RAPD markers in wild and cultivated Vitis vinifera

by

M.S. GRANDO, L. DE MICHELI, L. BIASETTO and A. SCIENZA

Istituto Agrario, San Michele all'Adige, Italia

S u m m a r y : 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.

K e y w o r d s : 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. viniferassp. 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

G r a p e m a t e r i a l : 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.

D N A is olation: 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 λ DNA 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, 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 Bombino n. Chardonnay Chenin b. Furmint Garganega Hàrslevelù Kadarka Lambrusco f.f. Muscat of Alexandria Pinot n. Riesling r. Rkatsiteli Sangiovese Sauvignon b. Semillon Syrah Tocai friulano Trebbiano toscano | vs03 vs12 vs13 vs16 vs27 vs28 vs26 vs27 vs29 vs31 vs33 vs37 vs38 vs42 vs43 vs56 | Yeni Katla Kl-Hoerdt Sinni 2 Moille Sinni T Casale Giglio Bagnoli Sinni P Nigra Caucasica Sinni 5 Cumone 1 Sinni U Fenelle Nettuno Martignano | Germany Germany Italy Switzerland Italy Italy Italy Italy URSS Italy Italy Italy Switzerland Italy Italy Italy |

Correspondence to: Dr. M.S. GRANDO, Istituto Agrario, I-38010 San Michele all'Adige, Italy

After the cycling was completed, 12 μ l 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.

D a t a a n a l y s i s : 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.

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22



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.

Acknowledgement

Researches supported by the Italian Ministry of Agriculture in the framework of the project "Resistenze genetiche delle piante agrarie agli stress biotici ed abiotici".

References

- ANZANI, R.; FAILLA, O.; SCIENZA, A.; CAMPOSTRINI, F.; 1990: Wild grapevine (Vitis vinifera ssp. silvestris) in Italy: Distribution characteristics and germplasm preservation. Proc. 5th Intern. Symp. Grape Breeding, 12-16 Sept. 1989, St. Martin/Pfalz, FRG. Vitis, Special Issue, 97-113.
- DOYLE, J. J.; DOYLE, J. L.; 1990: Isolation of plant DNA from fresh tissue. Focus 12, 13-15.
- FAILLA, O.; ANZANI, R.; SCIENZA, A.; 1992: La vite selvatica in Italia: diffusione, caratteristiche e conservazione del germoplasma. Vignevini 19 (1/2), 37-46.

- GRANDO, M. S.; DE MICHELI L.; SCIENZA, A.; 1994: Characterization of Vitis germplasm using random amplified polymorphic DNA markers. Gen. Res. Crop Evol. (submitted).
- KOLLER, B.; LEHMANN, A.; MCDERMOTT, J. M.; GESSLER, C.; 1993: Identification of apple cultivars using RAPD markers. Theoret. Appl. Genet. 85, 901-904.
- MATTIVI, F.; SCIENZA, A.; FAILLA, O.; VILLA, P.; ANZANI, R.; TEDESCO, G.; GIANAZZA, E.; RIGHETTI, P.; 1990: Vitis vinifera - a chemotaxonomic approach: Anthocyanins in the skin. Proc. 5th Intern. Symp. Grape Breeding, 12-16 Sept. 1989, St. Martin/Pfalz, FRG.Vitis, Special Issue, 119-133.
- --; VALENTI, L.; MASTROMAURO, F.; SCIENZA, A.; 1993: Impiego del profilo antocianico nella classificazione della vite selvatica italiana (*Vitis v. silvestris*): confronto con i vitigni coltivati (*Vitis v. sativa*). Vignevini 20 (10), 40-45.
- SCIENZA, A.; 1983: Notizie ed importanza di alcuni recenti ritrovamenti di Vitis vinifera silvestris GMELIN in Italia. Not. Ortoflorofrutticolt. 6, 297-310.
- --; ANZANI, R.; FAILLA, O.; MATTIVI, F.; VILLA, P. L.; TEDESCO, G.; RIGHETTI, P. G.; ETTORI, C.; MAGENES, S.; GIANAZZA, E.; 1990: Vitis vinifera - a chemotaxonomic approach: Seed storage proteins. Proc. 5th Intern. Symp. Grape Breeding, 12-16 Sept. 1989, St. Martin/Pfalz, FRG. Vitis, Special Issue, 24-28.
- TEDESCO, G.; CARGNELLO, G.; ZANARDINI, E.; VILLA, P.; MANINI, F.; GIANAZZA, E.; 1990: Vitis vinifera - a chemotaxonomic approach: Pollen wall proteins. Proc. 5th Intern. Symp. Grape Breeding, 12-16 Sept. 1989, St. Martin/Pfalz, FRG. Vitis, Special Issue, 114-118.
- VALENTI, L.; MATTIVI, F.; MASTROMAURO, F.; ANZANI, R.; SCIENZA, A.; 1993: Caratterizzazione della Vitis v. silvestris italiana attraverso i pigmenti antocianici. Vignevini 20 (9), 42-48.
- WELSH, J.; McCLELLAND, M.; 1990: Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18, 7213-7218.
- WILLIAMS, J. G. K.; KUBELIK, A. R.; LIVAK, K. J.; RAFALSKI, J. A.; TINGEY, S. V.; 1990: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18, 6531-6535.

Received September 16, 1994