

Research Note

Characterization of *Vitis vinifera* cultivars by Random Amplified Polymorphic DNA markers

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During the past two years an alternative strategy based on PCR technology has been described (WILLIAMS *et al.* 1990) and carried out successfully to differentiate cultivars of several plant species (CARLSON *et al.* 1991; KLEIN-LANKHORS *et al.* 1991; PARRAN *et al.* 1991; WILDE *et al.* 1992). This procedure, named RAPD for Random Amplified Polymorphic DNA, used single decamer primers of arbitrary sequence to amplify DNA fragments of polymorphic length. We have tested this method for the intraspecific characterization of *Vitis vinifera* and we report here the results obtained on 8 cultivars.

DNA extractions were performed from clean, young and fully expanded leaves according to DOYLE and DOYLE (1987) with 1 % of polyvinylpyrrolidone (PVP) in the extraction buffer. Extracted DNA was purified using glass-max DNA isolation spin cartridge system purchased by Gibco Brl. Yield of DNA were measured using the HOECHST dye assay method with a TKO 100 minifluorimeter (Hofer Scientific Instrument); Random primers were provided by Operon Technologies Inc. and were not further purified.

For RAPD analysis the protocol reported by WILLIAMS *et al.* (1990) was followed with minor modifications. Amplification reactions were carried out in 25 µl buffer containing 10 mM Tris pH 8.3, 50 mM KCl, 4 mM MgCl₂, 200 µM each of dATP, dGTP, dCTP, dTTP, 0.2 µM primer and 0.5 unit of Taq Polymerase (CETUS). Amplification was performed in a Perkin Elmer CETUS DNA thermocycler programmed for 45 cycles of 1 min at 94 °C for denaturing, 1 min at 36 °C for annealing, 2 min at 72 °C for extension.

More than 50 decamers of arbitrary sequences have been screened as primers on total DNA of grape cultivars in way to generate polymorphic PCR products. Many of them allowed the amplification of 2-15 DNA fragments sized from 0.1 to 5 kb. Under described standard conditions the electrophoretic patterns were highly reproducible.

Among a set of successful primers, some determined complex profiles with numerous and not discrete bands which were difficult to interpret. Others generated simple and readable patterns but only few of them were discriminant between cultivars. The results obtained with two primers OPA 01 (CAGGCCCTTC) and OPA 18 (AGGTGACCGT) are presented in Figure A and B. OPA 01 allowed to obtain five "phenotypes" among 8 tested cultivars. Two pairs, namely Cabernet Sauvignon and Cabernet franc (Figure A, lanes 3 and 4) and Gamay and Chenin (Figure A, lanes 7 and 8), could not be differentiated. The OPA 18 primer gave more numerous amplified products (Figure B). It yielded to the differentiation of the 8 cultivars. However, two of them, Syrah and Chenin (Figure B, lanes 2 and 8) differed only by a weak but constant band (arrow) of their electrophoretic patterns. In fact only cross comparisons between patterns obtained with the two primers OPA 01 and OPA 18 led to a reliable identification of each cultivar.

OPA 01 and OPA 18 have also been tested for clones discrimination within the same variety (data not shown) but without success. Experiments with a wide range of decamer primers are in progress. They indicate that each grape variety and certainly some clones within the same cultivar could be characterized by RAPD markers. As already demonstrated for other cultivated plants these markers could also be very useful for genetic mapping of *Vitis vinifera*.

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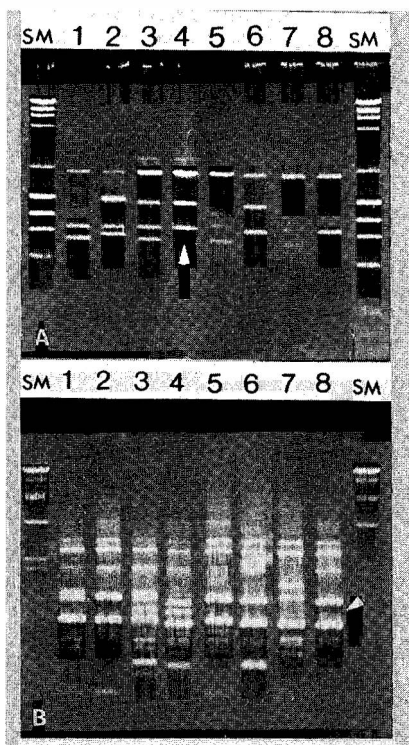


Figure: Amplification patterns of polymorphic DNA from 8 cultivars of *Vitis vinifera* using single arbitrary 10-mer oligonucleotide primers, OPA 01 (A) and OPA 18 (B). Lanes 1-8 correspond respectively to Pinot noir, Syrah, Cabernet Sauvignon, Cabernet franc, Chardonnay, Grenache, Gamay noir, Chenin. SM: HindIII digested lambda DNA+HaeIII digested Phi x 174 DNA. Agarose gel concentration 1.2 %. On figure A note that electrophoretic profiles of the two Cabernet cultivars (lanes 3 and 4) are identical even if the smallest band is very weak in lane 4 (arrow).

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