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RAPD markers in wild and cultivated *Vitis vinifera*

by

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Summary: Some *Vitis vinifera* cultivars and *V. vinifera* ssp. *silvestris* individuals have been subjected to the RAPD analysis in order to estimate the genetic diversity existing within this germplasm. 44 decamer primers of arbitrary sequence have been used for PCR and reproducible band profiles have been obtained. The distribution of the individualized polymorphic DNA markers has not turned out to be different in a remarkable way between cultivated and wild grapevines but this RAPD approach provides for some characteristics useful to analyze genetic relationships even within the *Vitis vinifera* species.

Key words: genetic diversity, PCR, Vitis vinifera ssp. silvestris.

Introduction

Some years ago a research on the diffusion and characterization of the Italian populations of *Vitis vinifera* ssp. *silvestris* was set up. Among one of its aims there was also the analysis of the relationships between wild and cultivated grapevines (SCIENZA 1983).

The individualized and collected germplasm of *V. vinifera* ssp. *silvestris* has been described by means of the usual ampelographic method and through ampelometric analyses (ANZANI *et al.* 1990, FAILLA *et al.* 1992). Some genotypes have also been characterized according to the electrophoretic patterns of the seed storage proteins (SCIENZA *et al.* 1990) and pollen wall proteins (TEDESCO *et al* 1990) as well as according to the anthocyanin profile of grape skins (MATTIVI *et al.* 1990, and 1993, VALENTI *et al.* 1993).

Since the molecular analysis methods based upon PCR (Polymerase Chain Reaction), recently applied also to the DNA of grapevine, can give a practically unlimited series of primary characters (GRANDO *et al.* 1994), we suggest in this paper the attempt of using RAPD markers (Random Amplified Polymorphic DNA) (WELSH and MCCLELLAND 1990, WILLIAMS *et al.* 1990) as an approach to the study of the genetic diversity of wild and cultivated grapevines.

Materials and methods

Grape material: All plant material was obtained from the germplasm collection maintained at our Institute. Young fully expanded leaves were harvested, immediately frozen in liquid nitrogen and stored at -80 °C until DNA extraction. The genotypes used for this analysis are listed in the Table.

DNA isolation: DNA was extracted and purified according to the hexadecyltrimethylammonium bromide (CTAB) method of DOYLE and DOYLE (1987) for fresh tissue. To estimate DNA concentration, samples of grape DNA were compared with dilutions of known concentration of λDNA after agarose gel electrophoresis and ethidium bromide staining.

Amplification reaction conditions: 44 random decamer oligonucleotides (from sets A, E and U, Operon Technologies Inc. Alameda CA, USA), were used as single primers for the amplification of RAPD sequences. The polymerase chain reaction (PCR) was performed in a volume of 25 µl containing 5 µl of the DNA extract (approximately 15 ng), 10 mM Tris-HCl pH 9, 50 mM KCl, 2 mM $MgCl_2$, 0.1 mM each dATP, dCTP, dGTP and dTTP (Boehringer), 0.28 µM primer and 0.3 U Taq DNA polymerase (Super Taq P.H. Stehelin & Cie AG, Basel, Switzerland). Amplification was performed in a Perkin Elmer Cetus Gene Amp PCR System 9600 programmed as follows (KOLLER *et al.* 1993): 2 cycles of 30 s at 94 °C, 30 s at 36 °C, 120 s at 72 °C; 20 cycles of 20 s at 94 °C, 15 s at 36 °C, 15 s at 45 °C, 90 s at 72 °C; 19 cycles of 20 s at 94 °C (increased 1 s/cycle), 15 s at 36 °C, 15 s at 45 °C, 120 s at 72 °C (increased 3 s/cycle) followed by 10 min at 72°C.

Table

List of cultivars and individuals of *Vitis vinifera* used for the RAPD analysis

CULTIVATED	WILD		
	CODE		ORIGIN
Armenia	vs03	Yeni Katla	Germany
Bombino n.	vs12	Kl-Hoerdt	Germany
Chardonnay	vs13	Sinni 2	Italy
Chenin b.	vs16	Moille	Switzerland
Furmint	vs17	Sinni	Italy
Garganega	vs18	Veano 6	Italy
Hàrslevelù	vs24	Sinni T	Italy
Kadarka	vs25	Casale Giglio	Italy
Lambrusco f.f.	vs26	Bagnoli	Italy
Muscat of Alexandria	vs27	Sinni P	Italy
Pinot n.	vs29	Nigra	Germany
Riesling r.	vs31	Caucasica	URSS
Rkatsiteli	vs33	Sinni 5	Italy
Sangiovese	vs37	Cumone 1	Italy
Sauvignon b.	vs38	Sinni U	Italy
Semillon	vs42	Fenelle	Switzerland
Syrah	vs43	Nettuno	Italy
Tocai friulano	vs56	Martignano	Italy
Trebbiano toscano			

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After the cycling was completed, 12 µl of the RAPD products were separated by electrophoresis in 1.5 % agarose gels run in 0.5 x TBE (0.045 M Tris-borate and 1.0 mM EDTA pH 8), visualized with ethidium bromide and photographed under UV with Polaroid 667 films.

Data analysis: Each amplified band was named by the primer used and its size in bp. RAPD profiles were scored visually. Data were recorded as the presence or absence of an amplification fragment of a given length. Only those major fragments whose presence was unambiguous were retained.

Genetic similarity between each pair of accessions and the dendrogram tree were calculated using NTSYS-pc package 1.8 developed by Rohlf (Exeter Software, New York, USA).

Results and discussion

The DNA amplification conditions we used, have produced DNA fragments of discrete size with all tested primers. Yet, the electrophoretic profiles have turned out to be quite complex and often show not very consistent polymorphic bands. Anyway, a good reproducibility of the system has been noted, both concerning the number, and the intensity of the amplification products (Fig. 1). The 2 groups of primers belonging to sets A and E have shown a clear difference in their capacity of generating polymorphic DNA fragments. Among the 20 primers of set E no RAPD markers with a clear position in gel have been determined, whereas set A has given most of the polymorphic products used for statistical analysis. From set U primers 1, 2, 10 and 14 have been tested. The latter has given 2 poymorphic bands.

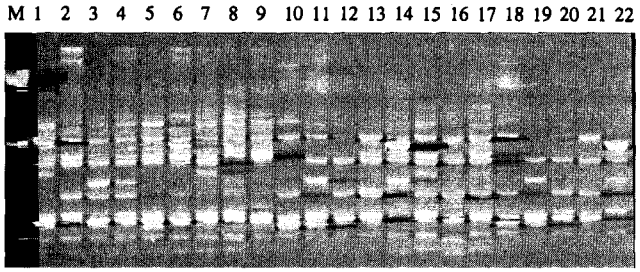

Fig. 1: RAPD profiles generated by primer U14 (5'TGGGTCCCTC3'). For some accessions the DNA products of two independent reactions are shown. **1** Tocai f., **2** Sangiovese, **3** Sauvignon b., **4** Chenin b., **5** Semillon, **6** Hàrslevelù, **7** Syrah, **8** Armenia, **9** Rkatsiteli , **10-18** vs 27, **11-19** vs 31, **12-20** vs 56, **13-21** vs 12, **14-22** vs 24, **15** vs 29, **16** vs 33, **17** vs 16, **M** Molecular size DNA Ladder (Gibco BRL).

The comparison between genotypes has thus been based upon the distribution of the following RAPD markers: $A1_{965}$, $A1_{860}$, $A7_{830}$, $A8_{1020}$, $A9_{520}$, $A9_{450}$, $A10_{1570}$, $U14_{930}$ and $U14_{585}$ (Figs. 1, 2, 3). The dendrogram originated from this first comparative analysis of RAPD profiles (Fig. 4) has not pointed out remarkable differences between cultivated and wild grapevines. Nevertheless, it can be noted that some vines ascribed to the *occidentalis* NEGR. group are clearly separated from the other genotypes whereas the RAPD profiles of *V.vinifera* ssp. *silvestris* acessions are very often more similar or even identical to those of the cultivars ascribed to the *proles pontica* NEGR. group.

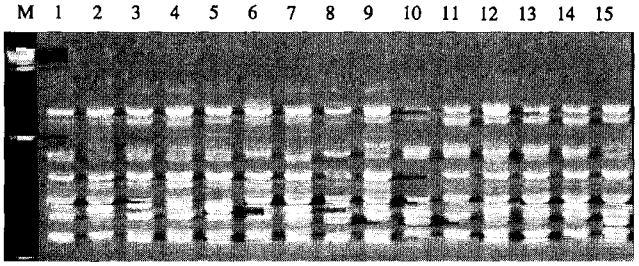

Fig. 2: RAPD profiles generated by primer A01 (5'AGGCCCTTC3'). **1-9** and **M**: see Fig. 1; **10** vs 56, **11** vs 12, **12** vs 24, **13** vs 29, **14** vs 33, **15** vs 16.

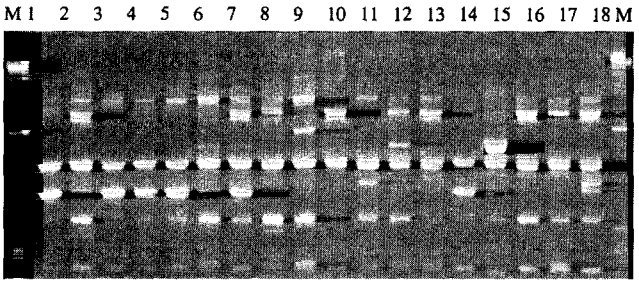

Fig. 3: RAPD profiles generated by primer A07 (5'GAAACGGGTG 3'). **1-9** and **M**: see Fig. 1; **10** vs 27, **11** vs 31, **12** vs 56, **13** vs 12, **14** vs 13, **15** vs 24, **16** vs 29, **17** vs 33, **18** vs16.

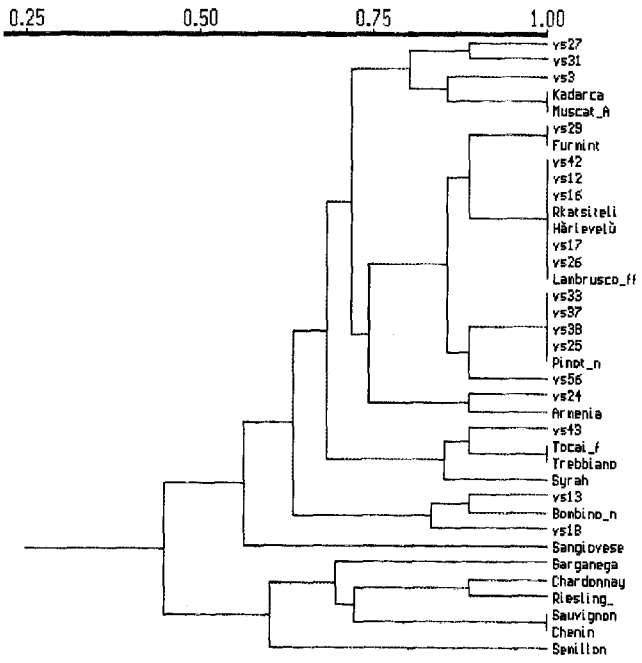

Fig. 4: Phylogram tree based on RAPDs data showing the relationships among *Vitis vinifera* wild and cultivated genotypes.

Conclusions

Through this first screening of oligo-nucleotide decamers of arbitrary sequence for a RAPD analysis of the germplasm of cultivated and wild *V. vinifera*, some

polymorphisms of the DNA amplification products have been selected. Although for some DNA markers a greater homogeneity in the accessions of the *V. vinifera* ssp. *silvestris* group has been noted, no remarkable differences between cultivated and wild vines have been found.

Yet, the different capacity of generating polymorphic markers as shown by the different sets of primers used and the good quality of the analysis procedure used, encourage us to go on using the screening system in order to base the comparison between accessions on larger portions of *V. vinifera* genome.

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