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High genetic differentiation of grapevine rootstock varieties determined by molecular markers and artificial neural networks

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ABSTRACT. The genetic differentiation of grapevine rootstock varieties was inferred by the Artificial Neural Network approach based on the Self-Organizing Map algorithm. A combination of RAPD and SSR molecular markers, yielding polymorphic informative loci, was used to determine the genetic characterization among the rootstock varieties 420-A, Schwarzmann, IAC-766 Campinas, Traviú, Kober 5BB, and IAC-572 Jales. A neural network algorithm, based on allelic frequency, showed that the individual grapevine rootstocks (n = 64) were grouped into three genetically differentiated clusters. Cluster 1 included only the Kober 5BB rootstock, Cluster 2 included rootstocks of the varieties Traviú and IAC-572, and Cluster 3 included 420-A, Schwarzmann and IAC-766 plants. Evidence from the current study indicates that, despite the morphological similarities of the 420-A and Kober 5BB varieties, which share the same genetic origin, two new varieties were generated that are genetically divergent and show differences in performance.

Keywords: clustering methods; RAPD-SSR loci; self-organizing map algorithm.

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Introduction

The use of resistant rootstocks has become an important strategy to control phylloxera (*Daktulosphaira vitifoliae*) in viticulture. Most vineyards in Brazil are planted by employing rootstock for grafting from the producing cultivars in the field (Roberto, Kanai, & Yano, 2004). Marialva, known as the capital of table grapes, in the north-western region of Paraná State, Brazil, has more than 1,000 hectares of producing vines, all formed with grafted plants. The most commonly used rootstocks are IAC-766-Campinas, IAC-572-Jales, Kober 5BB, 420-A, Schwarzmann, and Traviu (Kishino, Carvalho, & Roberto, 2007). The rootstock Kober 5BB, a cross between *Vitis berlandieri* x *V. riparia*, is widely used worldwide. It had been employed in the Marialva region prior to IAC766. Kober 5BB is remarkable in studies on factors influencing the grafting success and the compatibility of grape rootstocks (Vršič, Pulko, & Kocsis, 2015; Suriano et al., 2016). Although there are different agronomic characteristics between 420A and 5BB Kober (40% divergence; Keller, 2010), the Kober 5BB rootstock can be confused with 420-A before its flowering. The 420-A also originated from a cross between *Vitis berlandieri* x *V. riparia* but is less vigorous and less productive. According to Camargo (1998), the correct identification of rootstocks is highly relevant since 420-A will result in a performance below expectations.

Schwarzmann belongs to the group of hybrids between *V. riparia* x *V. rupestris* and has edaphic aptitudes similar to 101-14 Mgt. This group of complex hybrids includes IAC-766-Campinas, obtained by Santos Neto in 1958 (Santos-Neto, 1973). IAC-766-Campinas shows high strength rates and is well adapted to São Paulo's edaphoclimatic conditions (Pommer, Passos, Terra, & Pires, 1997). Traviú is also part of this group of hybrids and was produced from a cross between *V. riparia* x (*V. cordifolia* x *V. rupestris* 106-8 Mgt). Its adaptation is similar to 104-14 Mgt, although it prefers cooler soils (Sousa, 1969). The IAC-572-Jales rootstock is the result of a cross between *V. caribaea* × (*V. riparia* × *V. rupestris* 101-14 Mgt). Since the early 1990s, use of the IAC-572-Jales rootstock has been widespread under the label, 'Virus-free Tropical Vine' (Pommer et al., 1997).

Ampelography is the most common method used to identify rootstock varieties. In spite of its large and obvious contribution to Brazilian viticulture, during the classification process, ampelography can introduce variables that are not controlled or that depend on the environment (Wolfe, 1976). Further, since grafted rootstocks are never allowed to develop leaves in vineyards, this feature makes correct ampelographic identification difficult. The development of methods that use unchangeable parameters under all circumstances is highly relevant for a straightforward way to differentiate rootstocks. Molecular characterization in rootstocks is mainly employed to compare other *V. vinifera* cultivars from collections in the largest germplasm banks of *Vitis*. Molecular markers, such as restriction fragment length polymorphism (RFLP) (Bourquin, Otten, & Walter, 1991; Bourquin, Tournier, Otten, & Walter, 1992; Guerra & Meredith, 1995), inter simple sequence repeat (ISSR) (Alizadeh, Singh, Jhang, & Sharma, 2008), amplified fragments length polymorphism (AFLP) (Ergül, Aras, & Söylemezoğlu, 2010; Sabir, Doğan, Tangolar, & Kafkas, 2010), simple repeated sequences (SSR) (Sefc, Regner, Glössl, & Steinkellner, 1998; Dzhambazova et al., 2007; Laucou et al., 2011; Jahnke, Majer, Varga, & Szöke, 2011; Andre et al., 2012; Sant'Ana et al., 2012; Emanuelli et al., 2013; Barankova, 2014), and some methods based on retrotransposons (Guo, Guo, Hou, & Zhang, 2014), have been used to identify polymorphic loci for the measurement of genetic divergence or similarity between rootstock varieties.

The rootstock identification process must be as accurate as possible since the rootstock affects the vegetative growth, yield and quality of the grape bunches. Vines undergo significant edaphoclimatic interference and respond differently, according to the canopy grafted (Cookson & Ollat, 2013). Since the identification of rootstocks using phenological parameters is not completely accurate and has caused problems for growers, the current study uses two types of molecular markers to assess the genetic relationship among six rootstock varieties: Kober 5BB, 420-A, Schwarzmann, IAC-766-Campinas, Traviu, and IAC-572-Jales, extant in the North and Northwest regions of the state of Paraná, Brazil. Polymorphisms of randomly amplified DNA segments (RAPD) and polymorphisms in SSR loci, also denoted as microsatellites, are very useful for successfully identifying the six rootstock varieties for further use in breeding programs. One important hypothesis in the current study is that RAPD and microsatellite markers may differentiate the often confused varieties of rootstock 420A and Kober-5BB. The genetic differentiation of grapevine rootstocks was inferred by an artificial neural network approach based on the Self-Organizing Map algorithm.

Material and methods

The young leaves of 15 healthy plants from rootstock varieties 420-A, Schwarzmann, IAC-766 Campinas, Traviú, and IAC-572 Jales were collected from the experimental area of the *Cooperativa de Rolândia* (Corol), in Rolândia, State of Paraná, Brazil. The rootstock variety Kober 5BB was obtained from the *Centro Nacional de Pesquisa de Uva e Vinho* (Embrapa) in Bento Gonçalves, State Rio Grande do Sul, Brazil. Varieties 420-A, IAC-766 Campinas, IAC-572 Jales and Kober 5BB are more commonly used in the region of Marialva for the Italia, Rubi, Benitaka and Brasil cultivars of *V. vinifera*. Partially expanded leaves, free of contaminants, were collected from the six rootstock varieties. Samples were individually stored in labeled plastic screen bags to avoid the mixture of varieties, maintained on ice (4°C) and transferred to the laboratory. They were then frozen in liquid nitrogen and stored at -80°C until DNA extraction.

DNA was extracted according to the protocol originally described by Thomas & Scott (1993), with minor modifications, which included 100 mg of leaves from individual rootstocks instead of 2.0 g of leaves. Reactions were carried out in 2-mL microcentrifuge tubes. The quality of the isolated DNA was determined by electrophoresis with a 0.8% agar gel using TAE (0.04 M Tris-acetate, 0.001 M EDTA) buffer, according to Hoisington, Khairallah, & González-Léon (1994). UV quantification by visual comparison with known quantities of lambda DNA (Invitrogen) averaged from 20 to 150 ng μL^{-1} per sample. DNA from the six grapevine rootstock varieties (420-A, Schwarzmann, IAC-766 Campinas, Traviú, Kober 5BB, and IAC-572 Jales) was evaluated by 12 and 14 primers for microsatellites and RAPD, respectively.

Microsatellite primer selection and amplification reactions

A set of 12 microsatellite primers developed for *Vitis vinifera*, named VMC (Di Gaspero, Peterlunger, Testolin, Edwards, & Cipriani, 2000), VVMD (Bowers, Dangl, Vignani, & Meredith, 1996; Bowers et al., 1999), SCU (Scott et al., 2000) and UDV (Di Gaspero et al., 2005) (Table 1), were previously selected (VMC4D4, VVMD6, VVMD7, VVMD8, VVMD28, SCU14VV, UDV01, UDV11, UDV32, UDV34, UDV108 and UDV127) and used with

DNA samples from the rootstocks IAC-766 Campinas (11 plants), IAC-572 Jales (11 plants), 420-A (11 plants), Schwarzmann (10 plants), Traviú (10 plants) and Kober 5BB (11 plants). The 12 primers were polymorphic, and they were used for all 64 plants.

Polymerase chain reaction (PCR) was performed with the Techne TC-512 thermal cycler. Amplifications were performed by a touchdown PCR program (Don, Cox, Wainwright, Baker, & Mattick, 1991) with 20 μL volumes containing 30 ng of genomic DNA; reaction buffer 1x (10 mM Tris-HCl, pH 8.8); 2.0 mM $MgCl_2$; 0.8 μM each of dATP, dGTP, dCTP, and dTTP; 0.4 μM of each primer (F and R primers); 1 unit of Taq polymerase (Invitrogen); and Milli-Q water to bring the reaction to the final volume. Electrophoresis was performed in a 4% MS-8 agar gel with 0.5 x TBE buffer (44.5 mM Tris-borate and 1 mM EDTA) at 60 V, for 4 hours. After electrophoresis, gels were stained with ethidium bromide at 0.5 μg mL^{-1} and images were taken by a Molecular Image LOCCUS L-PIX - HE with Picasa 3. The size of the PCR fragments was determined by a 100-bp DNA Ladder (Invitrogen).

RAPD primer selection and amplification reactions

Primers from the OPA, OPB, OPC, OPF, OPI, OPL, OPM and OPP kits (Operon Technologies Inc. Alameda CA USA) were initially screened from one plant of the six rootstock varieties to select the polymorphic primers. The appearance of clear and score-able segment patterns was observed for 17 primers (OPB-1, OPB-3, OPB-4, OPB-5, OPB-7, OPB-8, OPB-10, OPB-11, OPB-15, OPB-17, OPB-18, OPC-2, OPC-4, OPC-7, OPP-8, OPP-11, and OPP-17). The polymorphic primers (OPB-1, OPB-3, OPB-4, OPB-5, OPB-7, OPB-8, OPB-10, OPB-11, OPB-15, OPB-17, OPB-18, OPC-2, OPC-4, and OPC-7) were used with DNA samples (64 plants) from rootstocks IAC-766 Campinas, IAC-572 Jales, 420-A, Schwarzmann, Traviú and Kober 5BB.

The amplification reactions were carried out according to Williams, Kubelik, Livak, Rafalski, and Tingey (1990), with minor modifications, in an aseptic chamber in a 20-μL volume that contained 30 ng of genomic DNA; 10 mM Tris-HCl, pH 8.8; 3.0 mM $MgCl_2$; 50 mM KCl; 0.1% Triton X-100; 0.1 mM each of dATP, dGTP, dCTP, and dTTP; 0.2 μM primer; and 1 unit of Taq DNA polymerase (Invitrogen). Ten-mer primers (Operon Technologies Inc. Alameda CA USA) were used in the amplification reactions, performed in duplicate with a Techne TC-512 Thermocycler using the following PCR program conditions: denaturation for 5 min. at 96°C; 45 cycles of 94°C for 45 s, 35°C for 1 min., and 72°C for 90 s; and a final extension time of 72°C for 7 min.

Table 1. Nucleotide sequences of the microsatellite primers, simple sequence repeated for each primer, number of alleles (NA) detected by each primer in the rootstock varieties 420-A, Schwarzmann, IAC-766 Campinas, Traviú, IAC-572 Jales and Kober 5BB.

Primer	Nucleotide Sequence (5'→ 3')	Simple Sequence Repeated	NA
UDV34	AAGAGACCAAGGATAGATCAAACA(F) AAATGCAACGGGAGATGGTA(R)	$(TG)_n(AG)_n(AT)_n(AGAGG)_n$	4
VMC4D4	GTCTTGTAATGGAACCAACTGC(F) AGATTGACCTGGACCTGAAACT(R)	$(GCT)_9$	3
UDV127	CGTCAATCGCTTGTAATCTGC(F) TGAGCATTTTGCTCCTTCATT(R)	$(CA)_n$	4
UDV32	CATGCGTATGTGTTAGAGAGCA(F) CATGGCATGTGCTTTGTTAT(R)	$(AC)_n(AT)_n$	4
VVMD8	TAACAAACAAGAAGAGGAAT(F) AGCACATCCACAACATAATG(R)	$(TC)_{12.5}(TA)_8$	3
UDV01	TCATTTTCTTGATCGAAGTCCA(F) TGAGCATCAAAACAGGAAGC(R)	$(TA/TG)_n$	4
UDV11	TTTATGGCAACCCTCCAATC(F) TTGATGGTCCACTGGAACTG(R)	$(CT/CA)_n$	4
VVMD6	ATCTCTAACCCTAAAACCAT(F) CTGTGCTAAGACGAAGAAGA(R)	$(CT)_4C(CT)_2TTAG(CT)TAAT(CT)_6C(CT)_2C(CT)_2$	4
SCU14VV	CTGCACTTGAATACGAGCAGGTC(F) TGTTATATGATCCTCCCCCTCCTC(R)	$(GAA)_n$	3
UDV108	TGTAGGGTTCCAAAGTTCAGG(F) CCTTTTTATATGTGGTGGAGCA(R)	$(CT)_n(CA)_n(GT)_n$	4
VVMD28	AACAATTCATGAAAAGAGAGAGAGAGA(F) TCATCAATTTCGTATCTCTATTTGCTG(R)	$(CT)_8$	3
VVMD7	AGAGTTGCGGAGAACAGGAT(F) CGAACCTTCACACGCTTGAT(R)	$(CT)_{14.5}$	5

Amplification products were separated by electrophoresis in a 2% agar TBE (0.045 M Tris-borate/0.001 M EDTA) gel at 60 V for 5 hours. Gels were stained with ethidium bromide (0.5 mg·mL^{-1}) and the images were captured in a High Performance Ultraviolet Transilluminator - Edas 290, using the Kodak 1D 3.5 program. A 1-kb DNA Ladder (Invitrogen) was used as a size marker.

Data analysis

The total number of bands (N), number of polymorphic bands (A), Nei´s gene diversity (H), Shannon's information index (I), gene flow (Nm) and genetic differentiation coefficient between populations (G_{ST}) for RAPD were determined by POPGENE 1.32 (Yeh, Boyle, & Xiyan, 1999). The fixation index (F_{ST}) for each SSR and between varieties was calculated by GenAlEx 6.5 (Peakall & Smouse, 2012).

The genetic differentiation of grapevine rootstock varieties was inferred by an artificial neural network (ANN) approach of the Self-Organizing Map algorithm (SOM; Kohonen, 1998) in MATLAB (2010). The SOM algorithm consisted of two layers of artificial neurons, i. e., the input and output layer neurons (Peña-Malavera, Bruno, Fernandez, & Balzarini, 2014). The input layer is composed of *p* neurons (where p corresponds to the number of molecular markers) connected to all grapevine rootstock varieties. On the other hand, the output layer is composed of *n* nodes (neurons), which correspond to genetically differentiated clusters. The clusters were constructed using competitive learning (Chaudhary, Bhatia, & Ahlawat, 2014) in which the neuron activation is a function of distance (based on Euclidean distance) between neuron weight and input data. The learning process of SOM was carried out with allelic frequencies calculated in GenAlEx 6.5. Additionally, the principal coordinate analysis (PCoA) and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) were used to confirm the SOM results. UPGMA was performed according to default settings in the PHYLIP program (Felsenstein, 1989), and PCoA was implemented in GenAlEx. Nei's genetic distance, calculated among the six varieties of grapevine rootstocks, was employed to create the UPGMA dendrogram. Further, the confidence degree at the nodes was assessed by a Bootstrapping method with 10,000 replicates. The principal coordinate analysis was based on the standardized covariance of genetic distances calculated for co-dominant markers (option Distance, sub-option Genetic), following Mora et al. (2015). The Davies-Bouldin index (DB index; Davies & Bouldin, 1979) was computed using functions from the ClusterSim library of the R project, in order to test the accuracy of the procedures (UPGMA and SOM).

Results and discussion

The 14 RAPD markers yielded 203 reproducible fragments (Table 2), with an average of 14.5 bands per primer. Further, 172 were polymorphic (85%), with an average of 12.3 bands for each primer. On the other hand, 43 alleles were observed for SSR markers, with a variation between 3 and 4 alleles per locus.

Nei´s gene diversity index (H) varied from 1.86 (*Vvmd08*) to 3.67 (*Udv32*) for the SSR markers and from 0.14 (OPB-15) to 0.39 (OPB-03) for the RAPD markers (Table 3). Shannon´s information index (I) showed higher rates for SSR than for RAPD markers, where the lowest ranged from 0.81 (*Vvmd08*) to 0.21 (OPB-15) (SSR and RAPD, respectively), and the highest from 1.34 (*Udv32*) to 0.56 (OPB-03) (SSR and RAPD, respectively) (Table 2).

The genetic differentiation coefficient between populations (G_{ST}) for RAPD markers ranged from 0.54 (OPC-07 to OPB-03) to 0.93 (OPB-15), averaging 0.7 (Table 3). In the case of SSR markers, the fixation index (F_{ST}) varied from 0.09 (*Udv127*) to 0.41 (*Vvmd08*), averaging 0.19 (Table 3). Gene flow (Nm) showed higher rates for SSR than for RAPD markers, with an average of 1.29 and 0.37, respectively. The Nm for SSR ranged from 0.36 (*Vvmd08*) to 2.5 (*Udv127*), while that for RAPD varied from 0.15 (OPB-01) to 0.93 (OPB-15).

PIC ranged from 0.32 (*Vvmd08*) to 0.68 (*Udv32*) for SSR markers, averaging 0.54, while it varied from 0.46 (OPB-18) to 0.75 (OPB-10) for RAPD markers, averaging 0.6 (Table 3). The PIC rates of two SSR (*Udv34* and *Udv32*) and six RAPD markers (OPB-01, OPB-03, OPB-04, OPB-07, OPB-10 and OPB-17) exceeded 0.6, indicating their potential to detect differences among grapevine rootstock varieties (Babu, Agrawal, Mahajan, & Gupta, 2009).

The neural network via the SOM algorithm, based on the SSR and RAPD markers´ allelic frequencies, showed that the 6 varieties of grapevine rootstock were grouped into three genetically differentiated clusters (Figure 1). Cluster 1 included only Kober 5BB rootstock, Cluster 2 included the rootstocks of Traviú and IAC-572, and Cluster 3 included 420-A, Schwarzmann and IAC-766. The SOM results were

consistent with those of the Unweighted Pair-Group Method with Arithmetic Averages (UPGMA) and with those obtained with PCoA (first two PCs) in which the rootstocks were divided into three clusters (Figures 2 and 3, respectively). Since the first PC of PCoA explained 40% of the variation in the samples, Cluster 3 could be separated from Clusters 1 and 2 (Figure 2). The UPGMA clustering method (Figure 3) was strongly supported by bootstrapping (with rates between 84 and 100%), indicating high confidence levels. The clustering results from the neural network procedure agreed with those of the PCoA. This aspect was highlighted by Ferreira, Scapim, Maldonado, and Mora (2018) in an SSR-based genetic analysis. The UPGMA dendrogram showed that the IAC-766 variety was grouped individually and, on the other hand, that the Kober 5BB was grouped with Traviú and IAC-572. However, the DB index value was relatively lower in the SOM clustering (0.346) than the UPGMA method (0.361), indicating a higher precision in the clustering performed with artificial neural networks. These results agree with those indicated by Ferreira et al. (2018) and Peña-Malavera et al. (2014), who report that the UPGMA method may create highly unbalanced clusters, which produces an increase in the error rates of the UPGMA compared with both SOM and PCoA procedures. The clustering analysis using competitive learning-based neural networks (via SOM) is proposed as an alternative method to analyze population structure and has a good adaptation to multi-allelic data (Peña-Malavera et al., 2014; Ferreira et al., 2018), is computationally faster than MCMC methods (Nikolic, Park, Sancristobal, Lek, & Chevalet, 2009) and does not consider the assumption of Hardy-Weinberg equilibrium in the population under study (Ferreira et al., 2018). Moreover, the artificial neural networks has the advantage of being non-parametric, does not require detailed information about the physical processes to be modeled and is tolerant of data loss (Azevedo et al., 2015). Interestingly, our results confirm that the artificial neural networks provide precise results in the identification of genetically differentiated groups.

Table 3 shows F_{ST} and Nei´s genetic distances, calculated from SSR and RAPD markers, respectively. The results from the two methods were similar. The lowest rates were between varieties 420-A, Schwarzmann and IAC-766 and suggested that they belonged to a genetically differentiated group. On the other hand, the highest rates occurred between Kober 5BB and the other varieties. The rootstock Traviú formed a separate group. These results were similar to those from SOM and PCoA analyses (Figures 1 and 2). Varieties 420-A, Schwarzmann and IAC-766-Campinas formed a group, albeit with different origins, whereas 420-A and Kober 5BB varieties, which have the same origin, i. e., they are derived from crosses between *V. berlandieri* x *V. riparia*, showed a high Nei´s genetic distance rate (0.46) (Table 3) and belong to separate groups (Figure 3). Consequently, Nei´s genetic distances, calculated by both molecular markers, revealed that the highest or lowest rates of genetic distance among rootstocks (IAC-766 Campinas, IAC-572 Jales, 420-A, Schwarzmann, Traviú and Kober 5BB) are not related to the parental crosses (Table 1) from which the rootstocks arose. RFLP markers also revealed that 420A was notably distinct from Kober 5 BB (Guerra & Meredith, 1995), despite having the same origin.

Evidence in the current study indicates that, in spite of the morphological similarities of the 420-A and Kober 5BB varieties and the same genetic origin, the selection processes of genotypes led to the generation of two new varieties, genetically divergent and showing different performances. The 420-A variety features a low vigor rate, limited diffusion and difficult rooting (Nachtigal & Camargo, 2005). When a producer uses the 420-A rootstock instead of the cultivar Kober 5BB, the vine’s productivity is much lower than expected. The different performances of 420-A and Kober 5BB varieties may be the result of the differential selection of the ancestral genome during the generation of the two varieties. An analysis of 20 grapevine rootstock accessions from Bulgaria and Cyprus grapevine (*Vitis* spp.) collections, characterized by microsatellites, were also reported in separate clusters (Dzhambazova et al., 2007).

Somatic crossing-over may also explain the genetic divergence at the DNA level between the 420-A and Kober 5BB varieties. The occurrence of somatic crossing-over with changes in the DNA sequence of *V. vinifera* cultivars has been proposed by Oliveira-Collet, Collet, and Machado (2005). The occurrence of somatic recombination may also explain the observations by Crespan (2004), who noted a higher probability in observing genetically different plants in older grape varieties. It is possible that most somatic mutations that occur during plant growth may have no effect on phenotype, although they may be identified at the molecular level. Transposition mutations during vegetative propagation may also influence genome structure and gene function. As the grapevine is a perennial plant that may be strongly stressed during its long productive growth, retrotransposons may contribute toward the grapevine´s genomic plasticity.

Characterizations of transposable elements, which comprise 41.4% of the grapevine genome (Jaillon et al., 2007), have been highlighted in a review by Pelsy (2010), who also described the somatic chimerism, which is quite common in grapevines, inducing distinct DNA profiles, different from those of the parent plant, besides cellular rearrangements in the chimera.

The SSR and RAPD markers in the current study were crucial in revealing genetic differentiation among the six rootstocks (420-A, Schwarzmann, IAC-766 Campinas, Traviú, IAC-572 Jales and Kober 5BB) and principally as methods to identify and differentiate rootstocks Kober 5BB and 420-A. Thus, the sometimes confused Kober 5BB and 420-A varieties may be differentiated at the molecular level. Genetic divergence, revealed by the organization of the three well-defined and highly consistent groups (Figure 3), is a promising method to test the compatibility of the rootstocks with the main fine table grapes (Italy, Rubi, Benitaka, Brasil, and Black Star) cultivated in Marialva. A DNA sequence analysis of retrotransposons IRAP and REMAP has demonstrated that Italia, Rubi, Benitaka, Brasil and Black Star are genetically divergent cultivars (Strioto et al., 2019). Thus, the different compatibilities of different rootstocks with the Italy, Rubi, Benitaka, Brasil and Black Star cultivars are expected. Their productive performances may be tested with the rootstocks showing high or low genetic distances. Importantly, knowledge of the genetic distance between rootstocks is highly useful for performing compatibility tests with different table grape cultivars.

Table 2. Parameters revealing the discriminatory capacity of RAPD and SSR markers and the genetic variability within rootstocks IAC-766 Campinas, IAC-572 Jales, 420-A, Schwarzmann, Traviú and Kober 5BB.

Marker	N	A	PIC	H	I	P_{ST}	Nm
RAPD							
OPB - 01	12	10	0.69	0.31	0.46	0.77	0.15
OPB - 03	15	15	0.61	0.39	0.56	0.54	0.43
OPB - 04	17	17	0.66	0.29	0.46	0.72	0.20
OPB - 05	12	10	0.60	0.25	0.39	0.76	0.16
OPB - 07	16	13	0.61	0.33	0.48	0.64	0.29
OPB - 08	14	13	0.50	0.35	0.52	0.59	0.59
OPB - 10	12	7	0.75	0.23	0.34	0.67	0.24
OPB - 11	18	17	0.62	0.38	0.55	0.90	0.90
OPB - 15	11	4	0.53	0.14	0.21	0.93	0.93
OPB - 17	16	15	0.72	0.34	0.50	0.68	0.24
OPB - 18	12	9	0.46	0.27	0.41	0.67	0.25
OPC - 02	15	13	0.57	0.29	0.43	0.72	0.19
OPC - 04	16	13	0.55	0.28	0.42	0.68	0.24
OPC - 07	17	16	0.54	0.31	0.46	0.54	0.42
SSR							
Udv34	4	4	0.67	3.58	1.32	0.14	1.60
Vmc444	3	3	0.46	2.31	0.91	0.27	0.68
Udv127	4	4	0.58	2.78	1.13	0.09	2.50
Udv32	4	4	0.68	3.67	1.34	0.16	1.33
Vvmd08	3	3	0.32	1.86	0.81	0.41	0.36
Udv001	4	4	0.56	2.66	1.11	0.22	0.89
Udv011	4	3	0.57	2.61	1.11	0.13	1.67
Vvmd06	4	4	0.59	2.72	1.13	0.24	0.77
Scu14vv	3	3	0.46	2.30	0.93	0.14	1.58
Udv108	4	4	0.56	2.81	1.16	0.20	1.03
Vvm428	3	3	0.52	2.64	1.03	0.13	1.70
Vvm407	3	3	0.54	2.77	1.05	0.16	1.32

N: total number of bands; A: number of polymorphic bands; H: Nei's gene diversity; I: Shannon's information index; P_{ST}: Genetic differentiation coefficient between populations and Fixation index for RAPD and SSR, respectively; Nm: Gene flow.

Table 3. Genetic differentiation among varieties of grapevine rootstocks IAC-766 Campinas, IAC-572 Jales, 420-A, Schwarzmann, Traviú and Kober 5BB, based on F_{ST} rates (above the diagonal) and Nei´s Genetic Distances (below the diagonal).

	420-A	Schwarzmann	Traviú	IAC-766	IAC-572	Kober 5BB
420-A	-	0.22	0.34	0.27	0.42	0.49
Schwarzmann	0.12	-	0.41	0.28	0.53	0.56
Traviú	0.27	0.28	-	0.34	0.49	0.45
IAC-766	0.19	0.15	0.23	-	0.41	0.46
IAC-572	0.32	0.37	0.37	0.28	-	0.57
Kober 5BB	0.46	0.43	0.34	0.37	0.43	-

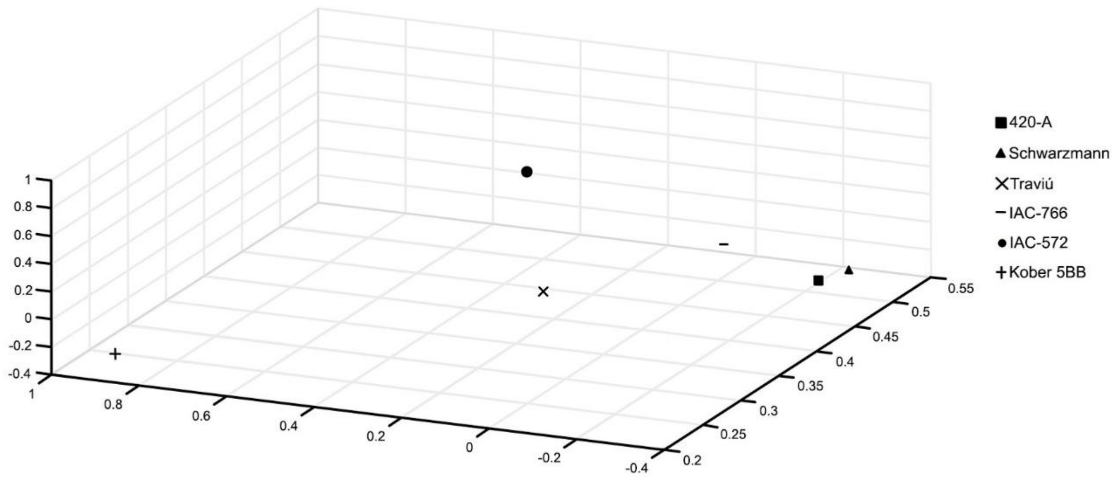

Figure 1. Results from the clustering procedure, based on an artificial neural network analysis with RAPD and SSR markers assessed in 6 varieties of grapevine rootstocks, which evidenced three genetically differentiated groups.

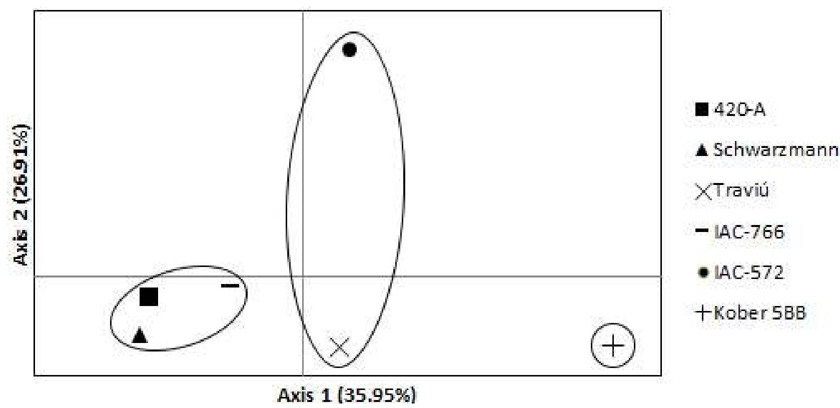

Figure 2. Principal coordinate analysis of genetic distances with RAPD and SSR primers in 6 varieties of grapevine rootstocks. Circles denote the genetically differentiated clusters, according to the artificial neural network model.

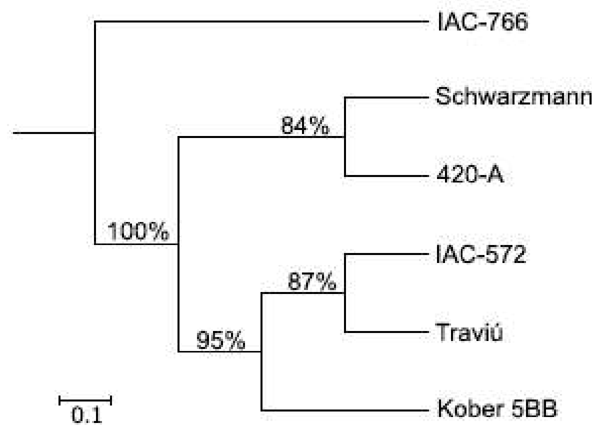

Figure 3. UPGMA dendrogram based on Nei's genetic distances, built with SSR and RAPD markers, assessed for the 6 varieties of grapevine rootstocks. Values at the nodes indicate the proportions of bootstrap runs (10,000) in a particular node.

Conclusion

The genetic divergence among six rootstocks, namely, 420-A, Schwarzmann, IAC-766 Campinas, Traviú, IAC-572 Jales and Kober 5BB, is not dependent on the parental crosses that originated each rootstock variety. The lowest genetic distance rate has been registered between rootstocks Schwarzmann (*V. riparia* × *V. rupestris*) and 420-A (*V. berlandieri* × *V. riparia*) and between rootstocks Schwarzmann and IAC-766 (106-8 Mgt × *V. caribaea).* The morphologically similar rootstocks 420-A and Kober 5BB are genetically divergent

despite the same genetic origin (*V. berlandieri* × *V. riparia*). SSR and RAPD markers revealed that the sometimes confused Kober 5BB and 420-A varieties may be differentiated at the molecular level.

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