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Identification of Grape (*Vitis*) Rootstocks Using Sequence Characterized Amplified Region DNA Markers

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Abstract. **Five sequence characterized amplified region (SCAR) DNA markers were reevaluated at substantially higher annealing temperatures than originally reported; four were polymorphic among nine rootstocks tested. Four new informative SCAR markers also are reported, based on redesigning primers from previously cloned random amplified polymorphic DNA (RAPD) markers. Based on the eight polymorphic markers, rootstocks MG 420A, MG101-14, Richter 99, Couderc 3309, and Kober 5BB were distinguishable. Riparia Gloire and Couderc 1616 could be distinguished from the others, but not from one another, and SO4 and 5C also could be distinguished from the others, but not from one another.**

Traditional methods of identifying grape cultivars have relied on morphological characters whose expression is affected by developmental and environmental factors. Limited polymorphism has hampered the use of biochemical markers such as isozymes (Parfitt and Arulsekar, 1989; Subden et al., 1987). The need for practical and objective means for identifying grape cultivars has encouraged researchers to investigate DNA-based techniques (Botta et al., 1995; Bourquin et al., 1993, 1995; Bowers et al., 1993; Guerra and Meredith, 1995; Striem et al., 1990; Thomas et al., 1994). We recently generated six polymer chain reaction (PCR) markers from cloned random amplified polymorphic DNA (RAPD) fragments for identifying winegrape rootstocks (Xu et al., 1995). The markers were designed as sequence characterized amplified region (SCAR) DNA markers (Paran and Michelmore, 1993), and three of the six were found to be informative. The SCAR markers were developed because the original RAPDs were not sufficiently reproducible for use in genetic typing. Here, five of the SCAR markers have been reevaluated at substantially higher annealing temperatures, and four were polymorphic among the nine rootstocks tested. Further, four new informative SCAR markers also are described, based on redesigning primers from the previously cloned RAPD fragments.

The reliability of RAPD markers has been subject to controversy. Workers have shown that the RAPD assay is unusually sensitive to changes in reaction conditions and that the banding patterns are DNA polymerase-dependent (discussed in Xu et al., 1995). To overcome these difficulties, sequence-specific, RAPD-derived markers, such as SCARs (Paran and Michelmore, 1993) and allele-specific associated primers (ASAPs) (Yu et al., 1995), have been developed. This approach was found useful in the present study.

Materials and Methods

Rootstock origins (Galet, 1979) are MG 420A (*Vitis berlandieri* x *V. riparia*), Richter 99 (*V. berlandieri* x *V. rupestris*), 5C (*V. berlandieri* x *V. riparia*), SO4 (*V. berlandieri* x *V. riparia*), Couderc 3309 (*V. riparia* tomentose x *V. rupestris*), MG 101-14 (*V. riparia* x *V. rupestris*), Couderc 1616 [*V. solonis* (*riparia-rupestris-candicans*) x *V. riparia*], Kober 5BB (*V. berlandieri* selection), and Riparia Gloire (*V. riparia* selection). DNA extraction procedures, RAPD PCR amplification conditions, agarose gel electrophoresis, cloning of RAPD markers, DNA sequencing, and design of sequence-specific primers have been described (Xu, 1995; Xu et al., 1995). Southern hybridization analysis confirmed that the correct RAPD fragments had been cloned (data not shown for marker $OPG05_{1420}$) (Xu et al., 1995). Each marker is designated by the name of the original RAPD primer (UBC = Univ. of British Columbia, OP = Operon Technologies), followed by the size of the SCAR fragment (subscript). The corresponding SCAR primers are designated similarly with a U (upper) or L (lower), and sometimes a number, following the subscript. Annealing temperatures were chosen empirically and were 11 to 13C higher than the optima suggested by computer analysis (Oligo version 4.0; National Biosciences, Plymouth, Minn.). The five markers, $UBC204_{1140}$, $UBC231_{1840}$, $UBC231_{1660}$, $UBC251_{1440}$, and $OPG05_{500}$, are the same as in Xu et al. (1995), except that the latter is designated $OPG05_{550}$ in the earlier publication, and all were evaluated in the present study at higher than the original annealing temperatures. Markers $OPG06_{1440}$ and $OPG02_{1320}$ (Xu et al., 1995) were evaluated using newly designed internal primers. Marker $OPG02_{430}$ is half the size of the original marker, $OPG02_{860}$, (Xu et al., 1995) because the new internal primers generate a significantly smaller fragment. Marker $OPG05_{1420}$ has not been described before.

PCR mixtures contained 10 mM Tris-HCl, 50 mM KCl, pH 9.0, 0.1% Triton X-100, 1 unit of *Taq* DNA polymerase (Promega Corp., Madison, Wis.), 100 µM of each dNTP, 0.2 µM of each primer, 1.5 mM $MgCl_2$, and ≈5 ng genomic DNA in a final volume of 20 µl. Reaction mixtures were overlaid with mineral oil (E.R. Squibb & Sons, Princeton, N.J.) in an EasyCycler thermocycler (Ericomp, San Diego) and heated for 5 min at 94C, followed by 35 cycles of 30 sec at 94C; 1 min at the annealing temperatures specified in Table 1, and 2 min at 72C; followed by a final 5 min at 72C. All reaction mixtures were prepared in a laminar flow hood, and control reactions containing all components, except genomic DNA, were performed with each set of amplifications. After amplification, about half of each reaction product was electrophoresed, and the agarose gels were stained with ethidium bromide and viewed on an ultraviolet transilluminator.

Results and Discussion

Nine sequence-specific primer pairs derived from nine cloned RAPD markers were evaluated (Tables 1 and 2). Primer pairs $OPG05_{1420}$U/L, $UBC231_{1840}$U2/L2, and $UBC251_{1440}$U/L generated a product only from the rootstocks from which the original RAPD markers had been cloned, giving rise to markers specific to MG101-14, Richter 99, and Couderc 3309, respectively. Primer pairs $OPG02_{1320}$U/L, $UBC231_{1660}$U/L, and $OPG06_{1440}$U/L gave rise to products from rootstocks in addition to those from which the original RAPD markers had been cloned. Nevertheless, the results were polymorphic and, thus, informative. Amplifications involving primer pairs $OPG02_{430}$U2/L2 and $OPG05_{500}$U/L also generated amplification products from rootstocks, in addition to those from which the original RAPD markers had been cloned. However, they also generated apparent length variants that provided additional polymorphisms. Although Southern analysis established homology among the variants and the expected bands (data not shown), allelism could not be demonstrated for lack of a pedigree. Amplification using primer pair $UBC204_{1140}$U/L resulted in a loss of polymorphism because all rootstocks gave rise to the formerly polymorphic RAPD band. However, this marker may still have utility because it may be polymorphic among rootstocks not tested in this study.

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Table 1. Sequence-specific primer pairs derived from cloned RAPD markers.[z]

Code[y]	Primer pairs	Sequence (5'-3')	Annealing temp (°C)
1	$OPG02_{430}U2$	GAA TCG TCA GAG CCA TAA CC	66
	$OPG02_{430}L2$	ATT GCC TAA CAC TGT CCA AG	
2	$OPG02_{1320}U$	GAT TAG GAG TTG TGG GAT GA	64
	$OPG02_{1320}L$	AGG TGT AGC CTC AAT GAG AA	
3	$OPG05_{500}U$	CTG AGA CGG AGA GCT AAA AAA TAA	60
	$OPG05_{500}L$	CTG AGA CGG ACA CTA TTT CAC ACA	
4	$OPG05_{1420}U$	AAA TCA CGG CCA ATC TTG TC	70
	$OPG05_{1420}L$	GGG CCT AAA GCT GGT CAT TC	
5	$UBC204_{1140}U$	TTC GGG CCG TGT CAC ATG AAT TCC	66
	$UBC204_{1140}L$	TTC GGG CCG TTG ACT TTG AGG CAA	
6	$UBC231_{1840}U2$	ACC GGT ATG GTA CAG ATG CT	62
	$UBC231_{1840}L2$	GGG AGT TCC AAC AAG ATT TA	
7	$UBC231_{1660}U$	TCC ACG GAC GGG TTG GGA TAG ATT	66
	$UBC231_{1660}L$	TCC ACG GAC GAA TTC CAG GGG GTC	
8	$UBC251_{1440}U$	CTT GAC GGG GTA TGC TGG GCT GAG	66
	$UBC251_{1440}L$	CTT GAC GGG GGA GGG TTT GAA	
9	$OPG06_{1440}U$	GAT TGA GGT AAC TTG ACT GA	62.5
	$OPG06_{1440}L$	GTT AGG TGG AAA GAT GAA AG	

[z]The subscripts preceeding the "U" (upper) and "L" (lower) indicate the size of the marker in base pairs.
[y]Numbers used to identify primers in Table 2. Underlined sequences are derived from the original RAPD primers. $OPG05_{500}U/L$ was designated $OPG05_{550}U/L$ in Xu et al. (1995).

Table 2. Grape rootstock–sequence characterized amplified region (SCAR) marker matrix.

	SCAR primer pairs[z]								
Rootstock	1	2	3	4	5	6	7	8	9
MG 420A	+/+/+[y]		+/+[x]		+				
Richter 99	+	±	+		+	±	+		+
Couderc 3309		+	+		+			±	
MG 101-14		+	+	±	+				+
Kober 5BB			+/+[x]		+		+		+
SO4	+		+		+		+		+
5C	±		+		+		±		±
Riparia Gloire			+		+				
Couderc 1616			+		±				

[z]Refer to Table 1 for sequences of the coded primers. "+" indicates the presence of the marker when rootstock DNA was used in an appropriate DNA amplification reaction. "±" (underlining) indicates that the original RAPD marker was cloned from the specified rootstock. Internal sequence-specific primers derived from a monomorphic RAPD fragment of 922 bp cloned from SO4 generated the predicted band of 650 bp in all rootstocks at an annealing temperature of 64C. The primer sequences are (5'-3') $UBC234_{650}U1$, CTC CCA CAC TTT CAG ACA AC and $UBC234_{650}L1$, AAC CAT CCA TCC TAA CCA TA.
[y]"+/+/+" indicates that in addition to the 430-bp product, two apparent length variants of ≈460 and 490 bp were amplified.
[x]"+/+" indicates that in addition to the 500-bp product, an apparent length variant of ≈550 bp was amplified.

Based on the eight polymorphic markers, rootstocks MG 420A, MG101-14, Richter 99, Couderc 3309, and Kober 5BB can be unambiguously identified; Riparia Gloire and Couderc 1616 can be distinguished from the others, but not from one another; and SO4 and 5C also can be distinguished from the others, but not from one another. These results suggest that sequence-specific PCR primers derived from cloned RAPD markers will be useful in typing grape rootstocks.

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