Vitis 34 (2), 109–112 (1995)

Comparison of *Vitis Berlandieri* x *Vitis riparia* rootstock cultivars by restriction fragment length polymorphism analysis

by

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S u m m a r y : Patterns produced by RFLP analysis of genomic DNA extracted from nine *Berlandieri* ∞ *riparia* rootstock cultivars were compared. Of the six grape DNA probes used, several combinations of three probes were sufficient to clearly distinguish all nine rootstocks. Calculation of a similarity index for each pair revealed that 420 A was notably distinct from the other members of the group (D=0.56 compared to an average similarity of 0.77 among all the hybrids), while the Teleki hybrids (SO 4, 5 C, 5 BB, 125 AA, Cosmo 2, Cosmo 10) were generally very similar to each other (average D=0.85). No differences were observed between 5 A and 5 BB, consistent with previous reports that at least some 5 A vines are identical to 5 BB.

K e y w o r d s : RFLP, DNA profile, Vitis berlandieri, Vitis riparia, grapevine rootstock, identification.

Introduction

The *Berlandieri* × *riparia* hybrids are arguably the most important group of grape rootstocks in use today (GALET 1988). They are the result of crosses and subsequent selection aimed at combining resistance to phylloxera with lime tolerance and other desirable horticultural properties (BECKER 1968) (Fig. 1). The morphological similarity among these cultivars makes them difficult to distinguish ampelographically and necessitates rigorously accurate planting records in collections, foundation plantings and nurseries in order to prevent errors. One such mistake was recently detected in the rootstock collection in the Department of Viticulture and Enology at the University of California at Davis (WALKER and BOURSIQUOT 1992) but others have undoubtedly occurred. Because of their worldwide importance in commercial viticulture, objective and reliable means for identifying these stocks are essential.

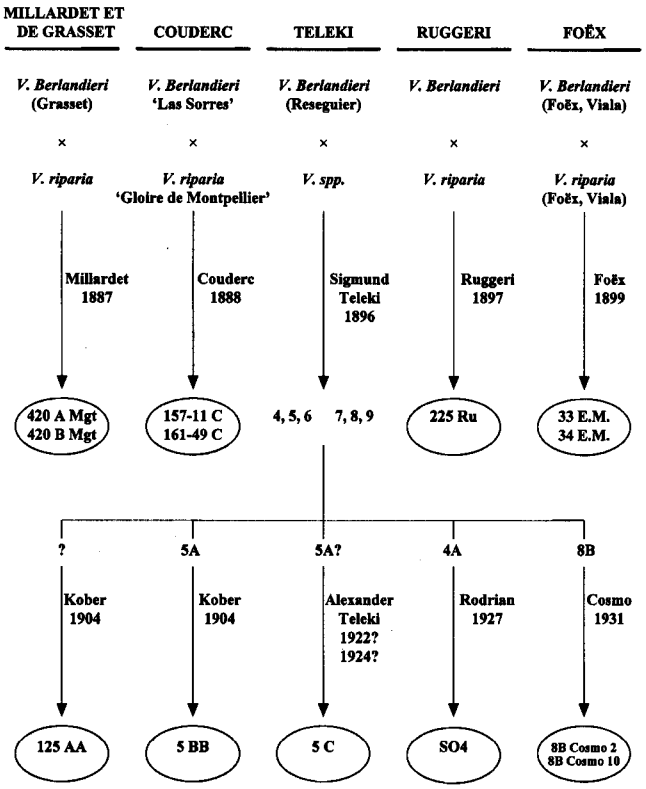

Fig. 1: Pedigrees of the *Berlandieri* × *riparia* hybrids. Compiled from BECKER 1968, COSMO 1979, GALET 1988, PONGRACZ 1983 and TELEKI 1927.

Isozyme analysis is a relatively simple and inexpensive laboratory method for distinguishing rootstocks and has been shown to differentiate many rootstock cultivars, including *Berlandieri* × *riparia* hybrids (ALTUBE *et al.* 1991; BOURSIQUOT and PARRA 1992; WALKER and LIU 1995). However, the susceptibility of isozyme patterns to modification by all factors that affect gene expression (e.g., season, temperature, tissue, developmental stage) suggests that DNA polymorphism patterns, being more stable, may be more reliable for resolving identity in particularly important situations, such as in national collections.

Although DNA polymorphism has been used to distinguish grape cultivars, the emphasis has been on scion cultivars of *V. vinifera* (BOWERS *et al.* 1993; THOMAS *et al.* 1993; THOMAS and SCOTT 1993) rather than rootstocks. BOURQUIN *et al.* (1991, 1992) used restriction fragment length polymorphism (RFLP) analysis to differentiate 16 rootstocks, including four *Berlandieri* × *riparia* hybrids, with three DNA probes. The objective of this work was to differentiate the main commercial rootstocks of the *Berlandieri* × *riparia* family by RFLP analysis.

Materials and methods

P l a n t m a t e r i a l : Young leaves and shoot tips were collected from the vineyards of the Department of Viticulture and Enology, University of California at Davis. Nine hybrids (SO 4, Teleki 5 C, Teleki 5 A, Kober 5 BB, Kober 125 AA, 8B Cosmo 2, 8B Cosmo 10, 420 A Millardet

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et De Grasset, Ruggeri 225 A) and one accession of each of the parental species (*Vitis Berlandieri* 'La Font', *Vitis riparia* 'Grand Glabre') were analyzed. For each accession, multiple DNA preparations were made from a single vine. (Details regarding Davis vineyard locations and original sources are available upon request.) Tissue was maintained at -80 °C before DNA extraction.

DNA extraction: Genomic DNA was extracted by the CTAB (hexadecyltrimethylammonium bromide) method, according to SAGHAI-MAROOF *et al.* (1984) with some modifications. The powdered tissue (2 g) was added to 20 ml of extraction buffer (2 % CTAB; 1.4 M NaCl; 100 mM Tris-HCl; 20 mM EDTA; 1 % β-mercaptoethanol; pH 8), incubated for 1 h at 60 °C and then extracted with chloroform/isoamyl alcohol (24:1)(v:v).

The first CTAB extraction was followed by a second one with 4 ml of 10 % CTAB (BOWERS *et al.* 1993). This step proved very effective in further eliminating contaminants. The chloroform/isoamyl alcohol extractions were repeated until the interface was totally removed (2 to 4 times). The DNA was precipitated with one volume of cold isopropanol; soaked briefly in 75 % ethanol 0.2 M NaAc, and then 75 % ethanol 0.1 M NH_4Ac; washed with 75 % ethanol and then 90 % ethanol; and finally resuspended in TE (10 mM Tris-HCl; 1 mM EDTA; pH 8). After RNase (150 µg/ml for 3 h at 37 °C) and proteinase K (125 µg/ml for 1 h at 50 °C) treatments, the DNA was extracted once again, precipitated and washed as before. DNA concentrations were estimated by fluorimetry because CTAB interferes with absorbance at 260 nm. The DNA yield was 20 to 40 µg per gram of frozen tissue.

Probe preparation: The probes were selected in our laboratory from a genomic library of *Vitis vinifera* cv. Thompson Seedless (BOWERS *et al.* 1993). Plasmid DNA was isolated by alkali lysis and purified with polyethylene glycol (SAMBROOK *et al.* 1989). The inserts were isolated by digestion with *Pst* I, agarose gel electrophoresis, excision of the band and centrifugation through filter paper (WEICHENHAN 1991). Probes were labeled with ^{32}P-α-dCTP according to FEINBERG and VOGELSTEIN (1983).

DNA digestion, electrophoresis and hybridization: DNA (8 µg/100 µl) was digested with 40 units of *Eco*RV or *Hind*III (Boehringer Mannheim or Gibco BRL) for 3 h. Digestion was sometimes incomplete and a second digestion was carried out in the presence of bovine serum albumin (0.1 µg/µl) and spermidine (0.2 µg/µl).

Electrophoresis was performed in 20 × 24 × 0.5 cm 1 % LE agarose (Seakem) at 35 V for 24 h with 3 µg of DNA per lane. Southern blotting was performed with Zetaprobe nylon membranes (Bio-Rad) and the manufacturer's protocol. Prehybridization and hybridization were in 0.25 M Na_2HPO_4, 1 mM EDTA and 7 % SDS at 60 °C, for 1 h and overnight, respectively. The membranes were washed with 2XSSC, 0.1 % SDS for 15 min at room temperature, followed by another 15 min at 60 °C, and a final wash with 1XSSC 0.1 % SDS for 15 min at 60 °C. The dried membranes were exposed to Kodak X-OMAT AR film with one intensifier screen at -80 °C for 3-10 d.

Results and discussion

Four of the probes (4G3, 3B4, 7B4, 1A10) proved much more useful in differentiating the cultivars than the other two (7D3, 6E8) (Tab. 1). The two restriction enzymes gave comparable results, with *Hind*III producing two more band patterns than *Eco*RV with the probe 1A10, and *Eco*RV producing one more pattern with the probe 7D3. Several combinations of three of the four most informative probes were sufficient to distinguish all of the cultivars. One such combination (3B4/*Eco*RV + 7B4/*Hind*III + 4G3/*Eco*RV) is illustrated in Fig. 2. Others include 4G3/*Eco*RV + 3B4/*Eco*RV + 1A10/*Eco*RV and 3B4/*Eco*RV + 7B4/*Hind*III + 1A10/*Hind*III. Multiple DNA samples from the same accession produced identical patterns (data not shown). We have previously shown that RFLP patterns produced with the protocol and probes used in this study are stable and reproducible (BOWERS *et al.* 1993).

In order to examine the similarity among the hybrids, a similarity index, D, was calculated for each pair (Tab. 2). D represents the proportion of shared bands and is calculated as two times the number of bands shared by geno-

Table 1

RFLP patterns observed for *Berlandieri* × *riparia* hybrids. For each probe and enzyme combination, identical patterns are indicated by the same letter

DNA probe:	4G3		3B4		7B4		1A10		7D3		6E8	
Restriction enzyme:	*Eco*RV	*Hind*III	*Eco*RV	*Hind*III	*Eco*RV	*Hind*III	*Eco*RV	*Hind*III	*Eco*RV	*Hind*III	*Eco*RV	*Hind*III
V. Berlandieri La Font	A	A	A	A	A	A	A	A	A	A	A	A
V. riparia Grand Glabre	B	B	B	B	B	B	B	B	B	B	B	B
SO 4	C	C	C	C	C	C	C	C	C	B	C	B
5 C	D	D	D	D	D	D	D	D	C	B	C	B
5 BB	D	D	C	C	D	D	C	C	D	B	C	B
5 A	D	D	C	C	D	D	C	C	D	B	C	B
420 A	E	E	E	E	E	E	D	E	E	C	D	C
Cosmo 2	D	D	F	C	C	C	E	F	D	B	C	B
Cosmo 10	C	C	F	C	C	C	C	C	D	B	C	B
125 AA	C	C	D	F	F	F	E	F	D	B	C	B
Ruggeri 225	D	D	F	C	D	D	D	G	D	B	C	B

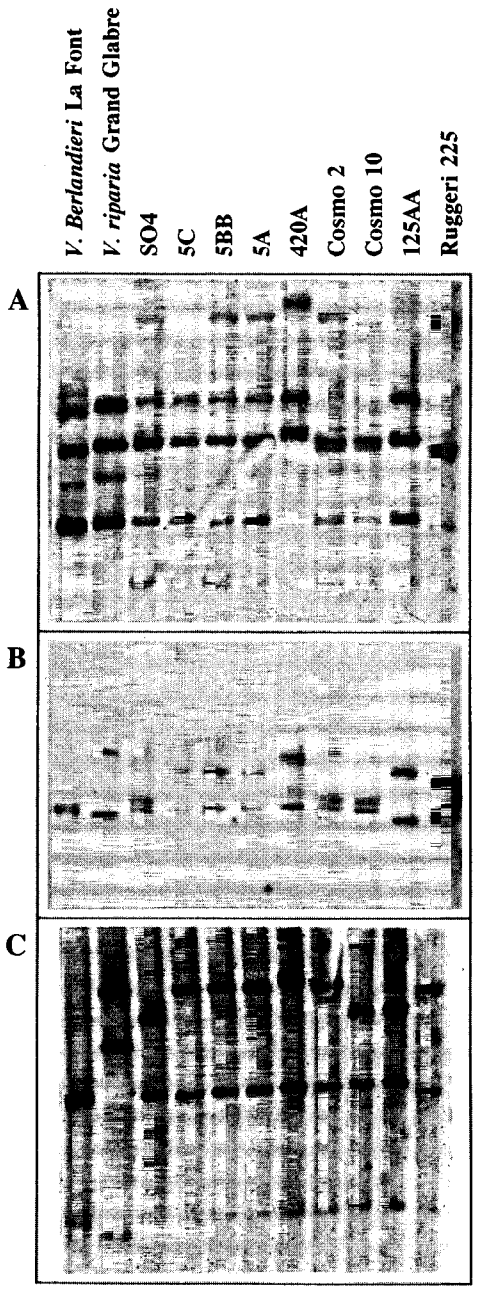

Fig. 2: RFLP patterns of *Berlandieri* × *riparia* rootstock hybrids produced with three DNA probes: A) 3B4 (*Eco*RV), B) 7B4 (*Hind*III) and C) 4G3 (*Eco*RV).

types A and B, divided by the total number of bands present in A and B.

One cultivar, 420 A Millardet et de Grasset, was distinct from the others, with an average similarity index of only 0.56 (compared to an overall average similarity of 0.78 among the other hybrids). This hybrid could be separated from the rest of the group with any of the probes tested. 420 A was the only representative of the Millardet et de Grasset collection examined in this study and its parents may have been genetically quite different from those used by other breeders. The *riparia* parent is known to have been morphologically unusual (dark green and deeply trilobed leaves) and, consistent with the RFLP results, 420 A is the most easily recognizable of the *Berlandieri* × *riparia* hybrids, by virtue of its trilobed basal leaves and purple nodes extending to the end of the shoot (GALET 1988).

At the other extreme were the pair with the highest similarity (D=1), 5 A and 5 BB, which could not be distinguished with any of the DNA probe/enzyme combinations tested. These two cultivars are also morphologically indistinguishable (WALKER and BOURSIQUOT, unpublished) and could not be differentiated by isozyme analysis with 8 different enzyme systems (WALKER and LIU 1995). Since three separate vines were originally designated as 5 A by Teleki and at least one of these was later called 5 BB by Kober (GALET 1988), our results are consistent with the possibility that the 5 A and 5 BB vines analyzed in this study are actually the same genotype.

The rootstocks SO 4 and 5 C are very similar morphologically. In fact, several SO 4 accessions in the Davis collection were incorrectly labeled as 5 C for many years, before being corrected by WALKER and BOURSIQUOT (1992) after detailed ampelographic study and isozyme analysis. Although SO 4 and 5 C had a high similarity index (D=0.83), any of the four most informative probes could distinguish them.

The high similarity between Cosmo 2 and Cosmo 10 (D=0.94) is not surprising since both were selected from Teleki 8 B, originally propagated from a group of five morphologically similar seedlings (GALET 1988), and may be closely related, perhaps even siblings. Similarities between other pairs of the Teleki hybrids, such as SO 4 and

Table 2

Similarity values for pairwise comparisons of DNA profiles

V. riparia	0.52									
SO 4	0.48	0.67								
5 C	0.65	0.63	0.83							
5 BB	0.64	0.73	0.86	0.90						
5 A	0.64	0.73	0.86	0.90	1.00					
420 A	0.52	0.50	0.58	0.79	0.61	0.61				
Cosmo 2	0.52	0.68	0.85	0.81	0.88	0.88	0.51			
Cosmo 10	0.60	0.71	0.92	0.75	0.86	0.86	0.54	0.94		
125 AA	0.64	0.67	0.83	0.87	0.89	0.89	0.56	0.80	0.83	
225 Ru	0.55	0.61	0.73	0.86	0.88	0.88	0.53	0.88	0.82	0.81
	Berl	*rip*	SO 4	5 C	5 BB	5 A	420 A	Co 2	Co 10	125 AA

Cosmo 10 (D=0.92) and 5 BB and 5 C (D=0.90) are also not surprising, considering the common heritage of these rootstocks.

Vitis species are dioecious and outcrossing in nature and thus individual accessions within a species are genetically different and produce different RFLP patterns (GOLMAN and MEREDITH, in preparation). As expected, some bands produced by the hybrids were not seen in either of the *V. Berlandieri* or *V. riparia* accessions analyzed. If the specific accessions used as parents were to be analyzed, one would expect each hybrid to exhibit only bands produced by one or the other of its two parents. Unfortunately, the actual accessions originally used in the crosses (which would have been expected to account for all bands seen in the hybrids) were not available to us. The accessions of *V. riparia* and *V. Berlandieri* were relatively dissimilar to the hybrids (average similarity indices of 0.65 and 0.60, respectively) and to each other (D=0.52), as expected.

The results of this study show that the *Berlandieri* × *riparia* rootstock cultivars are for the most part genetically quite similar, as might be expected considering the common heritage of these hybrids and their strong morphological similarity. However, this study also shows that, despite their similarity, RFLP analysis can clearly distinguish all 9 cultivars analyzed. The identical results obtained for 5 A and 5 BB confirm previous reports that these are indeed the same genotype. Although rootstock cultivars can also be distinguished by the simpler and faster method of isozyme analysis, the greater stability of DNA polymorphisms argues for their use in those situations in which correct identification is most critically important, such as in major collections which are used as identification references by others. Current advances in DNA polymorphism analysis, notably the development of microsatellite markers, are increasing the speed and reliability with which identification can be resolved by the detection of genotypic differences at the DNA level.

Acknowledgement

This research was supported by a grant to BIBIANA GUERRA from the Instituto Nacional de Investigaciones Agrarias, Spain.

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Received November 7, 1994