Obtaining interspecific hybrids, and molecular analysis by microsatellite markers in grapevine

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Abstract – The objective of this work was to assess the potential of interspecific hybridization of *Vitis labruscana* and *Muscadinia rotundifolia* by using artificial cross-pollinations. Microsatellite markers were used to confirm interspecific hybridizations and the identity of the parental genotypes. In crosses in which *M. rotundifolia* was used as the female parent, no true hybrids were obtained. In the reciprocal crosses, 114 seedlings were identified as true *V. labruscana* x *M. rotundifolia* hybrids. Self-pollination occurred in direct and in reciprocal crosses. The crossings between 'Bordo' x 'Carlos', 'Magnolia', 'Regale' and 'Roanoke', and between 'Isabel' x 'Bountiful', 'Carlos', 'Magnolia', 'Regale' and 'Roanoke' were confirmed. The 15 markers evaluated showed that two *M. rotundifolia* parental genotypes had the same fingerprint profile, indicating a likely planting error. The success of hybridization depends mainly on the species and on the cultivar used as the female parent. Microsatellite markers are efficient to confirm the paternity of interspecific F1 hybrids and to determine the correct identity of *M. rotundifolia* cultivars.

Index terms: *Muscadinia rotundifolia*, *Vitis labruscana*, hybrid identification, interspecific crosses, SSRs.

Obtenção de híbridos interespecíficos e análise molecular por marcadores microssatélites em videira

Resumo – O objetivo deste trabalho foi avaliar o potencial da hibridação interespecífica entre *Vitis labruscana* e *Muscadinia rotundifolia* por meio de polinizações cruzadas artificiais. Marcadores microssatélites foram utilizados para confirmar as hibridizações interespecíficas e a identidade dos parentais. Nos cruzamentos em que *M. rotundifolia* foi utilizada como parental feminino, nenhum híbrido verdadeiro foi obtido. Nos cruzamentos recíprocos, 114 plântulas foram identificadas como verdadeiros híbridos de *V. labruscana* x *M. rotundifolia*. Ocorreu autopolinização nos cruzamentos diretos e nos recíprocos. Foram confirmados os cruzamentos 'Bordo' x 'Carlos', 'Magnolia', 'Regale' e 'Roanoke', e 'Isabel' x 'Bountiful', 'Carlos', 'Magnolia', 'Regale' e 'Roanoke'. Os 15 marcadores avaliados revelaram que dois parentais de *M. rotundifolia* apresentaram o mesmo perfil genético, o que indica provável erro de plantio. O sucesso da hibridização depende principalmente da espécie e da cultivar utilizada como parental feminino. Os marcadores microssatélites são eficientes para confirmar a paternidade de híbridos interespecíficos F1 e para determinar a correta identidade de cultivares de *M. rotundifolia*.

Termos para indexação: *Muscadinia rotundifolia*, *Vitis labruscana*, identificação híbrida, cruzamentos interespecíficos, SSRs.

Introduction

The use of rootstocks in viticulture is a widespread practice. Since the invasion by phylloxera [*Daktulosphaira vitifoliae* (Fitch, 1856)], grape growers began grafting susceptible *Vitis vinifera L.* fruiting cultivars onto rootstocks bred from resistant North American *Vitis* species, particularly *V. rupestris* Scheele, *V. riparia* Michx., *V. berlandieri* Planch. and *V. labruscana* L.H. Bailey. However, in addition to phylloxera resistance, a good rootstock must show adaptability to the local climate and soil conditions, besides easy rooting, affinity with the scion, good vegetative growth, longevity and resistance or tolerance to pests and pathogens (Reynolds & Wardle, 2001).

Rootstock usage in the wine regions of Southern Brazil is dominated by different North American hybrid cultivars. Some of these cultivars are considered traditional, such as Solferino, SO4, Kober 5BB and 101-14 Mgt (*V. berlandieri* x *V. riparia*), whereas others were more recently introduced, such as Paulsen 1103 and R99 (*V. berlandieri* x *V. rupestris*). Although these rootstocks have been recommended due to their resistance to *Fusarium oxysporum* Schlecht., they are the most susceptible to the main soil pest of this region,

the Brazilian ground pearl [*Eurhizococcus brasiliensis* Hempel (Hemiptera: Margarodidae)] (Botton & Colleta, 2010; Botton et al., 2010).

The muscadine genotypes [*V. rotundifolia* Michx. Syn. *M. rotundifolia* (Michx.) Small] are resistant to the Brazilian ground pearl (Botton & Colleta, 2010). This species has been classified as having the highest level of resistance to grape pests and disease (Kellow et al., 2002) and has consequently been used in many breeding programs worldwide to create resistant rootstocks. Although *M. rotundifolia* (2n = 40) is not used as a rootstock, due to its graft incompatibility with commercial cultivars of *Vitis* species (2n = 38) and to its inability to form roots from dormant cuttings (Goldy & Onokpise, 2001), interspecific hybrids showing compatibility were created with *Vitis* species (Olmo, 1986). Some hybrids from these crosses, including VR039-16 and VR043-43 (*V. vinifera* x *M. rotundifolia*), released by the grape breeding program at the University of California, Davis, CA, USA (Walker et al., 1991), are currently the main rootstocks used for management of the Brazilian ground pearl (Dalbó et al., 2007). However, 'VR043-43' is no longer recommended for use in phylloxera-infested sites, since the resistance of its rootstock to Brazilian ground pearl was recently placed in doubt (De Césaro, 2008). Its full sibling 'VRO39-16' is only recommended for use in grapevine fanleaf virus-infected sites, as its long-term resistance to phylloxera is also questionable (Smith, 2010). Therefore, given that phylloxera resistance should be in the background of all new rootstocks and that the use of *V. vinifera* in rootstock development has almost invariably been disappointing, it is important to develop rootstocks with broader Brazilian ground pearl resistance and durable resistance to phylloxera, which can be obtained through breeding.

The hybridization of North American species with *M. rotundifolia* is an alternative to hybridization with *V. vinifera*. Among the many North American species, *V. labruscana* hybrids are well adapted to the wine regions of Southern Brazil. These hybrids are commonly planted with their own roots and show some resistance to soil pathogens, such as *F. oxysporum* (Garrido et al., 2004), besides adequate phylloxera resistance. However, these hybrids do not exhibit the high level of resistance to *E. brasiliensis* that *M. rotundifolia* cultivars do.

In breeding, molecular markers have been used as a tool to reduce the time required to develop a new cultivar. Among the various uses of molecular markers, the identification of true hybrids is an extremely useful tool in breeding programs. Therefore, the use of molecular markers to identify plants from crosses is highly important, so that plants from self or undesirable crosses can be identified and discarded in the F1 generation (Cordeiro et al., 2006).

The objective of this work was to assess the potential of interspecific hybridization of *V. labruscana* and *M. rotundifolia* by using artificial cross-pollinations.

Materials and Methods

All crosses were done at the Estação Experimental do Canguiri, of Universidade Federal do Paraná, Pinhais, PR, Brazil (25°25'S, 49°08"W, at 930-m altitude), and at the Estação Experimental de Videira, Videira, SC, Brazil (27°0'5"S, 51°7'60"W, at 750-m altitude) and at the Estação Experimental de Campos Novos, Campos Novos, Santa Catarina, Brazil (27°23'60"S, 51°12'0"W, at 947-m altitude), of Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (Epagri). The controlled crosses were made in both directions between *M. rotundifolia* cultivars: Bountiful, Carlos, Magnolia, Magoon, Regale and Roanoke, and *V. labruscana* cultivars: Bordo, Goethe, Isabel, Marta and Niagara Rosada, in 2008 and 2009. Sixty crosses were made. The number of controlled artificial pollinations varied among the crosses depending on the availability of flowers (Tables 1 and 2). Simple sequence repeat (SSR) markers were used to confirm the paternity of the putative hybrids and to authenticate the identity of the parental genotypes.

The controlled crosses were performed according to Leão & Borges (2009). The female parents were emasculated before anthesis and bagged with paper bags to avoid contamination from unwanted pollen. The pollen was collected from flower clusters before anthesis in the morning, eliminating open flowers. The anthers were separated from the calyptra with a sieve, placed in petri dishes and dried at room temperature (20 to 25°C) for three days. The pollen was placed in small bottles, labeled and stored in a desiccator with silica gel at 4°C. Pollination was done with a brush for three consecutive days when the stigmas of the female

parents were receptive, and the inflorescences were covered with paper bags.

Due to uneven maturation, the berries were harvested individually from January to April in both years. The seeds were extracted by forcing the fruits through a sieve, washed with water to remove the pulp, treated with a fungicide solution of Cercobin 2 g L^{-1} and then placed in moist media of sterilized vermiculite in petri dishes and stratified at 4°C for a period of 75 days to break dormancy. Seeds were planted in seedling trays containing a steam-sterilized commercial substrate of Plantmax. When the first true leaves of seedlings appeared, they were transferred to individual pots (125 cm^3), containing a mixture of 2 Plantmax:1 soil :1 humus:1 vermiculite, and kept in the greenhouse.

Lyophilized leaf tissue of the parental genotypes and putative F1 hybrids was homogenized with DNA extraction buffer in plastic bags using a Homes 6 mechanical homogenizer (Bioreba, Longmont, CO, USA). DNA was extracted with a modified CTAB (hexadecyltrimethylammonium bromide) procedure (Riaz et al., 2004). In the final step, DNA pellets were suspended in 100 µL 1X Tris-EDTA buffer and stored at -20°C. The integrity of the DNA was visualized on 1.2% agarose gel.

To analyze the extent of polymorphism of SSR markers in the parental genotypes, a set of 20 SSRs were tested on the *M. rotundifolia* and *V. labruscana* cultivars. A quality score, A, B, C or D, was given to each marker: Score A was given to four primer pairs (UDV41, UDV43, VrZAG62 and VVIN75) of good quality, displaying clear single band patterns easily scorable in both parents; score B to four medium quality markers (VVIM11, VVIC72, VVMD5 and VVMD31), displaying smears and fainter bands occasionally difficult to read; score C to three markers (UDV76, UDV111 and VVIP16), displaying multiband profiles; and score D to ten markers (UDV35, UDV111, VVIV05, VVIV21, UDV32, UDV35, UDV67, UDV124, VVIB68 and NVMCNG), producing smears, non-reproducible bands or no products. The four markers with score A were run on the entire populations to confirm the hybrid origin of the progenies. To verify the identity of the parental genotypes used in the crosses, the molecular profile of five *M. rotundifolia* cultivars was compared to the Grape DNA Identification Reference Database (Foundation Plant Services, University of California, Davis, CA, USA) with 15 SSR markers. However, 'Roanoke' was not analyzed due to its absence in the reference database. DNA fingerprinting of the *V. labruscana* cultivars was done in a previous study by Schuck et al. (2009). The SSR markers used in the present study were VVS2, VVMD5, VVMD7, VVMD27, VVMD31, VVMD32, VrZag62, VrZag79, VMC4f3.1, VMC8g9, VMC7f2, VMC3df, VMC5a1, VMC5h2 and UDV108. PCR conditions used were described by Riaz et al. (2004). PCR reactions were carried out in 10 µL reaction mixtures containing 5 pmol of each primer, 2.5 mmol L^{-1} of each dNTP, 1 µL 10X gold PCR buffer (Perkin Elmer Inc., Wellesley, MA, USA), 0.5 unit AmpliTaq Gold DNA polymerase (Perkin Elmer Inc., Wellesley, MA, USA), 2 mmol L^{-1} of $MgCl_2$ solution and 10 ng of genomic DNA. Temperature cycling for PCR was carried out on either a Peltier Thermal Cycler-200 (MJ Research, Inc., Waltham, MA, USA) or on a Bio-Rad iCycler (Bio-Rad Laboratories, Hercules, CA, USA). The following cycling program was used: denaturation of DNA and activation of Taq DNA polymerase at 95°C for 10 min; 35 cycles of amplification distributed in 45 s at 94°C, 45 s at 56°C and 1 min at 72°C; final extension of 10 min at 72°C; and cooling at 4°C. To separate amplification products, PCR reactions were mixed with denaturing dye (98% formamide, 10 mmol L^{-1} EDTA, 0.05% bromophenol blue and xylene cyanol) and heated at 94°C for 2 min before loading on a 5% polyacrylamide sequencing gel. Gels were run at constant 70 W for 2–3 hours depending on allele sizes. Samples were visualized by silver staining with a commercial kit (Promega, Madison, WI, USA). Assuming Mendelian inheritance of the SSR loci, each of the analyzed progeny genotypes was considered to be a hybrid when one of the two SSR alleles amplified with each locus was common to the alleles in the maternal genotype and the other was the same as one of the SSR alleles found in the paternal genotype. All gels were scanned and stored in a digital archive.

Results and Discussion

In 2008, 30 cross combinations were made between six *M. rotundifolia* cultivars; Bountiful, Carlos, Magnolia, Magoon, Regale and Roanoke, and five *V. labruscana* cultivars: Bordo, Goethe, Isabel, Marta and Niagara Rosada. A total of 14,775 flowers were pollinated with *V. labruscana* pollen and 217 berries

(1.5% fruit set) were obtained. From these berries, 427 seeds were extracted and 236 germinated (Table 1). The number of berries obtained compared to the total flowers pollinated was extremely low, indicating that the crosses were very inefficient. Therefore, crosses using *M. rotundifolia* as the seed parent were not repeated in 2009. In 2008 and 2009, the reciprocals *V. labruscana* x *M. rotundifolia* were also made, and 9,398 *V. labruscana* flowers were pollinated producing 416 berries (4.4% fruit set), 1,040 seeds and 342 seedlings (Table 2).

DNA fingerprinting with the SSR loci UDV41, UDV43, VrZAG62 and VVIN75 indicated that, whenever *M. rotundifolia* was used as the female and *V. labruscana* as the male parent, the crosses were a complete failure, producing no true hybrids but only direct descendants of *M. rotundifolia*. From 342, in the reciprocal crosses, 114 seedlings, originated from nine

Table 1. Number of pollinated flowers, fruit set and number of surviving seedlings from the crosses between six *Muscadinia rotundifolia* cultivars, as the female parent, and five *Vitis labruscana* cultivars, as the male parent, at the Estação Experimental do Canguiri, Pinhais, PR, Brazil, and at the Estação Experimental de Videira, SC, Brazil, in 2008.

Vitis labruscana cultivar as male parent	Nº of emasculations	Nº of berries	Fruit set (%)	Nº of seeds	Nº of seedlings
	M. rotundifolia 'Bountiful' as female parent				
Bordo	720	16	2.2	39	32
Goethe	560	1	0.2	1	1
Isabel	560	22	3.9	47	38
Marta	720	12	1.7	29	25
Niagara Rosada	560	7	1.3	13	10
	M. rotundifolia 'Carlos' as female parent				
Bordo	50	0	0.0	0	0
Goethe	300	6	2.0	17	14
Isabel	150	0	0.0	0	0
Marta	100	4	4.0	8	6
Niagara Rosada	100	3	3.0	10	5
	M. rotundifolia 'Magnolia' as female parent				
Bordo	1,200	13	1.1	12	1
Goethe	1,100	7	0.6	11	9
Isabel	1,300	10	0.8	13	2
Marta	1,000	6	0.6	19	9
Niagara Rosada	1,300	12	0.9	8	3
	M. rotundifolia 'Regale'[(1)] as female parent				
Bordo	780	33	4.2	74	31
Goethe	645	6	0.9	12	4
Isabel	720	19	2.6	37	13
Marta	630	15	2.4	31	12
Niagara Rosada	480	10	2.1	14	5
	M. rotundifolia 'Roanoke' as female parent				
Bordo	360	4	1.1	8	3
Goethe	360	2	0.6	3	1
Isabel	360	1	0.3	2	2
Marta	360	6	1.7	14	4
Niagara Rosada	360	2	0.6	5	6
Total	14,775	217	1.5	427	236

[(1)]The results obtained in the crosses between *Vitis labruscana* cultivars and 'Magoon' were added to those of 'Regale', since the genetic profile of 'Magoon' matched with 'Regale' at all 15 markers, indicating that the genotype was misnamed at the time of introduction and that the same genotype was planted with different names.

Table 2. Number of pollinated flowers, fruit set and number of surviving seedlings from the crosses between five *Vitis labruscana* cultivars, as the female parent, and six *Muscadinia rotundifolia* cultivars, as the male parent, at the Estação Experimental de Campos Novos and Videira, SC, Brazil, in 2008 and 2009.

Muscadinia rotundifolia cultivar as male parent	Nº of emasculations	Nº of berries	Fruit set (%)	Nº of seeds	Nº of seedlings
	V. labruscana 'Bordo' as female parent				
Bountiful	65	0	0.0	0	0
Carlos	325	21	6.5	48	7
Magnolia	520	52	10.0	120	54
Regale[(1)]	625	55	8.8	114	52
Roanoke	715	10	1.4	35	30
	V. labruscana 'Goethe' as female parent				
Bountiful	94	0	0.0	0	0
Carlos	105	0	0.0	0	0
Magnolia	119	0	0.0	0	0
Regale[(1)]	143	0	0.0	0	0
Roanoke	86	0	0.0	0	0
	V. labruscana 'Isabel' as female parent				
Bountiful	250	4	1.6	5	1
Carlos	300	33	11.0	71	16
Magnolia	550	70	12.7	135	31
Regale[(1)]	1,501	41	2.7	87	21
Roanoke	450	7	1.6	16	9
	V. labruscana 'Marta' as female parent				
Bountiful	150	0	0.0	0	0
Carlos	150	0	0.0	0	0
Magnolia	100	0	0.0	0	0
Regale[(1)]	150	1	0.6	2	1
Roanoke	100	2	2.0	4	0
	V. labruscana 'Niagara Rosada' as female parent				
Bountiful	150	0	0.0	19	0
Carlos	525	25	4.8	145	62
Magnolia	450	52	11.6	110	36
Regale[(1)]	675	45	6.6	129	22
Roanoke	100	0	0.0	0	0
Total	9,398	416	4.4	1,040	342

[(1)]The results obtained in the crosses between *Vitis labruscana* cultivars and 'Magoon' were added to those of 'Regale', since the genetic profile of 'Magoon' matched with 'Regale' at all 15 markers, indicating that the genotype was misnamed at the time of introduction and that the same genotype was planted with different names.

different crosses, were identified at all four markers as true *V. labruscana* x *M. rotundifolia* hybrids (Table 3).

In the crosses in which 'Isabel' was the female parent, 65 true hybrids were identified (Figure 1). All the *M. rotundifolia* cultivars used to pollinate 'Isabel' generated true hybrids, except 'Magoon'. The largest population of *V. labruscana* x *M. rotundifolia* consists of 27 seedlings (87.1% true hybrids) and is a cross of 'Isabel' x 'Magnolia'. From the cross 'Isabel' x 'Regale', 20 seedlings were evaluated and 17 F1 hybrids were confirmed. The cross 'Isabel' x 'Carlos' yielded 16 seedlings, of which 11 were true hybrids (68.8%). The highest percentage of hybrid formation was observed in the crosses 'Isabel' x 'Roanoke' and 'Isabel' x 'Bountiful', with 100% true hybrids. From these crosses, only nine and one seedlings were analyzed, respectively (Table 3).

Table 3. Percentage of interspecific *Vitis* hybrids from the crosses between two *Vitis labruscana* cultivars, as female parent, and five *Muscadinia rotundifolia* cultivars, as male parent, confirmed by SSR markers (UDV41, UDV43, VrZAG62 and VVIN75).

Muscadinia rotundifolia cultivar as male parent	Nº of plants evaluated	Nº of true hybrids	True hybrids (%)
	V. labruscana 'Bordo' as female parent		
Carlos	7	3	42.9
Magnolia	54	16	29.6
Regale	22	15	68.9
Roanoke	30	15	50.0
Total	113	49	43.4
	V. labruscana 'Isabel' as female parent		
Bountiful	1	1	100
Carlos	16	11	68.8
Magnolia	31	27	87.1
Regale	20	17	85.0
Roanoke	9	9	100.0
Total	77	65	84.4
Total	190	114	60

The crosses between the female parent 'Bordo' with *M. rotundifolia* cultivars produced 49 true hybrids (43.4%) (Figure 2). Of the six cultivars used as the male parent with 'Bordo', two ('Bountiful' and 'Magoon') did not produce progenies with the SSR profile expected for F1 interspecific hybrids. The percentage of hybrid formation was highest in the crosses 'Bordo' x 'Regale' and 'Bordo' x 'Roanoke', with 68.9 and 50% true hybrids, respectively. The lowest percentage (29.6%) of true hybrids originated from the cross 'Bordo' x 'Magnolia'. Out of the 54 individuals analyzed, 16 were confirmed as true hybrids. From the cross 'Bordo'

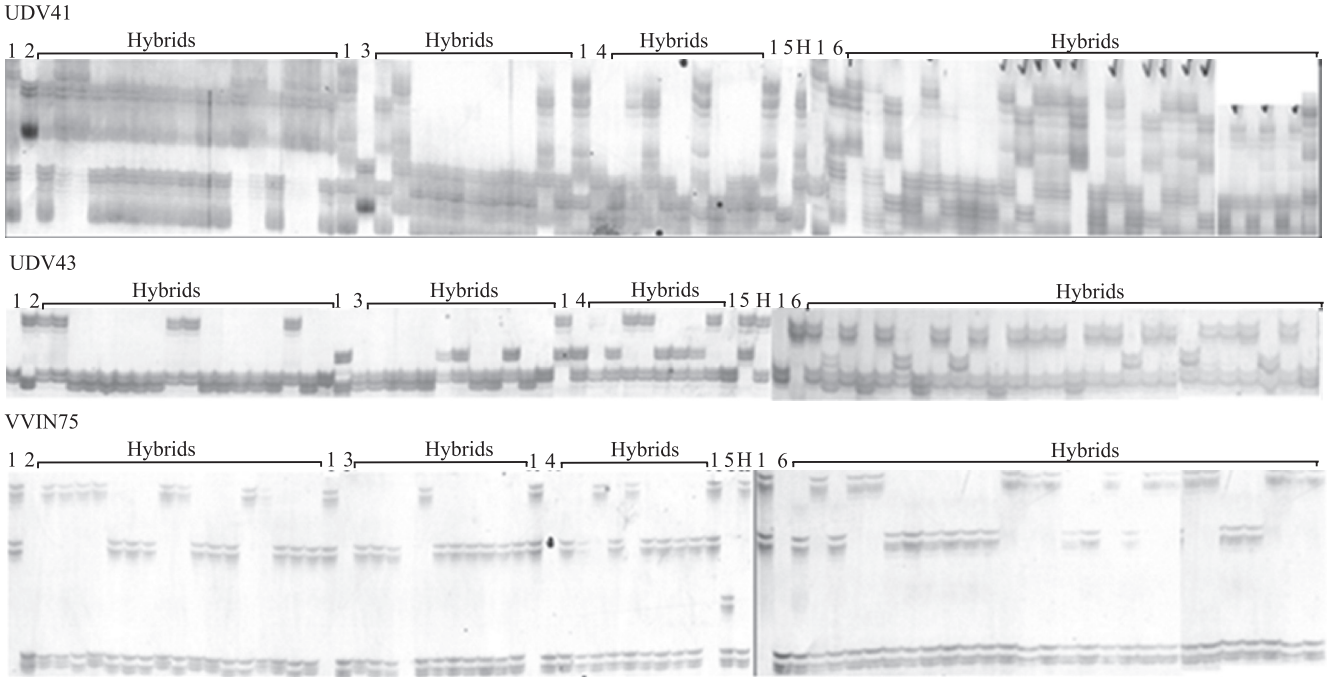

Figure 1. PCR amplification profile of the SSR markers UDV41 and UDV43 (Di Gaspero et al., 2005) and VVIN75 (Merdinoglu et al., 2005) in 'Isabel' (1), 'Regale' (2), 'Carlos' (3), 'Roanoke' (4), 'Bountiful' (5) and 'Magnolia' (6) followed by the true hybrids derived from each cross. H, hybrid.

x 'Carlos', seven F1 plants were evaluated and three hybrids were confirmed (42.9%) (Table 3).

Though only five *V. labruscana* genotypes were used as female parents in the present study, a marked difference was observed between them in their crossability with *M. rotundifolia*. These results clearly indicate that the success of the interspecific crosses depends not only on the species and on the direction of the cross, but also on the genotypes of the species involved in the hybridization. Other authors showed that the *V. vinifera* cultivars used as the female genotype influenced the crossability with *M. rotundifolia* (Bouquet, 1980).

The chromosome difference between *Vitis* spp. (2n = 38) and *M. rotundifolia* (2n = 40) is considered the main reason for the low success of the crosses in both directions (Goldy & Onokpise, 2001). However, in crosses involving *M. rotundifolia* as the maternal parent, the difficulties in obtaining hybrids are higher than in the reciprocal (Bouquet, 1980; Olmo, 1986). The failure in obtaining hybrid seedlings when *M. rotundifolia* serves as the female parent can be attributed to the inability of *Vitis* spp. pollen tubes to successfully penetrate *M. rotundifolia* styles and to fertilize the egg cells (Lu & Lamikanra, 1996).

In addition to the genetic incompatibility between the species, phenological factors also limit the success of reciprocal crosses. In Southern Brazil, *V. labruscana* blooms from September to October, and *M. rotundifolia* from November to January. Because of this difference, in the present study, *V. labruscana* cultivars were double pruned, during their regular blooming period, to force them to bloom again so that fresh pollen from *M. rotundifolia* could be used in the flowers of *V. labruscana*.

Although fresh pollen was used to make these 60 crosses, crosses in both directions produced a high proportion of non-hybrids, all of which were the result of self-fertilization. Even though paper bags were placed over the pollinated flower clusters and the adjacent clusters on the same plant were eliminated to avoid contamination from unwanted pollen, wind could have deposited pollen on the emasculated clusters during the few seconds that the protective bags were removed to perform pollination. Therefore, bagging of flowers does not guarantee the lack of undesired pollinations (Neal & Anderson, 2004). Moreover, shoot position and bunch location on the shoot in the developing canopy also influence flowering (Vasconselos et al.,

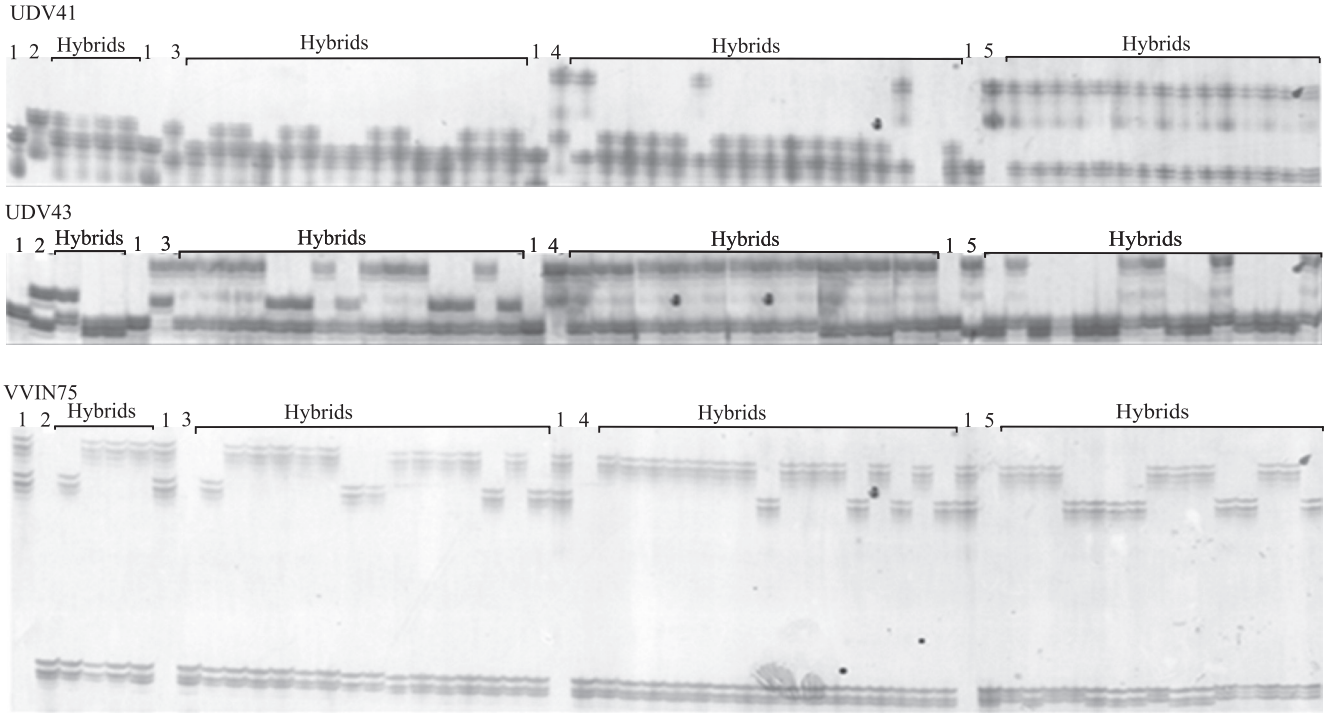

Figure 2. PCR amplification profile of the SSR markers UDV41 and UDV43 (Di Gaspero et al., 2005) and VVIN75 (Merdinoglu et al., 2005) in 'Bordo' (1), 'Carlos' (2), 'Roanoke' (3), 'Magnolia' (4) and 'Regale' (5) followed by the true hybrids derived from each cross. H, hybrid.

2009). In the present study, the inflorescences used in the crosses were from the mid cane of the shoots.

The average high temperatures reported for both years (2008/2009) in the three regions where the crosses were made (Pinhais, PR – 31°C; Campos Novos, SC – 26.5°C; and Videira, SC – 31°C) may have induced early pollen maturation of the flower clusters near the end of the canes of the same plant and in the neighboring inflorescences of the same vine located a few meters away. This could have accelerated pollen drying and increased the chances of pollen being carried by the wind and being deposited on unprotected flowers of *M. rotundifolia* and *V. labruscana* before they bloomed. In addition, the role of insects as pollination agents cannot be rejected.

The use of microsatellite markers for the identification of true hybrids in the early stage of the selection process has shown to be a very useful tool. A high proportion of selfed plants were identified, which were discarded in the F1 generation, allowing for significant savings in resources, particularly time and space. In species with long breeding cycles, such as *Vitis* spp., the conventional process of hybrid identification by morphological differences is slow. Molecular tools may overcome these difficulties and open the way to new strategies for more efficient breeding.

The identification of the *V. labruscana* genotypes was part of a previous study, and the SSR profiles of the cultivars Bordo, Goethe, Isabel, Niagara Rosada and Marta were the same as those available in the data banks for the same cultivars using SSR markers VVS2, VVMD5, VVMD7, VVMD27, VrZag62 and VrZag79. Therefore, these cultivars were correctly identified (Table 4) (Schuck et al, 2009).

To confirm the identity of the *M. rotundifolia* parental genotypes, five cultivars were genotyped at 15 SSR loci. Consistent and reliable profiles were obtained for the five cultivars at all SSR markers. The genetic profile of the *M. rotundifolia* cultivars Bountiful, Carlos, Magnolia and Regale were identical to those of the reference database at all 15 SSR loci. However, 'Magoon' did not match the reference profile at the same SSR loci, but matched with 'Regale', indicating that the genotype was misnamed at the time of introduction and that the same genotype was planted under different names (Table 4).

The correct identification of accessions is a basic requirement for the coherent management of germplasm repositories and for the use of the germplasm in ongoing breeding programs. It is essential to identify the existence of synonyms, homonyms and naming errors to avoid future propagation and breeding errors. These mistakes are difficult to detect based on morphological descriptors, since they are highly influenced by environmental and developmental factors (Sefc et al., 2001). In contrast, SSRs markers have been used to solve problematic naming and for genetic diversity assessment (Leão & Motoike,

Table 4. Allele sizes (bp) of *Muscadinia rotundifolia* and *Vitis labruscana* cultivars used as parents.

Locus	*M. rotundifolia*					*V. labruscana*[1]					Reference
	'Bountiful'	'Carlos'	'Magnolia'	'Magoon'	'Regale'	'Bordo'	'Goethe'	'Isabel'	'Marta'	'Niagara Rosada'	
VVS2	153–153	147–149	147–147	147–147	147–147	118–130	120–130	118–146	120–146	118–128	Thomas & Scott (1993)
VVMD5	230–246	230–246	230–230	226–230	226–230	234–234	230–236	236–236	234–234	234–234	Bowers et al. (1996)
VVMD7	235–235	243–245	243–245	243–243	243–243	235–239	235–247	235–249	235–249	235–241	Bowers et al. (1996)
VVMD27	199–199	197–215	185–215	185–197	185–197	179–181	181–183	175–179	179–181	175–181	Bowers et al. (1999)
VVMD31	246–250	168–170	170–210	170–210	170–210	201–213	203–209	201–213	201–203	201–201	Bowers et al. (1999)
VVMD32	249–301	249–301	249–301	249–301	249–301	248–248	246–252	248–272	248–248	248–272	Bowers et al. (1999)
VrZag62	215–223	199–215	199–215	199–199	199–199	200–202	190–204	200–202	200–202	200–202	Sefc et al. (1999)
VrZag79	255–255	257–287	259–287	255–259	255–259	247–247	239–247	237–247	247–264	237–259	Sefc et al. (1999)
VMC4f3.1	222–222	202–222	208–222	222–226	222–226	-[2]	-	-	-	-	Di Gaspero et al. (2000)
VMC8g9	138–140	136–140	138–140	135–138	135–138	-	-	-	-	-	Adam-Blondon et al. (2004)
VMC7f2	193–195	193–193	193–193	193–195	193–195	-	-	-	-	-	Adam-Blondon et al. (2004)
VMC3df	174–178	172–178	172–178	170–172	170–172	-	-	-	-	-	Adam-Blondon et al. (2004)
VMC5a1	163–170	163–170	170–170	170–170	170–170	-	-	-	-	-	Adam-Blondon et al. (2004)
VMC5h2	198–200	194–212	194–212	194–212	194–212	-	-	-	-	-	Adam-Blondon et al. (2004)
UDV108	208–208	208–208	202–202	202–220	202–220	-	-	-	-	-	Di Gaspero et al. (2005)

[1]Cultivars analyzed in previous study by Schuck et al. (2009). [2]SSRs not analyzed.

2011), for parentage analysis (Tapia et al., 2007) and to develop genetic maps (Riaz et al., 2004).

Conclusions

1. The hybridization success between *V. labruscana* and *M. rotundifolia* depends on the species and on the cultivar used as the female parent.

2. Cultivars of *V. labruscana* can be successfully crossed with pollen from *M. rotundifolia* cultivars, but all reciprocal combinations fail to produce true hybrids.

3. Crosses between *V. labruscana* cultivar Isabel x *M. rotundifolia* cultivars Bountiful, Carlos, Magnolia, Regale and Roanoke are possible, as well as crosses between *V. labruscana* cultivar Bordo x *M. rotundifolia* cultivars Carlos, Magnolia, Regale and Roanoke, indicating that these species can be used in breeding programs.

4. SSR markers are efficient to confirm the paternity of interspecific F1 hybrids and to determine the correct identity of *M. rotundifolia* cultivars.

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