ottonel | V. vinifera | Chasselas x Muscat de Saumur | France |
| Wanheibao | V. vinifera | Guibao x Christmas Rose | China |
| Guibao | V. vinifera | Helihip x Mycat Bha | China |
| Kyoho | Hybrid of V. vinifera and V. labrusca | Muscat hamburg x Peal of Csaba | China |
| Zaomeigui | V. vinifera | Muscat | Japan |
| Christmas Rose | V. vinifera | S44–35C x 9–170 | America |
| Meixiangbao | V. vinifera | Muscat ottonel x kyoho | China |
| Centennial seedless | V. vinifera | Gold x Q25–6 | America |
| Lihongbao | V. vinifera | Guibao x Centennial seedless | China |
| Jinghongbao | V. vinifera | Guibao x Centennial seedless | China |
| Zaokangbao | V. vinifera | Guibao x Centennial seedless | China |
| Jingya | Hybrid of V. vinifera and V. labrusca | Seedling from Black olympia | China |
| Black olympia | Hybrid of V. vinifera and V. labrusca | Kyoho x Jujing | Japan |
| Venus seedless | Hybrid of V. vinifera and V. labrusca | Alden x Y4600 | America |
| Mars seedless | Hybrid of V. vinifera and V. labrusca | Island belle x Ark139 | America |
| Otilla seedless | V. vinifera | Emperor x E4 | America |
| Blush seedless | V. vinifera | Emperor x Pirovano75 | America |
| Dawn seedless | V. vinifera | Emperor x Pirovano75 | America |
| Ruby seedless | V. vinifera | Emperor x Pirovano75 | America |
| Melissa seedless | V. vinifera | Emperor x Pirovano75 | America |
| Fantasy seedless | V. vinifera | Emperor x Pirovano75 | America |

Note: Genetic relationship or origin is unknown; ‘Pedigree’ and ‘origin’ help us to better understand the relationship between different species and the construction of molecular fingerprints.
BioSpectrometer Germany). Polymerase chain reaction (PCR) was performed using the diluted DNA solution in 20 ng/μL that was stored in −20 °C. We chose a total of 44 internationally well-known grape SSR primers derived from the NCBI public database and related references [19–21], 15 of which gave clear bands and had rich polymorphism (Table 2). The primers were synthesized by Bio-engineering (Shanghai) co., LTD.

The PCR was performed using 10 μL reaction mixtures containing 20 ng genomic DNA, 5 μL PCR Mix, 5 pmol of each primer, 1 U Taq DNA Polymerase (Promega) and 3 μL ddH2O. Reactions without DNA were used as negative controls. The PCR was carried out in a Biometra® PCR System with the following program: an initial denaturation step of 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 52–60 °C and 90 s at 72 °C, with a final extension at 72 °C for 10 min, 16 °C∞. The amplification products were visualized using capillary electrophoresis method to estimate their sizes.

### Genetic analysis

SSR data were scored as presence (1) or absence (0) of bands and each band was regarded as a locus, according to the statistics of 0/1 data matrix. The genetic similarity coefficients between different samples were calculated using the software Numerical Taxonomy and Multiware Analysis System, version 2.0 (NTSYS-pc2.10) [22]. Cluster analysis was done using the unweighted pair-group method with arithmetic means (UPGMA) [23].

Through polymorphism band order code conversion, a molecular identification system was built. The standard for the assignment was as follows: 1) 15 decimal numbers (0–9), corresponding to 15 pairs of SSR primers and the order listed in Table 2; 2) the amplification bands of each material and each primer are arranged in order of size; the fingerprint code indicates the order of all bands of this primer; 3) if a primer has more than 10 bands, the banding pattern is defined as 0; 4) if there are multiple bands corresponding to a primer, the smallest band order is taken as the fingerprint code of the primer.

### Results and discussion

#### Polymorphism analysis of 34 grape varieties

All the 34 samples were successfully amplified using the 15 SSR primers. The SSR analysis results of 12 wine grape varieties in Group I illustrated that a total number of 66 alleles, with 1–8 alleles per locus, average value of 4.4 alleles per locus, were genetically identified (Table 3). In Group II, 14 table grape varieties were analyzed. A total number of 54 alleles were genetically identified, also with 1–8 alleles per locus; and an average value of 3.6 alleles per locus (Table 4). In Group III, there were 12 seedless-table grape varieties. A total number of 54 alleles were genetically identified, with 1–7 alleles per

### Table 2. Nucleotide sequence of 15 microsatellite primers used for construction of a molecular fingerprinting system of 34 grapevine varieties.

| Primer | Forward sequence (5′–3′) | Reverse sequence (5′–3′) |
|--------|--------------------------|--------------------------|
| SCU04 vv | TGTCCTCTTCTTCCTCCCAAC | CAGTCGTCTACGTGACATGAGCC |
| SCU11 vv | AATGGAATGCAACGGATCTGCCC | AACGGCAGAAGAATCTCCCAAGG |
| SCU15 vv | GCCATAGCGACGCCAAGATACCA | TGGGAGTAGGGCCACCCCAACCTTC |
| SCU16 vv | CAAGAACGAAAAAGGCGACGAC | ACCGCTTCAAGGACACAGGGAC |
| UDV-046 | CGTCGTCTACGTCGTCTCAT | TGATACCAACAGTCTGATTTT |
| UDV-048 | GCACGTGTTGACGGATCTCCT | CCGTCTCTTACAGGACATC |
| UDV-060 | CCGCACTACACAAATCACAAA | TGCGTGAAACTGGGTGTTT |
| UDV-067 | CTAATGGCACTACATTCCAA | TGAGTGATGAGGACAGCTC |
| UDV-134 | GTAAGAAAGAATTGTTTGAGT | GCCGACGACAACCTTAAATCA |
| VVS1 | ACAATTGGAAACCGCGTGAGC | TTCCTTCAAGTATATTGTAGG |
| VVS3 | TGGCCCTACAATTAGTTCTACCTA | CCTCGATCTGGATATATTGTAGG |
| VVS4 | CCATCAGTGATAAACTAACCC | CCGCACTACCCATCTTAAATCA |
| VVS5 | TGGCCCTACAATTAGTTCTACCTA | CCGCACTACCCATCTTAAATCA |
| VVS6 | CCATCAGTGATAAACTAACCC | CCGCACTACCCATCTTAAATCA |
| VMC7b1 | CACGCAATCTTTCATTTCACAAA | CCCGAGCTGAAGCAGAGAC |
| VMD5 | CTAGGACTGCCAATCTTCA | TACCCCAAAATCATATATTCTAA |
| VMD19 | TGAAATATCAGCGCTCTCT | GTGTTGATGCTGCTCTT |

### Table 3. Comparison of genetic diversity among 12 wine grape varieties amplified by 15 SSR primers.

| Primer | Total number of amplified bands | Number of polymorphic bands | Percentage of polymorphism |
|--------|--------------------------------|-----------------------------|---------------------------|
| Scu04vv | 2 | 1 | 50% |
| Scu11vv | 4 | 4 | 100% |
| Scu15vv | 3 | 3 | 100% |
| Scu16vv | 1 | 1 | 100% |
| UDV-046 | 4 | 4 | 100% |
| UDV-048 | 5 | 5 | 100% |
| UDV-060 | 6 | 6 | 100% |
| UDV-067 | 5 | 5 | 100% |
| UDV-134 | 8 | 8 | 100% |
| VVS1 | 4 | 4 | 100% |
| VVS3 | 4 | 4 | 100% |
| VVS4 | 6 | 6 | 100% |
| VMC7b1 | 7 | 7 | 100% |
| VMD5 | 7 | 7 | 100% |
| VMD19 | 1 | 0 | 0% |
| Total | 66 | 64 | 97% |
alleles per locus was a little lower than in contrast earlier reports of 10 autochthonous cultivars (5.75) from Eastern Turkey [4], 25 autochthonous cultivars (8.67) from northeastern Turkey [26], 33 Slovenian cultivars (8.00) [27], 11 Romanian cultivars (7.90) [28], 50 Greek cultivars (7.90) [29] and 51 accessions from Bosnia and Herzegovina (7.82) [30]. The reason may be that the groups we had chosen were small, the varieties were few in each population, and all the cultivars in each group had similar traits.

**Genetic similarity coefficient analysis of 34 grape varieties**

The genetic similarity was estimated within the range of 0.5405–1.00 among the cultivars in Group I, 0.6271–0.9915 among those in Group II and 0.5902–0.9016 among the ones in Group III. Melot181, Melot343 and Zuoshan with 0.5405 similarity ratio, Jingya and Muscat ottonel with 0.6271, Mars seedless and Otilia seedless with 0.5902 were most distant; Melot181 and Melot343 were identical with a genetic similarity ratio of 1.00, Kyoho and Black Olympia with 0.9915, Jinhongbao and Lihongbao with 0.9016 in each group (Tables 6–8 and Figures 1–3). All these results indicated that the lowest genetic similarity coefficients were those from different species; the parents-offsprings varieties or sisters varieties had the highest genetic similarity in each group, which is in agreement with precious reports [14].

**Phylogenetic analysis of 34 grape varieties**

To reveal the genetic relationship of the grape varieties in three groups, we performed UPGMA cluster analysis to construct a dendrogram from the 15 SSR loci. In Group I, two hybrids of V. amurensis and V. vinifera, Gongniang 1 and Gongniang 2, and one V. amurensis Zuoshan were clustered in one group and separated from the other group, which was all V. vinifera varieties. The second V. vinifera varieties group contained nine varieties and this group was further divided into three subgroups. Three varieties (Cabernet sauvignon, Merlot181 and Merlot343) were grouped in one subgroup; two varieties (Muscat blanc and Muscat hamburg) with muscat flavour were included in another subgroup; and the third subgroup contained the remaining four varieties (Petit manseng, Petit verdot, Chinin blanc and Blue french) (Figure 1).

In Group II, three hybrids of V. vinifera and V. labrusca, Kyoho, Black Olympia and Jingya, and Meixiangbao, which is an offspring of Kyoho, were clustered together in one group and separated from the remaining V. vinifera varieties. The V. vinifera varieties group contained 10 varieties and was further divided into three subgroups. Centennial seedless with its three offsprings,

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**Table 4.** Comparison of genetic diversity among 12 table grape varieties amplified by 15 SSR primers.

| Primer   | Total number of amplified bands | Number of polymorphic bands | Percentage of polymorphism |
|----------|--------------------------------|-----------------------------|---------------------------|
| Scu04vv  | 2                              | 0                           | 0%                        |
| Scu11vv  | 5                              | 4                           | 80%                       |
| Scu15vv  | 3                              | 3                           | 100%                      |
| Scu16vv  | 1                              | 0                           | 0%                        |
| UDV-046  | 4                              | 4                           | 100%                      |
| UDV-048  | 3                              | 3                           | 100%                      |
| UDV-060  | 6                              | 6                           | 100%                      |
| UDV-067  | 3                              | 3                           | 100%                      |
| UDV-134  | 3                              | 3                           | 100%                      |
| VVS1     | 5                              | 5                           | 100%                      |
| VVS3     | 2                              | 2                           | 100%                      |
| VVS4     | 4                              | 4                           | 100%                      |
| VVS5     | 2                              | 2                           | 100%                      |
| VMC7b1   | 4                              | 4                           | 100%                      |
| VMD5     | 8                              | 8                           | 100%                      |
| VMD19    | 1                              | 1                           | 100%                      |
| Total    | 54                             | 48                          | 89%                       |

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**Table 5.** Comparison of genetic diversity among 12 seedless table grape varieties amplified by 15 SSR primers.

| Primer   | Total number of amplified bands | Number of polymorphic bands | Percentage of polymorphism |
|----------|--------------------------------|-----------------------------|---------------------------|
| Scu04vv  | 1                              | 0                           | 0%                        |
| Scu11vv  | 4                              | 4                           | 100%                      |
| Scu15vv  | 2                              | 2                           | 100%                      |
| Scu16vv  | 0                              | 0                           | 0%                        |
| UDV-046  | 4                              | 4                           | 100%                      |
| UDV-048  | 3                              | 3                           | 100%                      |
| UDV-060  | 5                              | 5                           | 100%                      |
| UDV-067  | 3                              | 3                           | 100%                      |
| UDV-134  | 3                              | 3                           | 100%                      |
| VVS1     | 5                              | 5                           | 100%                      |
| VVS3     | 2                              | 2                           | 100%                      |
| VVS4     | 4                              | 4                           | 100%                      |
| VVS5     | 2                              | 2                           | 100%                      |
| VMC7b1   | 4                              | 4                           | 100%                      |
| VMD5     | 8                              | 8                           | 100%                      |
| VMD19    | 1                              | 1                           | 100%                      |
| Total    | 54                             | 48                          | 89%                       |
Zaokangbao, Jinghongbao and Lihongbao, were divided in the first subgroup. The second subgroup contained four varieties, Guibao with its two offsprings, Zaohelibao and Wanhaibao, and Christmas rose, which is Wanhai- baoguo’s male parent. The remaining two varieties, Muscat ottonel (Meixiangbao’s male parent) and Zaomeigui (Zaohelibao’s male parent) were in the third subgroup. (Figure 2). In Group III, two hybrids of *V. vinifera* and *V. Labrusca*, Venus seedless and Mars seedless, were grouped together in one cluster, and separated from the remaining varieties. Another cluster contained 10 varieties and was further divided into two subgroups. Otilia seedless and Dawn seedless were included in the first subgroup. The second subgroup contained the
remaining eight varieties (Blush seedless, Ruby seedless, Fantacy seedless, Melissa seedless, Centennial seedless, Zaokangbao, Lihongbao and Jinghongbao) (Figure 3). In this study, 12 wine cultivars in Group I include some famous varieties (e.g. Cabernet-Sauvignon, Merlot), and some local varieties of China (e.g. Zuoshan, Gongniang 1 and Gongniang 2); 22 table grapes cultivars in Group II and III are most internationally important (e.g. Centennial seedless, Fantasy seedless), which also included 6 table varieties newly developed and their parents, which covered multiple species of grape and multiple characteristics of the same species. All these three groups, V. amurensisi, V. labrusca and V. vinifera, could be divided.

The results of clustering indicated that the grape varieties of different species could be distinguished clearly, which agreed with the classical eco-geographic grouping theory [15]. Although most Chinese local cultivars are genetically separated from foreign cultivars to form a group, it also illustrated that the origins of some Chinese local cultivars might be related to foreign cultivars. The separation might be the result of long-term
domestication or artificial selection pressure. Further research is still needed.

**Construction of molecular fingerprints of 34 grape varieties**

According to the amplification results of each primer and the assignment standard of allele, each of 34 varieties received a specific SSR molecular fingerprint code (Table 9). The 15 SSR molecular markers can make a clear distinction between each of the varieties tested (except the two clones Merlot 181 and Merlot 343), as well as between the relatives of the same parents and sister-varieties (Gongniang 1, Gongniang 2 and Zaokangbao, Lihongbao, Jinghongbao). It can also be applied to related appraisal, including the materials from different parents, different species or different subgenus.

In this study, we established the specific molecular identification of 34 grape germplasms. The sister varieties with the same parents, Lihongbao, Jinghongbao, Zaokangbao, and Gongniang 1, Gongniang 2, Jingya, the seedling of Black olympia, all could be effectively distinguished, but the two ‘Merlot’ lines ‘Merlot 181’ and ‘Merlot 343’ could not. Further work needs to address the molecular fingerprinting of these varieties using SSR molecules. The development of core molecular markers is one direction of our future research.

Using SSR markers as a tool for genetic mapping, cultivars identification, genetic diversity investigations, parentage analysis, as well as molecular fingerprint construction are highly accepted throughout the world [31]. The SSR analysis data reported here might provide worthy information for further grape protection, exploitation and utility, grape selection and breeding research in the whole world. Moreover, the established SSR data could enable researchers to preserve the valuable genetic cultivars for variety improvement, offer reference for the future studies on grape cultivars.

In this work, we used 15 pairs SSR markers selected from 44 pairs, but still did not cover the different linkage groups of the whole grape genome [32]. Complete and

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**Table 9. Molecular fingerprint codes for 34 grape varieties.**

| Accession     | Molecular fingerprint code | Accession     | Molecular fingerprint code |
|---------------|-----------------------------|---------------|-----------------------------|
| Gongniang 1   | 13126722464302              | Blue French   | 153124826124882             |
| Gongniang 2   | 13112926244305              | Merlot 181    | 152143822224352             |
| Zuo shan      | 13114545454545              | Merlot 343    | 152143822224352             |
| Muscat blanc  | 13143777774352              | Chenin blanc  | 15211472294352              |
| Muscat        | 12211272624352              | Petit Manseng | 15211472284351              |
| Cabernet      | 1311372244352               | Petit verdot  | 15211374224353              |
| Sauvignon     | 15211472634354              | Meixiangbao   | 152113827904852             |
| Muscat ottone | 15311372274352              | Jingya        | 141124826044754             |
| Centennial    | 12212672364354              | Kyoho         | 1221113726914852            |
| seedless      |                             |               |                             |
| Guibo         | 122144827234354             | Lihongbao     | 152124757264354             |
| Zaomei gui     | 141124826044354             | Black olympia | 141134826044354             |
| Jinghongbao   | 15212375374354              | Wanheibao     | 15211482634354              |
| Christmas     | 153116824224354             | Zaokangbao    | 122124754334354             |
| Rose          | 13212472234354              | Dawn seedless | 12312372134354              |
| Venus         | 132112556964054             | Ruby seedless | 122110924734354             |
| Mars seedless | 122116921364354             | Melissa seedless | 122146751934351          |
| Blush         | 122112457964354             | Fantasy seedless | 152124727354354        |
| Otilia        |                             |               |                             |
| seedless      |                             |               |                             |

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**Figure 3. Dendrogram of 12 seedless table grape varieties based on 15 SSR markers.**
irrefutable identification needs a larger number of loci [4]. The main purpose of our research was to perform a preliminary study of the genetic diversity and explore the molecular fingerprinting system of grape varieties. Thus, our work will be of benefit for future research in the grape research community.

Conclusions
Fifteen SSR primer pairs were selected to identify 34 grape varieties including 14 wine grape varieties, 22 table grape varieties of which 12 were seedless, six newly-developed table grape germplasms and their parents in China. The polymorphic band patterns among the 34 grapevine varieties were used to perform cluster analysis using UPGMA methods based on genetic similarity. All the three groups of grape varieties were clustered into two main groups, one is the V. vinifera and the other is the hybrid of V. vinifera and V. Labrusca or hybrid of V. amurensisi and V. vinifera. The SSR molecular fingerprint coding of each variety was established, even when there are sister varieties, or one is another variety’s seedling. This research indicated that the SSR markers are useful for identification, analysis of genetic diversity of grape, molecular fingerprint database construction and also for grape breeding in the future.

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Disclosure statement
No potential conflict of interest was reported by the authors.

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References
[1] Srivhostaen A, Charoenchai C, Urairong H. Application of microsatellite markers for identification of wine grape varieties in Thailand. Asia-Pac J Sci Technol. 2016;21 (1):97–110.
[2] Dokupilová I, Šturdík E, Mihálík D. Characterization of vine varieties by SSR markers. Acta Chim Slovaca. 2013;6 (2):227–234.
[3] Bowers J, Dangl GS, Vignani R, et al. Isolation and characterization of new polymorphic simple sequence repeat loci in grape (Vitis vinifera L.). Genome. 1996;39(4):628–633.
[4] Eydyuran SP, Erciisi S, Akin M, et al. Genetic characterization of autochthonous grapevine cultivars from Eastern Turkey by simple sequence repeats (SSRs). Biotechnol Biotechnol Equip. 2016;30(1):26–31.
[5] Thomas M, Scott N. Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites (STTs). Theor Appl Genet. 1993;86(8):985–990.
[6] Di Gaspero G, Peterlunger E, Testolin R, et al. Conservation of microsatellite loci within the genus Vitis. Theor Appl Genet. 2000;101(1):301–308.
[7] Arnold C, Rossetto M, McNally J, et al. The application of SSRs characterized for grape (Vitis vinifera) to conservation studies in Vitaceae. Am J Bot. 2002;89(1):22–28.
[8] Sefc K, Regner F, Glössl J, et al. Genotyping of grapevine and rootstock cultivars using microsatellite markers. Vitis. 1998;37(1):15–20.
[9] Fatahi R, Ebadi A, Bassil N, et al. Characterization of Iranian grapevine cultivars using microsatellite markers. Vitis. 2003;42(4):185–192.
[10] Thomas MR, Cain P, Scott NS. DNA typing of grapevines: A universal methodology and database for describing cultivars and evaluating genetic relatedness. Plant Mol Biol. 1994;25(6):939–949.
[11] Crespan M, Bottai R, Milani N. Molecular characterization of twenty seeded and seedless table grape cultivars (Vitis vinifera L.). Vitis. 1999;38(3):87–92.
[12] Martinez L, Cavagnaro P, Masueli R, et al. SSR-based assessment of genetic diversity in South American Vitis vinifera varieties. Plant Sci. 2006;170(6):1036–1044.
[13] Schneider A, Carra A, Akkak A, et al. Verifying synonyms between grape cultivars from France and Northwestern Italy using molecular markers. Vitis. 2001;40(4):197–203.
[14] This P, Jung A, Boccacci P, et al. Development of a standard set of microsatellite reference alleles for identification of grape cultivars. Theor Appl Genet. 2004;107 (7):1448–1458.
[15] Aradhya MK, Dangl GS, Prins BH, et al. Genetic structure and differentiation in cultivated grape, Vitis vinifera L. Genet Res. 2003;81(03):179–192.
[16] Sefc K, Lopes M, Lefort F, et al. Microsatellite variability in grapevine cultivars from different European regions and evaluation of assignment testing to assess the geographic origin of cultivars. Theor Appl Genet. 2000;100(3–4):498–505.
[17] Štajner N, Korosek-Koruzza Z, Rusian D, et al. Microsatellite genotyping of old Slovenian grapevine varieties (Vitis vinifera L.) of the Primorje (coastal) winegrowing region. Vitis. 2008;47(4):201–204.
[18] Doulati-Baneh H, Mohammadi S, Labra M. Genetic structure and diversity analysis in Vitis vinifera L. cultivars from Iran using SSR markers. Sci Hortic-Amsterd. 2013;160:29–36.
[19] Botta R, Scott N, Eynard I, et al. Evaluation of microsatellite sequence-tagged site markers for characterizing Vitis vinifera cultivars. Vitis. 1995;34(2):99–102.
[20] Scott KD, Eggler P, Seaton G, et al. Analysis of SSRs derived from grape ESTs. Theor Appl Genet. 2000;100(5):723–726.

[21] Jing Z, Wang X, Cheng J. Analysis of genetic diversity among Chinese wild Vitis species revealed with SSR and SRAP markers. Genet Mol Res. 2013;12(2):1962–1973.

[22] Rohlf J. NTSYSpc: numerical taxonomy and multivariate analysis system version 2.0 user guide. Setauket (NY): Appl Bio Inc.; 1998.

[23] Sneath PH, Sokal RR. Numerical taxonomy. The principles and practice of numerical classification; San Francisco (USA): Freeman; 1973.

[24] Martín J, Borrego J, Cabello F, et al. Characterization of Spanish grapevine cultivar diversity using sequence-tagged microsatellite site markers. Genome. 2003;46(1):10–18.

[25] Tangolar S. Genetic analysis of grapevine cultivars from the eastern Mediterranean region of Turkey, based on SSR markers. Tarim Bilim Derg. 2009;15(1):1–8.

[26] Boz Y, Bakir M, Çelikkol B, et al. Genetic characterization of grape (Vitis vinifera L.) germplasm from Southeast Anatolia by SSR markers. Vitis. 2011;50(3):99–106.

[27] Štajner Na, Rusjan D, Korosec-Korula Z, et al. Genetic characterization of old Slovenian grapevine varieties of Vitis vinifera L. by microsatellite genotyping. Am J Enol Viticult. 2011;62:250–255.

[28] Gheorghe RN, Popescu CF, Pamfil D, et al. Genetic diversity of some Romanian grapevine cultivars as revealed by microsatellite markers. Rom Biotech Lett. 2010;15(2):26–31.

[29] Lefort F, Roubelakis-Angelakis KK. Genetic comparison of Greek cultivars of Vitis vinifera L. by nuclear microsatellite profiling. Am J Enol Viticult. 2001;52(2):101–108.

[30] Tomic L, Stajner N, Jovanović-Cvetković T, et al. Identity and genetic relatedness of Bosnia and Herzegovina grapevine germplasm. Sci Hortic-Amsterd. 2012;143:122–126.

[31] Tomic L, Javornik B, Stajner N. Characterization of grapevines by the use of genetic markers: The Mediterranean genetic code–grapevine and olive. Rijeka (Croatia): InTech; 2013.

[32] Riaz S, Dangl G, Edwards K, et al. A microsatellite marker based framework linkage map of Vitis vinifera L. Theor Appl Genet. 2004;108(5):864–872.