GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Comparison of a retrotransposon-based marker with microsatellite markers for discriminating accessions of *Vitis vinifera*

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Genet. Mol. Res. 11 (2): 1507-1525 (2012)
Received August 29, 2011
Accepted January 26, 2012
Published May 21, 2012
DOI http://dx.doi.org/10.4238/2012.May.21.8

ABSTRACT. Identification and knowledge concerning genetic diversity are fundamental for efficient management and use of grapevine germplasm. Recently, new types of molecular markers have been developed, such as retrotransposon-based markers. Because of their multilocus pattern, retrotransposon-based markers might be able to differentiate grapevine accessions with just one pair of primers. In order to evaluate the efficiency of this type of marker, we compared retrotransposon marker *Tvv*1 with seven microsatellite markers frequently used for genotyping of the genus *Vitis* (VVMD7, VVMD25, VVMD5, VVMD27, VVMD31, VVS2, and VZAG62). The reference population that we used consisted of 26 accessions of *Vitis*, including seven European varieties of *Vitis vinifera*, four North American varieties and hybrids of *Vitis labrusca*, and 15 rootstock hybrids obtained from

crosses of several *Vitis* species. Individually, the *Tvv*1 and the group of seven SSR markers were capable of distinguishing all accessions except 'White Niagara' compared to 'Red Niagara'. Using the Structure software, the retrotransposon marker *Tvv*1 generated two clusters: one with *V. vinifera* plus North American varieties and the other comprising rootstocks. The seven SSR markers generated five clusters: *V. vinifera*, the North American varieties, and three groups of rootstock hybrids. The percentages of variation explained by the first two components in the principal coordinate analysis were 65.21 (*Tvv*1) and 50.42 (SSR markers) while the Mantel correlation between the distance matrixes generated by the two types of markers was 42.5%. We conclude that the *Tvv*1 marker is useful for DNA fingerprinting, but it lacks efficiency for discrimination of structured groups.

Key words: *Vitis* spp; *Tvv*1; Simple sequence repeats; Genotyping; Germplasm; Molecular markers

INTRODUCTION

The first report on retrotransposons serving as molecular markers in plants was published by Pelsy (2007). The author described the use of the *Tvv*1 family of retroelements for studying the genetic diversity in 94 *Vitis* accessions. *Tvv*1 is a highly variable untranslated leader (UTL) region and constitutes a particular class of short long terminal repeat (LTR) retroelements (Pelsy and Merdinoglu, 2002). The copy number of retrotransposons is closely related to the size of the genome (SanMiguel et al., 1996); in maize, a plant species with a large genome, the contribution of retrotransposon elements to the genome size is suggested to be between 33 and 62% (Sanmiguel and Bennetzen, 1998). Furthermore, retrotransposons are ubiquitous and widely dispersed through plant genomes (Kumar and Bennetzen, 1999); the high level of error-prone retroviral replication promotes the accumulation of genetic variations (Casacuberta et al., 1997; Cordaux and Batzer, 2009). These characteristics make these elements a powerful tool for studying genetic diversity. The use of retrotransposon sequences such as *Tvv*1 as a source of informative markers might optimize the process of genotyping because of their ability of simultaneously sampling the genome at several loci.

*Tvv*1 is a member of the *Ty1 copia*-like elements. These elements are characterized by a highly variable UTL region, upstream of the open reading frame with several *Tvv*1 variants, representing a family with at least 28 copies of different sizes (Pelsy and Merdinoglu, 2002). The authors also verified that this internal region is flanked by 2 long terminal repeats in direct orientation. Pelsy (2007) compared the use of *Tvv*1 retrotransposon-based marker with a group of 14 nuclear simple sequence repeat (SSR) markers having a high level of allelic polymorphism, of which 3 belonged to the grape SSR core set indicated by This et al. (2004). The results showed that this marker can be used as a "DNA barcode" for *Vitis* identification.

Although, at present, the SSR markers are the frequently used molecular markers in *Vitis*, we believe that a retrotransposon-based marker can play a useful role in genetic evaluation of *Vitis* due to its high multiplex ability, especially with regard to the large-scale evaluation of multiple individuals.

This study evaluated several parameters of genetic diversity in 26 *Vitis* spp and compared the results of the *Tvv*1 retrotransposon-based marker against 7 standard SSR markers optimized for grape genotyping (Leão et al., 2009; Schuck et al., 2009).

MATERIAL AND METHODS

Plant material

Twenty-six varieties of grapes used for the study are described in Table 1. Each genotype represents a different *Vitis* species or their hybrids, and they are maintained in a field collection at the Experimental Farm of EPAMIG, Caldas, MG, Brazil.

Table 1. Grape accessions evaluated.

ID	Name	Origin	Specie	Pedigree
Scions				
1	Moscato	Italy	Hybrid	Couderc 13 x July Muscat
2	Chardonnay	France	*V. vinifera*	Pinot Noir x Gouais Blanc
3	Syrah	France	*V. vinifera*	Dureza x Mondeuse
4	Cabernet Sauvignon	France	*V. vinifera*	Cabernet Franc x Sauvignon Blank
5	Merlot	France	*V. vinifera*	Magdeleine Noire des Charentes x Cabernet Franc
6	Cabernet Franc	France	*V. vinifera*	Unknown
7	Sauvignon Blanc	France	*V. vinifera*	Traminer x unknown
8	Red Niagara	Brazil	*V. labrusca*	Mutation of Niágara Branca
9	White Niagara	USA	*V. labrusca*	Concord (*V. labrusca*) x Cassady (*V. vinifera* x *V. labrusca*)
10	Bordô	USA	Hybrid	Isabella x *V.labrusca*
11	Violeta	Brazil	Hybrid	BRS Rúbea x IAC 1398-21
Rootstocks				
12	Gravesac	France	Hybrid	161-49 x 3309
13	Kober 5BB	Austria	Hybrid	*V. berlandieri* x *V. riparia*
14	Rupestris du Lot	France	*V. rupestris*	*V. rupestris*
15	101-14	France	Hybrid	*V. riparia* x *V. rupestris*
16	R99	France	Hybrid	*V. berlandieri* x *V. rupestris* (*Berlandieri Las Sorres* x *Rupestris du Lot*)
17	420 A	Italy	Hybrid	*V. berlandieri* x *V. riparia*
18	1045 - Paulsen	France	Hybrid	*V. berlandieri* x (*V. rupestris* x *V. vinifera*)
19	161-49	France	Hybrid	*V. riparia* x *V. berlandieri*
20	R110	USA	Hybrid	*V. berlandieri* x *V. rupestris*
21	3309	Italy	Hybrid	*V. riparia* x *V. rupestris*
22	1103 Paulsen	France	Hybrid	*V. rupestris* x *V. berlandieri*
23	SO4	Germany	Hybrid	*V. berlandieri* x *V. riparia*
24	Traviú	France	Hybrid	*V. riparia* x (*V. rupestris* x *V. cordifolia*)
25	IAC766	Brazil	Hybrid	106-6 x *V. caribea*
26	IAC572	Brazil	Hybrid	[(*V. tiliaefolia* x *V. riparia* x *V. rupestris* ('101-14 Mgt')]

DNA extraction

Samples of 10 young leaves were collected in the field, transported on ice, and stored at -80°C until extraction of genomic DNA. DNA extraction was performed according to the method described by Doyle and Doyle (1990). After DNA isolation, the samples were treated with RNase-A and incubated at 37°C for 1 h to remove RNA. Next, the DNA samples were visualized on a 0.8% agarose gel stained with ethidium bromide to check DNA quality. The DNA concentration was estimated spectrophotometrically at a wavelength of 260 nm. Absorbance was measured at the wavelength of 280 nm to check for putative protein contaminations.

Retrotransposon-based marker *Tvv*1 profile

A primer pair was used to amplify the UTL regions of the *Tvv*1 elements. The forward primer was Pltr1 (5'-CCTAATTCAGGACTCTCAAT-3'), and the reverse primer was P17 (5'-CTAGAATTCTTACTCTCTTCC-3'). The forward primer was complementary to a consensus domain of the 5'-LTR, and the reverse primer was complementary to the beginning of the gap region. The PCR amplification protocol was as described by Pelsy and Merdinoglu (2002), except that 40 cycles were run instead of 30 cycles. After amplification, 5 µL PCR product was run on an 0.8% agarose gel to confirm the existence of a PCR product. Subsequently, vertical electrophoresis on a 6% denaturing polyacrylamide gel was performed at 60 W. The gel was run for 7 h because of the high expected size of the majority of bands. The gels were then stained with silver nitrate according to the method described by Creste et al. (2001), with all steps performed on a shaker inside a fume hood. The gel was dried overnight at room temperature and then photodocumented. Two sets of amplifications and electrophoresis were performed to assure the multilocus standard and reproducibility of all amplicons generated by this technique. The bands were scored as present (1) or absent (0) across all genotypes and tabulated as binary data.

SSR marker profile

In all, the following 7 SSR markers previously described as polymorphic for the genus *Vitis* were used: VVS2 (Thomas and Scott, 1993), VVMD5, VVMD7 (Bowers et al., 1996), VVMD25, VVMD27, VVMD31 (Bowers et al., 1999b), and VRZAG62 (Sefc et al., 1999). The sequences of these markers are shown in Table 2. In this study, 5 of the 6 primer pairs of the core set of the SSR project GENRES 081 (Dettweiler et al., 2000; This et al., 2004) and other primer pairs widely used in genotyping of the vine were used. The core set comprises the following SSR markers: VVS2, VVMD5, VVMD7, VVMD27, and VRZAG62; these markers are used for standard screening of grapevine collections in Europe.

Table 2. SSR primers used in the present study, including sequences, references and expected allele size range (bp).

Primer	Sequence (5'-3')	Reference	Allele size (bp)
VVS2-Forward	CAGCCGTAAATGTATCCATC	Thomas and Scott (1993)	129-155
VVS2-Reverse	AAATTCAAAATTCTAATTCAACTGG		
VVMD5-Forward	CTAGAGCTACGCCAATCCAA	Bowers et al. (1996)	226-246
VVMD5-Reverse	TATACCAAAAATCATATTCCTAAA		
VVMD7-Forward	AGAGTTGCGGAGAACAGGAT	Bowers et al. (1996)	232-263
VVMD7-Reverse	CGAACCTTCACACGCTTGAT		
VVMD25-Forward	TTCCGTTAAAGCAAAAGAAAAAGG	Bowers et al. (1999b)	243-275
VVMD25-Reverse	TTGGATTTGAAATTTATTGAGGGG		
VVMD27-Forward	GTACCAGATCTGAATACATCCGTAAGT	Bowers et al. (1999b)	173-194
VVMD27-Reverse	ACGGGTATAGAGCAAACGGTGT		
VVMD 31-Forward	CAGTGTTTTTCTTAAAGTTTCAAGG	Bowers et al. (1999b)	196-224
VVMD 31-Reverse	CTCTGTGAAAGAGGAAGAGACGC		
VRZAG62-Forward	GGTGAAATGGGCACCGAACACACGC	Sefc et al. (1999)	185-203
VRZAG62-Reverse	CCATGTCTCTCCTCAGCTTCTCAGC		

The amplification reactions were performed in a final volume of 30 µL containing 50 ng DNA, 6 µL 5X reaction buffer, 1.5 µL 1.5 mM $MgCl_2$, 0.5 µL 200 mM of each dNTPs, 0.5

mM of each primer (Sigma, USA), and 0.75 U Taq DNA polymerase (Go Taq Flexi; Promega, USA). The reactions were conducted in a thermal cycler gradient (Gradient Multigene; Labnet International, USA) programmed for an initial denaturation step of 5 min at 94°C, followed by 37 cycles of denaturation at 94°C for 50 s, annealing for 50 s (variable temperature), and extension at 72°C for 1 min. A final extension step was performed at 72°C for 5 min. The amplifications were performed using a touchdown system, with the primer annealing temperature decreasing 1°C per cycle during the first 5 amplification cycles performed at 62-57°C. The sixth cycle onward, the annealing temperature was set at 57°C. The final quality of the amplification products was confirmed by performing the same procedures described above for the retrotranposon-based marker.

The amplified products were run on 0.8% agarose gels and then subjected to 6% denaturing polyacrylamide gel electrophoresis at 60 W for a variable time (2.5-3.5 h) according to the expected allele size. The SSR bands were revealed by silver nitrate staining as described above. On the basis of the allele profiles generated using the 7 SSR markers for the 26 varieties of grapes, we constructed a matrix in which each allele of every locus was designated by its molecular weight, measured using the anchored known allelic profile, as suggested by This et al. (2004) and Cipriani et al. (2008). This allowed a more accurate measurement of the SSR allele size.

Data analysis

*Tvv*1 *retrotransposon-based marker*

The applicative Structure 2.3.1 (Pritchard et al., 2000) was used to determine the genetic structure of the 26 genotypes analyzed in this study; the Bayesian method was used, and the number of groups (k) was set as the adjustable parameter more liable. The non-admixture model with independent allele frequencies was applied using the Markov Chain Monte Carlo algorithm and run at a burn-in period of 5000 steps and a chain length of 50,000 replicates during analysis. Twenty simulations were performed for each value of k, with the k values ranging from 1 to 12. The Δk statistical test was performed using the Structure Harvester program (http://taylor0.biology.ucla.edu/struct_harvest/) on the basis of the criterion suggested by Evanno et al. (2005). The criterion is based on the mean and standard deviation of the log probability of the data (lnP(D)) obtained for each value of k during the 20 simulations. The Δk value was estimated for each k to obtain the greatest value. After the optimum Δk value was selected, the lower lnP(D) value was chosen from among the 20 simulations for each value of k. A graph for each replicate run was generated; each color generated represented a group of structured individuals.

The matrix of genetic dissimilarity generated on the basis of the distance between shared alleles (Chakraborty and Jin, 1993) and a phylogenetic tree developed using the clustering method of the nearest neighbor model (Saitou and Nei, 1987) were obtained using Powermarker Version 3.25 (Liu and Muse, 2005).

GenAlex 6 (Peakall and Smouse, 2006) was used to estimate the graphic dispersion by using the PCoA method. The same program was used to analyze genetic variability within and between groups by the analysis of molecular variance (AMOVA) method (Excoffier et al., 2005).

SSR markers

The Convert program (Glaubitz, 2004) was used to individually estimate the allele frequency for all loci. This program was also used to convert diploid SSR data to a Structure 2.3.1 format. Structure 2.3.1 was also used for the SSR data providing the most liable grouping with the 26 grape genotypes.

Powermarker Version 3.25 (Liu and Muse, 2005) was used for statistical analysis of the following parameters: polymorphic information content (PIC), gene diversity, heterozygosity, and number of alleles at each locus. This program was also used to develop a matrix of genetic dissimilarity based on genetic distance calculated using CS Chord distance (Cavalli-Sforza and Edwards, 1967) as well as a phylogenetic tree obtained using the clustering method of the nearest neighbor model (Saitou and Nei, 1987).

GenAlex 6 (Peakall and Smouse, 2006) was used to estimate the following parameters: probability of identity (for each locus and cumulative) (Waits et al., 2001) and graphic dispersion by the PCoA method. The same program was used to analyze the genetic variability within and between groups by using AMOVA and to generate the Mantel test correlation between the genetic similarities by using the data obtained from both classes of molecular markers tested in this study.

RESULTS AND DISCUSSION

SSR markers

The 7 SSR markers used in this study allowed the differentiation of 25 genotypes from among the 26 grapevine varieties. Only the varieties 'Red Niagara' and 'White Niagara' showed the same allelic profile; this was expected since both the genotypes had similar genetic origins.

The allele frequencies for each locus are shown in Supplementary Table 1. Among the 26 varieties tested, the most frequent alleles were VVMD25-234 (Freq = 0.36) and VVMD25-240 (Freq = 0.3462). The allele frequency distribution for each locus allowed the evaluation of the efficiency of each marker for differentiation of the grapevine varieties. Loci that had relatively equal allele frequency distributions allowed more efficient differentiation of varieties; this finding was in accordance to that reported by Tessier et al. (1999). In that study, 224 grapevine varieties were analyzed, and the authors found that the discriminating power of a marker depended on not only the number of patterns generated but also the distribution of allele frequencies. Hence, even if 2 markers generate the same number of patterns, they may have very different discriminating powers. Conversely, 2 markers that generate different numbers of patterns may have similar discriminatory powers.

Some genetic parameters derived from the results are shown in Table 3. For the 7 loci, a total of 89 alleles were obtained. The number of alleles per locus ranged from 10 (VVMD25) to 16 (VVMD7 and VVMD27), with an average of 12.71 alleles per locus. These values are significantly higher than those reported by other similar studies. Santana et al. (2008) reported an average of 8.67 alleles per locus after analyzing 65 samples corresponding to 35 genotypes of *V. vinifera*, while Bowers et al. (1999a) detected an average of 11 alleles per locus after analyzing 350 varieties of French grapes. Similarly, Sefc et al. (2000) analyzed a set of 164

varieties and found an average of 9.8 alleles per locus. The relatively low allele frequency in these studies can be attributed to the fact that all grape varieties tested were of European origin and belonged to the same species (*V. vinifera*), regardless of the large sample size.

Table 3. Genetic parameters for seven markers in 26 accessions analyzed.

Locus	Sample size	Allele number	Probability of identity	Heterozygosity	Gene diversity	PIC
VVS2	26	13	0.018	0.9231	0.9009	0.8926
VVMD5	26	11	0.049	0.6400	0.8176	0.8017
VVMD7	26	16	0.016	0.9231	0.9053	0.8983
VVMD25	26	10	0.079	0.7692	0.7774	0.7478
VVMD27	26	16	0.015	0.8077	0.9075	0.9006
VVMD31	26	12	0.032	0.7308	0.8661	0.8518
VZAG62	26	11	0.034	0.6000	0.8700	0.8569
Average	26	12.71	0.034	0.7705	0.8635	0.8500
Total	-	89	1.89×10^{-11}	-	-	-

PIC = polymorphism information content.

Therefore, although not very numerous, the range of grape varieties analyzed in this study showed a relatively high genetic diversity possibly because of the differences in the genetic origin of the species. These included grape varieties of European origin, scion varieties of North American origin, and 15 rootstock varieties, which were mainly hybrid varieties that originated from interspecific crosses. This et al. (2004) also found a high allele frequency; they analyzed 46 varieties of grapes and found an average of 17 alleles per locus. They suggested that the high allele frequency was attributed to the combination of varieties of *V. vinifera* and interspecific hybrid rootstocks.

In this study, the heterozygosity ranged from 0.6000 (VZAG62) to 0.9231 (VVS2 and VVMD7), with an average of 0.7705. During the commonly used vegetative propagation of grapevine, varieties are selected for quality and productivity at an earlier stage; this would have resulted in a high heterozygosity. In addition, grapevine genotypes are usually sensitive to inbreeding depression, and the best breeding performances are obtained with heterozygous individuals (Lopes et al., 1999).

VVMD27 (0.9006), VVMD7 (0.8983), and VVS2 (0.8926) showed the highest PIC, while VVMD25 (0.7478) had the lowest PIC value. The lowest information content for this locus is because the allele frequencies are not equally distributed, and the 3 major alleles account up to only 0.75 of allele frequency. A locus can have maximum information content when all the alleles have equal frequencies (Sefc et al., 1999; Tessier et al., 1999).

The lowest probability of identity (PI) values were observed for VVMD27 (0.015), VVMD7 (0.016), and VVS2 (0.18) and the highest PI values were obtained for VVMD25 (0.79) and VVMD5 (0.49). The loci with higher PIC values showed lower PI values and vice versa. Thus, a correlation was observed between the different parameters used to measure the information content of each locus, indicating that the data were fairly robust with respect to the characterization of genotypes. The SSR markers could efficiently distinguish the genotypes evaluated. Considering that only 7 loci were used, the total PI of 1.89×10^{-11} confirmed the high efficiency of these markers for the differentiation of the grapevine accessions studied.

The 7 SSR markers identified 13 exclusive alleles of *V. vinifera* accessions, 4 unique alleles of the North American varieties, and 38 exclusive alleles of rootstock accessions. Al-

though the 3 varieties included different numbers of individuals (7 *V. vinifera*; 4 North American, and 15 hybrid rootstocks), the absolute number of alleles for each group does not allow the comparison of the abundance of private alleles in each group. The value of R (number of exclusive alleles/number of individuals of determined population) for the rootstock varieties (2.53) was higher than that for the other varieties (*V. vinifera* = 1.85 and North American = 1). This indicated that the large number of alleles for rootstock varieties was due to not only the largest number of individuals included in this group but also to the high genetic diversity among the accessions comprising this group. Furthermore, the allele size range for each locus in rootstock accessions was clearly larger (Supplementary Table 2), as has been reported by Arrigo and Arnold (2007).

The genetic distance measured using CS Chord revealed a high level of genetic variation among the varieties tested. The genetic dissimilarity ranged from 0 to 0.9003, with an average of 0.7806. A lower genetic divergence was observed between the varieties 'White Niagara' and 'Red Niagara' (0), which showed identical allelic profiles for all loci analyzed. This result was expected since 'Red Niagara' originated from a spontaneous mutation of the 'White Niagara'. 'White Niagara' was introduced in Brazil in 1894 and in 1933 farmers identified a somatic mutation in this variety, and shortly afterward, this variety with pale red grapes was named 'Red Niagara' (Sousa, 1959). At present, 'Red Niagara' is a very popular variety cultivated in Brazil.

Reports suggesting that SSR markers can be used to distinguish 2 clones or identify a point mutation are very rare (Riaz et al., 2002; Hocquigny et al., 2004; Moncada et al., 2006). Therefore, distinguishing the genetic profiles of 'White Niagara' and 'Red Niagara' would require markers that are more broadly distributed in the genome than SSRs, such as single nucleotide polymorphisms that can be identified from genomic sequencing and expressed sequence tag data of grapevine or even more multiplex systems such as amplified fragment length polymorphism.

The computational program Structure was also used to analyze the genetic organization of the 26 grapevine varieties. According to the criterion of Evanno et al. (2005), the optimum Δk was obtained when $k = 5$, indicating that the maximum structure similarity was observed when the sample was divided into 5 allele groups. Figure 1 shows a high degree of structure similarity for the 5 groups formed. One group consisted of only *V. vinifera* varieties (red); the other group, the North American accessions (blue), and the remaining 3 groups (yellow, green, and pink), the rootstock accessions. The red group did not include 'Chardonnay' (a *V. vinifera* accession); however, this important accession had a membership coefficient of 0.398 with the red group. Although 'Chardonnay' grapes are a *V. vinifera* variety, they have a range of physiological traits that differ from those of the other genotypes included in this genus. Furthermore, this accession possesses great adaptability to water-deficit stress. These attributes would explain the diversity in the structure of this accession.

The 'Moscato' accession is a hybrid of 'Couderc 13' (1/2 *V. lincecumi*, 3/8 *V. vinifera*, and 1/8 *V. rupestris*) × 'July Muscat' (*V. vinifera*). This could explain the low membership coefficient (0.146) to the *V. vinifera* structure group. As reported by Bowers et al. (1999b), the accession 'Carbenet Sauvignon' inherits the SSR alleles from the 'Sauvignon Blanc' and 'Carbenet Franc' accessions in a Mendelian manner.

All the North American accessions clustered together in the blue group. When an 80% cutoff was used, the green group consisted of 5 rootstocks accessions, besides the 'Moscato'

hybrid. This group also included the 'Gravesac' accession, as well as its parents '3309' and '161-49'. Interestingly, comparison of the SSR profiles of these accessions revealed a Mendelian inheritance of alleles at all loci, providing molecular evidence for the historical pedigree of 'Gravesac'. In France, only 2 rootstocks were bred in the second half of the 20th century: 'Gravesac' and 'Fercal' (Laucou et al., 2009). 'Gravesac' was bred specially in acid soils. The 'Kober 5BB', an important rootstock, showed a 0.681 membership coefficient with this first structure group of rootstocks. The yellow group comprises only 3 well-clustered rootstock accessions ('SO4', 'Traviú', and 'IAC 766'). The pink group consisted of 5 well-clustered rootstocks (all from France) and 1 structure hybrid ('R110' from USA).

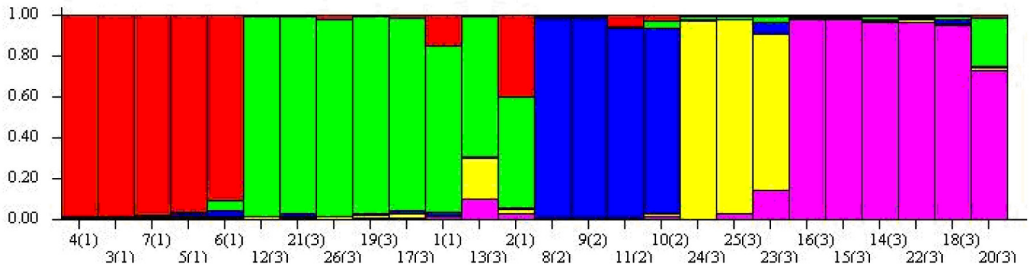

Figure 1. Analysis of the genetic structure of 26 grape accessions (On the x-axis, the numbers outside parentheses represent the 26 grapevine varieties studied. Where: (1) 'Moscato', (2) 'Chardonnay', (3) 'Syrah', (4) 'Cabernet Sauvignon', (5) 'Merlot', (6) 'Cabernet Franc', (7) 'Sauvignon Blanc', (8) 'Red Niagara', (9) 'White Niagara', (10) 'Bordô', (11) 'Violeta', (12) 'Gravesac', (13) 'Kober 5BB', (14) 'Rupestris du Lot', (15) '101-14', (16) 'R99', (17) '420 A', (18) '1045 Paulsen', (19) '161-49', (20) 'R110', (21) '3309', (22) '1103 Paulsen', (23) 'SO4', (24) 'Traviú', (25) 'IAC766', and (26) 'IAC572'. The numbers in parentheses represent the three groups of varieties, and (1) corresponds to the group of *Vitis vinifera* grapes, (2) the group of North American scions, and (3) the group of rootstocks accessions. The y-axis is a relative reference scale of coefficient of membership. Data generated by the 7 most often used *Vitis* SSR markers.

The neighbor joining tree (Figure 2) obtained using the CS Chord distance (1967) was very congruent with the Structure data, consisting of 5 branches, with each branch corresponding to a structured group. The 'Moscato' accession (ID 1) shared ancestral features with the green and red structure groups. Therefore, there was a clear early separation of this accession from the *V. vinifera* accession branch. The same behavior was observed for the accession 'Kober 5BB' (ID 13) that also shared ancestral features with the 3 structure groups. Hence, this accession was placed in the yellow group branch but was well separated from the 3 accessions included in this branch (IDs 23, 24, and 25). The remaining 3 branches (green, yellow, and pink) showed good agreement with the structure groups.

The PCoA showed the dispersion of all individuals of each group, and the North American scions were fairly separated from the other accessions (Figure 3). Nonetheless, it identified 2 additional groups: *V. vinifera* and rootstocks. Together, the 2 axes attributed to a 50.42% of the total variation. Among the *V. vinifera* accessions, 'Moscato' (a hybrid) was the most widespread accession, which was in agreement with the results of Structure analysis and neighbor joining tree method. A high variability was also observed in the rootstock group, suggesting the divergence of those accessions due to the interspecific crosses that constitute them. This great dispersion could also be explained by the high number of private alleles in this group.

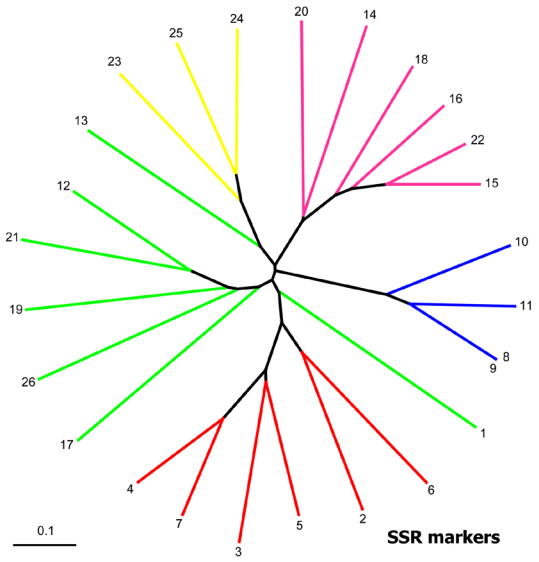

Figure 2. Dendrogram obtained by the method of nearest neighbor, based on CS Chord distance (1967), illustrating the phylogenetic relationships among 26 grapevine varieties and divided into five groups of genetic similarity, using the SSR markers. The names of the *Vitis vinifera* varieties are outlined in blue, North American scions in red and rootstocks in black. Where: (1) 'Moscato', (2) 'Chardonnay', (3) 'Syrah', (4) 'Cabernet Sauvignon', (5) 'Merlot', (6) 'Cabernet Franc', (7) 'Sauvignon Blanc', (8) 'Red Niagara', (9) 'White Niagara', (10) 'Bordô', (11) 'Violeta', (12) 'Gravesac', (13) 'Kober 5BB', (14) 'Rupestris du Lot', (15) '101-14', (16) 'R99', (17) '420 A', (18) '1045 Paulsen', (19) '161-49', (20) 'R110', (21) '3309', (22) '1103 Paulsen', (23) 'SO4', (24) 'Traviú', (25) 'IAC766', and (26) 'IAC572'.

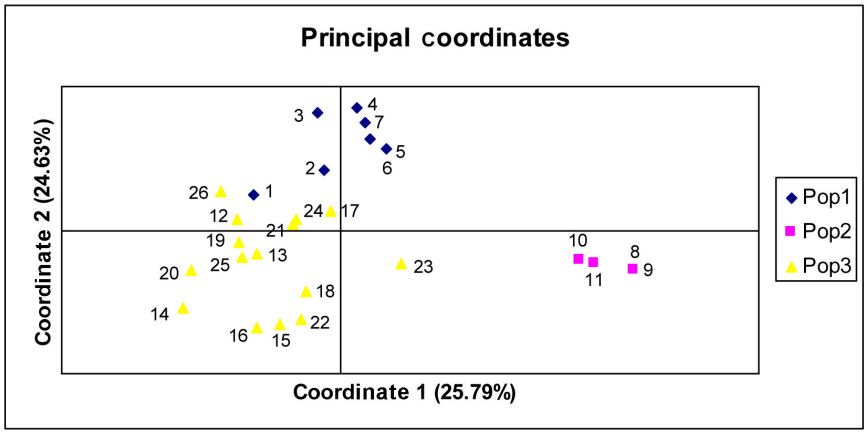

Figure 3. Principal coordinate analysis of 26 varieties of grapes using the 7 most often used *Vitis* SSR markers. The population 1 is composed by *Vitis vinifera* (ID 1, 2, 3, 4, 5, 6, 7, and 8). Population 2 is composed by North American varieties (ID 9, 10, 11, and 12). The population 3 is composed by rootstocks (ID 12 to 26). Where: (1) 'Moscato', (2) 'Chardonnay', (3) 'Syrah', (4) 'Cabernet Sauvignon', (5) 'Merlot', (6) 'Cabernet Franc', (7) 'Sauvignon Blanc', (8) 'Red Niagara', (9) 'White Niagara', (10) 'Bordô', (11) 'Violeta', (12) 'Gravesac', (13) 'Kober 5BB', (14) 'Rupestris du Lot', (15) '101-14', (16) 'R99', (17) '420 A', (18) '1045 Paulsen', (19) '161-49', (20) 'R110', (21) '3309', (22) '1103 Paulsen', (23) 'SO4', (24) 'Traviú', (25) 'IAC766', and (26) 'IAC572'.

The results of the AMOVA are shown in Table 4. In all, 70% of total variation was observed within groups and only 30% of total variation was observed between the groups. This indicates that the groups consist of varieties that have relatively high levels of dissimilarity. The high average dissimilarity between the rootstocks considerably contributed to the high value of genetic variance within the groups. In addition, the rootstock group included the largest number of varieties (15 of the 26 varieties) studied. This finding corroborates the theory that, in plants, genetic diversity is greater within populations than between populations.

Table 4. Analysis of molecular variance (AMOVA) of 26 grape varieties (7 *Vitis vinifera*, 4 North American scions, and 15 rootstocks) by simple sequence repeat (SSR) analysis.

Source of variation	d.f.	SS	MS	Estimated variance	Percentage of molecular variance
Between groups	2	47.191	23.596	2.429	30%
Within groups	23	127.954	5.563	5.563	70%
Total	25	175.145	29.159	7.992	100%

d.f. = degrees of freedom; SS = sum of squares; MS = mean square.

Retrotransposon-based marker

The retrotransposon-based marker profile generated 18 amplified DNA fragments from the 26 grape accessions; all the fragments were polymorphic. The 18 DNA fragments were capable of differentiating 25 accessions from among the 26 accessions included in this study. The 2 North American scion accessions ('Red Niagara' and 'White Niagara') had the same fragment size profile. This suggests that there is low genetic dissimilarity between these 2 varieties and markers covering much broader area of the genome would be required to identify the level of DNA polymorphism between the 2 genotypes. Generally, some bands appeared only for the rootstock accessions, while some others appeared only in a few genotypes of the scion accessions (*V. vinifera* and North American scion group). Therefore, such markers are very useful for fingerprinting purposes since they have the same power of genotype differentiation as SSR markers and produce reproducible results with very few PCR amplifications and gel electrophoresis, as has been previously reported by Pelsy (2007).

The Bayesian analysis performed using the Structure software showed that, according to the criterion of Evanno et al. (2005), the accessions could be stratified in 2 groups (50% cutoff) (Figure 4). One group comprises *V. vinifera* and North American scion accessions, and the second group comprises rootstock accessions. The accessions 'Bordô' (ID10) and 'Violeta' (ID11) were found to be Structure hybrids according to this marker and had a membership coefficient to the North American group of 0.759 and 0.514, respectively. Interestingly, the other accessions, irrespective of the grouping, showed a high degree of structure similarity, having at least 0.946 of membership coefficient. Compared to the SSR markers, the retrotransposon-based marker could efficiently discriminate North American varieties from rootstock varieties but was unable to distinguish 2 accessions within the North American and rootstock groups. This finding suggested that the retrotransposon-based marker was not appropriate for subgroup discrimination. Aradhya et al. (2003) suggested that the choice of a marker for evaluating the genetic structure of a population should be made on the basis of the codominant nature of the marker. Furthermore, Evanno et al. (2005) assumed that a codominant locus generally has the complete information corresponding to 10 dominant loci. The dominant nature of the *Tvv*1 marker conjugated with only 18 amplified loci could be the reason why the marker could not be used to study the genetic structure of the *Vitis* genotypes.

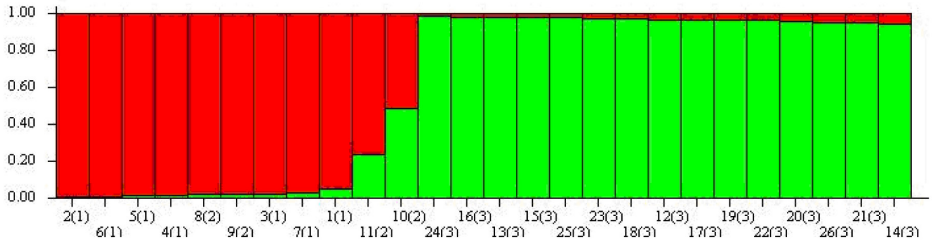

Figure 4. Analysis of the genetic structure of 26 grape accessions (On the x-axis, the numbers outside parentheses represent the 26 grapevine varieties studied. Where: (1) 'Moscato', (2) 'Chardonnay', (3) 'Syrah', (4) 'Cabernet Sauvignon', (5) 'Merlot', (6) 'Cabernet Franc', (7) 'Sauvignon Blanc', (8) 'Red Niagara', (9) 'White Niagara', (10) 'Bordô', (11) 'Violeta', (12) 'Gravesac', (13) 'Kober 5BB', (14) 'Rupestris du Lot', (15) '101-14', (16) 'R99', (17) '420 A', (18) '1045 Paulsen', (19) '161-49', (20) 'R110', (21) '3309', (22) '1103 Paulsen', (23) 'SO4', (24) 'Traviú', (25) 'IAC766', and (26) 'IAC572'. The numbers in parentheses represent the three groups of varieties, and (1) corresponds to the group of *Vitis vinifera* grapes, (2) the group of North American scions, and (3) the group of rootstocks accessions. The y-axis is a relative reference scale of coefficient of membership. Data generated by *Tvv*1 marker.

The neighbor joining tree was well separated into 2 parts, with accessions ID11 and ID10 located at the intermediate position on the tree (Figure 5). However, the tree had its own topology and was poorly congruent with the SSR neighbor joining tree. For example, the 'White Niagara' and 'Red Niagara' accessions were grouped with accessions 'Moscato' and 'Merlot'. According to the SSR data, 'Moscato' was close to the North American scion accessions, but 'Merlot' was slightly far from those accessions. Pelsy (2007) also encountered some disagreement between the tree topology generated by these 2 kinds of markers.

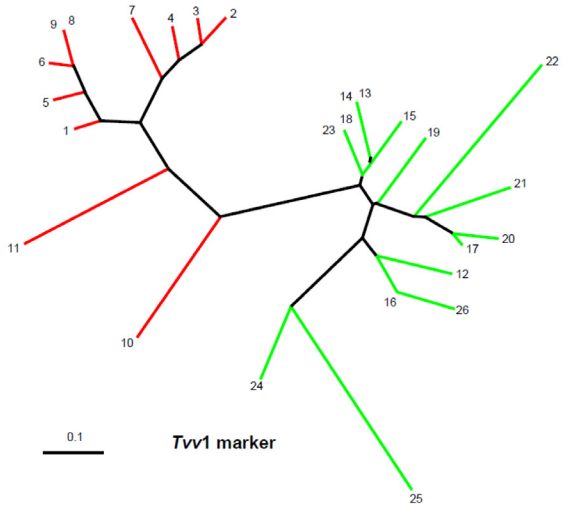

Figure 5. Dendrogram obtained by the method of neighbor joining tree, based on shared allele distance, illustrating the phylogenetic relationships among 26 grapevine varieties and divided into five groups of genetic similarity, using *Tvv*1 marker. The names of the *Vitis vinifera* varieties are outlined in blue, North American scions in red and the rootstocks in black. Where: (1) 'Moscato', (2) 'Chardonnay', (3) 'Syrah', (4) 'Cabernet Sauvignon', (5) 'Merlot', (6) 'Cabernet Franc', (7) 'Sauvignon Blanc', (8) 'Red Niagara', (9) 'White Niagara', (10) 'Bordô', (11) 'Violeta', (12) 'Gravesac', (13) 'Kober 5BB', (14) 'Rupestris du Lot', (15) '101-14', (16) 'R99', (17) '420 A', (18) '1045 Paulsen', (19) '161-49', (20) 'R110', (21) '3309', (22) '1103 Paulsen', (23) 'SO4', (24) 'Traviú', (25) 'IAC766', and (26) 'IAC572'.

The PCoA graph (Figure 6) showed very well-separated groups (scions and rootstocks). The first component suggested 51.25% of total variation. The first and second components together suggested 65.25% of variation; this value was considerably higher than that obtained by the SSR data. Although the first coordinate separated the rootstock accessions from scions (*V. vinifera* and North American scion groups), the 2 coordinates together could not separate the 2 scion groups, and a little dispersion was observed between the 2 scion groups.

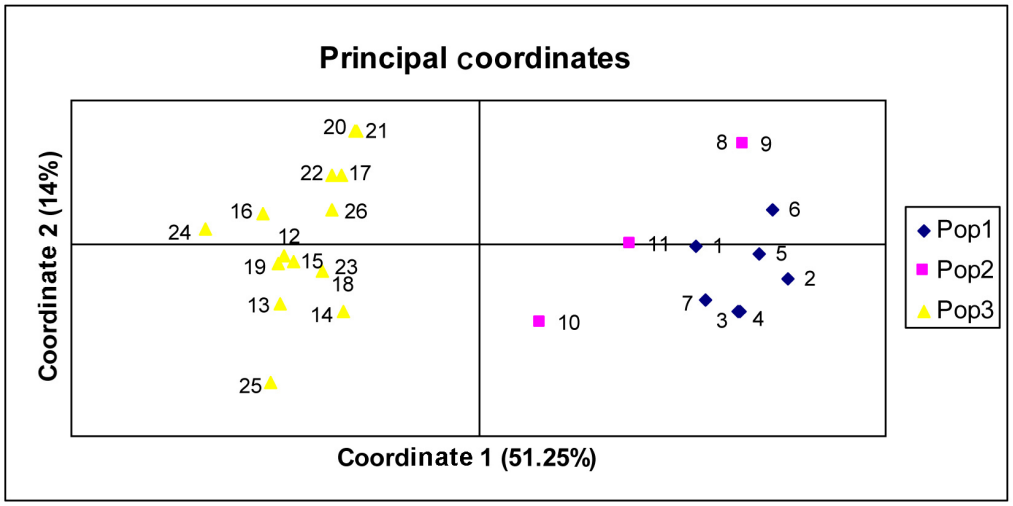

Figure 6. Principal coordinate analysis of 26 varieties of grapes using *Tvv*1 marker. The population 1 is composed by *Vitis vinifera* (ID 1, 2, 3, 4, 5, 6, and 7). Population 2 is composed by North American varieties (ID 8, 9, 10, and 11). The population 3 is composed by rootstocks (ID 12 to 26). Where: (1) 'Moscato', (2) 'Chardonnay', (3) 'Syrah', (4) 'Cabernet Sauvignon', (5) 'Merlot', (6) 'Cabernet Franc', (7) 'Sauvignon Blanc', (8) 'Red Niagara', (9) 'White Niagara', (10) 'Bordô', (11) 'Violeta', (12) 'Gravesac', (13) 'Kober 5BB', (14) 'Rupestris du Lot', (15) '101-14', (16) 'R99', (17) '420 A', (18) '1045 Paulsen', (19) '161-49', (20) 'R110', (21) '3309', (22) '1103 Paulsen', (23) 'SO4', (24) 'Traviú', (25) 'IAC766', and (26) 'IAC572'.

AMOVA revealed lower variance within the groups with the *Tvv*1 marker, making the variance between groups larger (Table 5). This finding highlighted the well-structured nature of the genotypes within the groups and the inability of the marker to detect small variance among individuals in a determined group.

Table 5. Analysis of molecular variance (AMOVA) of 26 grape varieties (7 *Vitis vinifera*, 4 North American scions, and 15 rootstocks) by *Tvv*1 marker analysis.

Source of variation	d.f.	SS	MS	Estimated variance	Percentage of molecular variance
Between groups	2	35.388	17.694	2.123	52%
Within groups	23	44.574	1.938	1.938	48%
Total	25	79.962	19.632	4.061	100%

d.f. = degrees of freedom; SS = sum of squares; MS = mean square.

The Mantel test performed by considering the genetic distance obtained using the 2 types of markers showed a correlation of 42.5% and probability of 0.01. In addition, the R^2 for the linear equation was not very high (0.1805; Figure 7). Pelsy (2007) also showed a low correlation between the data generated by using the *Tvv*1 marker against the SSR marker.

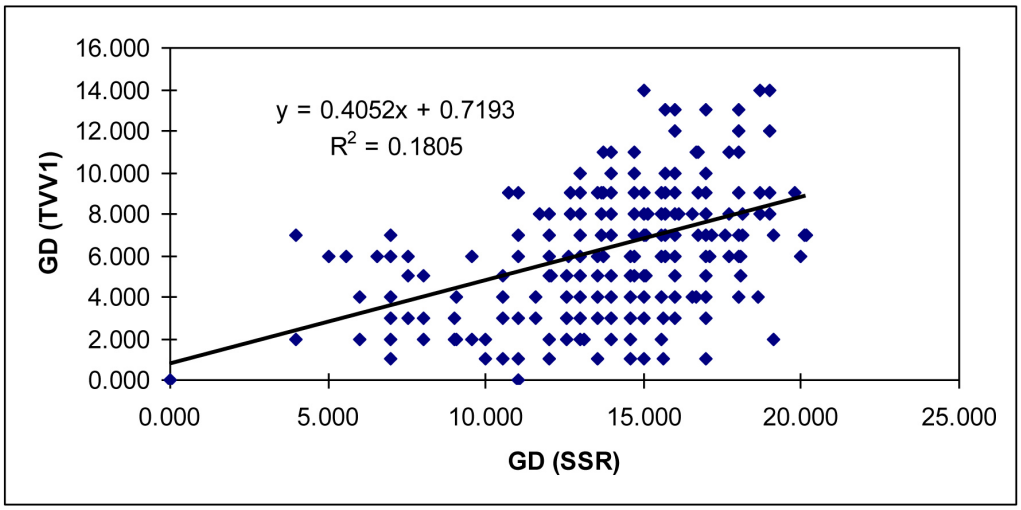

Figure 7. Mantel test correlation between genetic distance (GD) generated by *Tvv*1 marker and the 7 most common used simple sequence repeat (SSR) markers in *Vitis*.

In conclusion, the *Tvv*1 marker could efficiently discriminate the grape accessions but was unable to detect structured sub-groups. The 26 varieties of grapevine analyzed showed a relatively high genetic variability, as revealed by the data obtained using both the *Tvv*1 and SSR markers. This was mainly because of the inclusion of different species from the genus *Vitis*.

ACKNOWLEDGMENTS

The authors would like to acknowledge the financial support and scholarships from International Foundation for Science (IFS), Coordination for the Improvement of Higher Education Personnel (CAPES), Minas Gerais Research Foundation (FAPEMIG), Funding Agency of Studies and Projects (FINEP), National Council for Scientific and Technological Development (CNPq), and Brazilian Agricultural Research Corporation (EMBRAPA).

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Allele frequence comparison over populations, where Pop1 (*Vitis vinifera*), Pop2 (North American varieties), and Pop3 (Rootstocks).

Locus	Allele#	Allele size (bp)	Pop1	Pop2	Pop3	Overall	Private
VVMD7	1	229	0	0	0.0333	0.0192	Rootstocks
VVMD7	2	231	0	0	0.0667	0.0385	Rootstocks
VVMD7	3	233	0	0	0.1	0.0577	Rootstocks
VVMD7	4	235	0	0.5	0.1667	0.1731	-
VVMD7	5	239	0.6429	0	0	0.1731	*V. vinifera*
VVMD7	6	241	0	0.375	0	0.0577	North American varieties
VVMD7	7	243	0.0714	0	0	0.0192	*V. vinifera*
VVMD7	8	245	0	0	0.0667	0.0385	Rootstocks
VVMD7	9	247	0.0714	0	0	0.0192	*V. vinifera*
VVMD7	10	249	0.0714	0.125	0.0333	0.0577	-
VVMD7	11	251	0	0	0.1	0.0577	Rootstocks
VVMD7	12	257	0.0714	0	0.0667	0.0577	-
VVMD7	13	259	0	0	0.1	0.0577	Rootstocks
VVMD7	14	261	0	0	0.1	0.0577	Rootstocks
VVMD7	15	263	0.0714	0	0.0333	0.0385	-
VVMD7	16	265	0	0	0.1333	0.0769	Rootstocks
VVMD7	#	Samples:	7	4	15	26	
VVMD25	1	238	0.0714	0	0.4333	0.2692	-
VVMD25	2	240	0.3571	0.875	0.2	0.3462	-
VVMD25	3	242	0.1429	0	0	0.0385	*V. vinifera*
VVMD25	4	248	0	0	0.1333	0.0769	Rootstocks
VVMD25	5	250	0.2143	0.125	0.1	0.1346	-
VVMD25	6	252	0	0	0.0333	0.0192	Rootstocks
VVMD25	7	254	0	0	0.0333	0.0192	Rootstocks
VVMD25	8	256	0.2143	0	0	0.0577	*V. vinifera*
VVMD25	9	260	0	0	0.0333	0.0192	Rootstocks
VVMD25	10	266	0	0	0.0333	0.0192	Rootstocks
VVMD25	#	Samples:	7	4	15	26	
VVMD5	1	224	0.2857	0	0.0357	0.1	-
VVMD5	2	226	0.0714	0	0.0357	0.04	-
VVMD5	3	230	0.2143	0	0	0.06	*V. vinifera*
VVMD5	4	232	0.0714	0	0.0714	0.06	-
VVMD5	5	234	0.0714	1	0.3214	0.36	-
VVMD5	6	236	0.1429	0	0.0357	0.06	-
VVMD5	7	238	0.1429	0	0.0714	0.08	-
VVMD5	8	250	0	0	0.0357	0.02	Rootstocks
VVMD5	9	260	0	0	0.0357	0.02	Rootstocks
VVMD5	10	262	0	0	0.1071	0.06	Rootstocks
VVMD5	11	264	0	0	0.25	0.14	Rootstocks
VVMD5	#	Samples:	7	4	14	25	
VVMD27	1	171	0.1429	0	0	0.0385	*V. vinifera*
VVMD27	2	175	0.0714	0.25	0	0.0577	-
VVMD27	3	177	0.1429	0.25	0	0.0769	-
VVMD27	4	179	0	0.125	0	0.0192	North American varieties
VVMD27	5	181	0	0.375	0	0.0577	North American varieties
VVMD27	6	183	0.0714	0	0.1333	0.0962	-
VVMD27	7	185	0.4286	0	0.0667	0.1538	-
VVMD27	8	187	0.1429	0	0.0333	0.0577	-
VVMD27	9	189	0	0	0.1	0.0577	Rootstocks
VVMD27	10	191	0	0	0.0333	0.0192	Rootstocks
VVMD27	11	201	0	0	0.0667	0.0385	Rootstocks
VVMD27	12	203	0	0	0.0667	0.0385	Rootstocks
VVMD27	13	205	0	0	0.3	0.1731	Rootstocks
VVMD27	14	207	0	0	0.0667	0.0385	Rootstocks
VVMD27	15	209	0	0	0.1	0.0577	Rootstocks
VVMD27	16	215	0	0	0.0333	0.0192	Rootstocks
VVMD27	#	Samples:	7	4	15	26	

Continued on next page

Supplementary Table 1. Continued.

Locus	Allele#	Allele size (bp)	Pop1	Pop2	Pop3	Overall	Private
VVMD31	1	195	0	0	0.2333	0.1346	Rootstocks
VVMD31	2	197	0	0	0.2333	0.1346	Rootstocks
VVMD31	3	199	0	0	0.1333	0.0769	Rootstocks
VVMD31	4	201	0	0.875	0.1	0.1923	-
VVMD31	5	203	0.1429	0	0	0.0385	*V. vinifera*
VVMD31	6	205	0	0	0.0333	0.0192	Rootstocks
VVMD31	7	207	0.1429	0	0	0.0385	*V. vinifera*
VVMD31	8	209	0.2857	0	0.2	0.1923	-
VVMD31	9	211	0.0714	0	0	0.0192	*V. vinifera*
VVMD31	10	213	0.3571	0.125	0	0.1154	-
VVMD31	11	215	0	0	0.0333	0.0192	Rootstocks
VVMD31	12	219	0	0	0.0333	0.0192	Rootstocks
VVMD31	#	Samples:	7	4	15	26	
VVS2	1	123	0	0.5	0.0333	0.0962	-
VVS2	2	127	0	0	0.0333	0.0192	Rootstocks
VVS2	3	133	0.2143	0.25	0.0667	0.1346	-
VVS2	4	135	0	0.125	0.0667	0.0577	-
VVS2	5	137	0.0714	0	0.2667	0.1731	-
VVS2	6	139	0.2143	0	0	0.0577	*V. vinifera*
VVS2	7	141	0.0714	0	0.1333	0.0962	-
VVS2	8	143	0.0714	0	0.0667	0.0577	-
VVS2	9	145	0	0	0.0333	0.0192	Rootstocks
VVS2	10	147	0.0714	0	0.1	0.0769	-
VVS2	11	149	0	0	0.1	0.0577	Rootstocks
VVS2	12	151	0.2857	0.125	0	0.0962	-
VVS2	13	163	0	0	0.1	0.0577	Rootstocks
VVS2	#	Samples:	7	4	15	26	
VZAG62	1	176	0.0714	0	0.05	0.0526	-
VZAG62	2	186	0.2857	0	0	0.1053	*V. vinifera*
VZAG62	3	188	0	0	0.05	0.0263	Rootstocks
VZAG62	4	190	0.0714	0	0.1	0.0789	-
VZAG62	5	192	0.4286	0	0	0.1579	*V. vinifera*
VZAG62	6	194	0.0714	0	0.4	0.2368	-
VZAG62	7	196	0	0	0.1	0.0526	Rootstocks
VZAG62	8	198	0	0	0.3	0.1579	Rootstocks
VZAG62	9	200	0	0.5	0	0.0526	North American varieties
VZAG62	10	202	0.0714	0.5	0	0.0789	-
VZAG62	#	Samples:	7	2	10	19	

Supplementary Table 2. Genetic profile of 26 genotypes from EPAMIG grapevine panel screened by seven SSR loci.

Individual	VVMD7		VVMD25		VVMD5		VVMD27		VVMD31		VVS2		VZag62	
Vitis vinifera														
1	239	249	238	256	224	236	175	183	209	209	141	151	176	190
2	239	243	240	256	232	236	177	185	211	213	137	143	186	194
3	239	239	242	242	224	230	185	187	209	213	133	133	186	192
4	239	239	240	250	230	238	171	185	203	207	139	151	186	192
5	239	247	240	250	224	234	185	187	209	213	139	151	192	192
6	239	263	240	256	224	238	177	185	203	213	139	147	192	202
7	239	257	240	250	226	230	171	185	207	213	133	151	186	192
North American varieties														
8	235	241	240	240	234	234	175	181	201	201	123	133	?[1]	?
9	235	241	240	240	234	234	175	181	201	201	123	133	?	?
10	235	249	240	240	234	234	179	181	201	213	123	135	200	202
11	235	241	240	250	234	234	177	177	201	201	123	151	200	202
Rootstocks														
12	245	251	238	240	262	264	183	207	197	197	141	163	?	?
13	233	265	238	250	234	264	189	209	197	205	141	149	?	?
14	257	261	238	238	?	?	205	205	209	215	135	135	194	194
15	235	257	238	252	234	234	205	205	199	209	137	147	194	196
16	231	261	238	248	234	234	189	205	199	209	137	149	194	194
17	231	263	240	240	236	262	191	215	195	197	127	137	?	?
18	235	259	238	248	224	234	189	203	201	209	137	149	194	196
19	233	251	238	266	226	264	183	207	197	201	137	141	190	?
20	233	259	238	260	232	264	187	203	199	209	137	145	194	194
21	245	261	238	240	250	262	183	209	197	219	123	163	176	188
22	235	259	238	248	234	234	201	205	199	209	137	147	?	?
23	235	265	240	248	234	264	201	209	195	201	143	147	198	198
24	249	265	240	250	264	264	183	205	195	195	143	133	198	198
25	235	265	238	250	238	238	205	205	195	195	133	137	198	198
26	229	251	238	254	232	260	185	185	195	197	141	163	190	190

Data are reported in bp (allele size). "?" = null allele or missing data. (1) 'Moscato', (2) 'Chardonnay', (3) 'Syrah', (4) 'Cabernet Sauvignon', (5) 'Merlot', (6) 'Cabernet Franc', (7) 'Sauvignon Blanc', (8) 'Red Niagara', (9) 'White Niagara', (10) 'Bordô', (11) 'Violeta', (12) 'Gravesac', (13) 'Kober 5BB', (14) 'Rupestris du Lot', (15) '101-14', (16) 'R99', (17) '420 A', (18) '1045 Paulsen', (19) '161-49', (20) 'R110', (21) '3309', (22) '1103 Paulsen', (23) 'SO4', (24) 'Traviú', (25) 'IAC766', and (26) 'IAC572'.