

Research Note

Molecular characterization of grapevine cultivars of Riesling-type and of closely related Burgundies

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S u m m a r y : Ten cultivars representing Riesling-type vines and 12 cultivars of the Burgundy family were analyzed by RAPD-PCR. Employing computer programs, similarity degrees were calculated from the RAPD patterns obtained from a set of 20 dekamer primers and used to perform cluster analysis. The Riesling-type cultivars could be differentiated from each other and from the progenitor of a cross Pinot noir x Chambourcin (“Spezial” in Fig.1). With the exception of Aubin blanc, Aubin vert and Elbling white, the Riesling-type cultivars did not show any close relationship. From the Burgundies, Pinot noir, Pinot meunier, Pinot blanc and Pinot gris could not be differentiated. Neither could Chardonnay blanc be differentiated from Chardonnay musqué, nor Aligoté from Aligoté vert. Discrimination between the other cultivars was possible. Two Morillon samples from different origins were analyzed and showed clear differences in their RAPD patterns. Furthermore, they seemed to be more similar to Riesling white than to the Burgundies.

Key words : DNA, RAPD-PCR, Burgundy cultivar, Riesling cultivar, identification.

Introduction: Today, the number of different grapevine cultivars has been estimated to be in the range of 5000 to 15000. The origin of many of them and the relationship between some of the cultivars is uncertain. Distinction of closely related cultivars by ampelography often is difficult. Genomic analysis can help to solve these problems. RAPD (random amplified polymorphic DNA) analysis e.g. is one technique recently used with success to differentiate grapevine cultivars. It is rather cheap and easily performed, but nevertheless provides valuable information. Here we employed this method to investigate the distinguishability of several grapevine cultivars and their relationship.

Materials and methods: DNA was prepared from young, expanding leaves using the protocol described by THOMAS *et al.* (1993). RAPD analysis was performed with 20 dekamer primers (F01 to F20, Operon Technologies, Alameda, CA, USA) using the protocol described earlier (BÜSCHER *et al.* 1993; 1994). Amplification products were resolved by electrophoresis on 1.5% agarose gels made in 0.5 x TBE (44.5 mM Tris, 44.5 mM borate, 1 mM EDTA). Similarity degree values were calculated from the RAPD patterns with the help of the computer program “Rapid” developed by R.BLAICH at the institute. Cluster analysis based on these similarity indices was performed with a self-made computer program. This program uses the algorithm described by NÖBAUER and TIMISCHL (1979). The results are represented as dendrograms showing the similarities between the cultivars analyzed.

Results and discussion: The cultivars of the Riesling-type grapevines can be clearly distinguished by their RAPD patterns (Fig. 1). The cultivars Aubin vert, Aubin blanc and

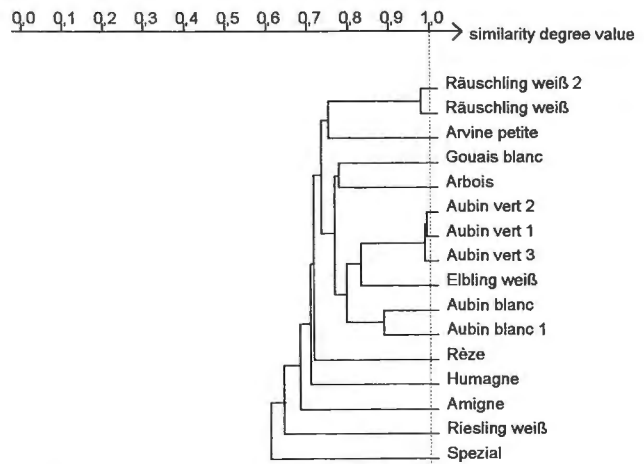


Fig. 1: Dendrogram of the Riesling-type grapevines. The similarity degree values were calculated using the bands generated by all 20 primers (F01 to F20) and with the help of the “Rapid” program. Cluster analysis was done with a self-made program (see Materials and methods for details).

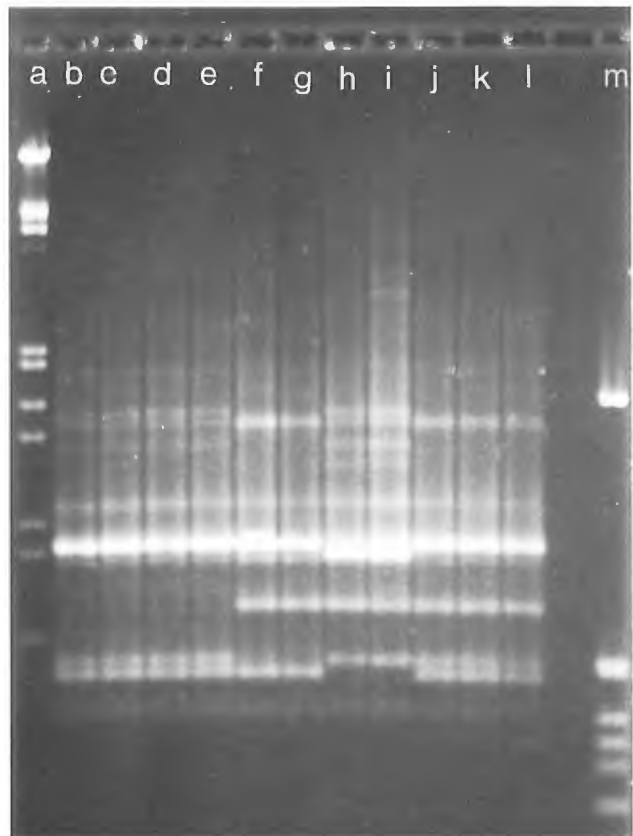


Fig. 2: Ethidiumbromide-stained 1.5% agarose gel showing resolved amplification products obtained with primer F04 (Operon Technologies) from some cultivars of the Burgundy group: a) λ DNA cut with *HindIII* and *EcoRI* as size standard; b and c) Aligoté; d and e) Aligoté vert; f and g) Auxerrois; h and i) Affenthaler; j and k) Chardonnay blanc; l) Chardonnay musqué; m) pBR322 cut with *HinfI* as size marker. Duplicate assays were prepared independently from two individual plants.

Elbling white appear rather closely related, in agreement with their morphological similarity (GALET 1990). Gouais blanc is clearly distinguishable from Elbling white, a result in contrast to GOETHE (1887). The other representatives of the Riesling-type vines do not seem to be closely related to each other. The progenitor of a cross Pinot noir x Chambourcin ("Spezial") is well separated from the Riesling-type vines and surely not closely related to Riesling.

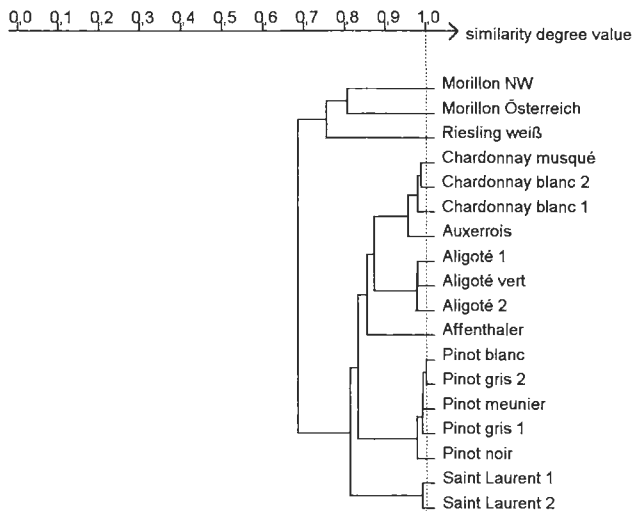


Fig. 3: Dendrogram of the Burgundy group. Calculation of similarity degrees and cluster analysis was performed as described in Fig. 1.

Quite in contrast to the Riesling-type cultivars, the Burgundy group is more homogeneous (Figs. 2 and 3). It was not possible to differentiate between the Pinot (Burgundy) varieties (Pinot noir, blanc, meunier, gris) themselves, due to their close relationship. These Burgundies probably differ only in single base changes. Thus it seems very difficult, however not impossible to find an appropriate primer able to discriminate them. Neither Chardonnay blanc could

be differentiated from Chardonnay musqué, nor Aligoté from Aligoté vert for similar reasons (genetic alteration by point mutations only). Auxerrois appeared closely related to Chardonnay, but was clearly different. Chardonnay could well be differentiated from the Pinots as well as Saint Laurent, Auxerrois and Morillon. This indicates, that these cultivars are not as closely related as the Pinots themselves. This result contrasts GALET (1990), MÜLLER (1930) and HILLEBRANDT *et al.* (1993). Especially Morillon exhibited significant differences in RAPD patterns to the other Burgundy cultivars. The similarity of Morillon and Riesling seems to be higher. Morillon therefore is considered not closely related to the Pinot cultivars. In addition, Morillon plants from different locations (Neustadt/Weinstraße, Germany and Klosterneuburg/Austria) exhibited different RAPD patterns. The name Morillon therefore refers to different cultivars. Affenthaler appeared to be similar to the Pinot cultivars, although it has been considered not related to the Pinots (HILLEBRAND *et al.* 1993).

- BÜSCHER, N.; ZYPRIAN, E.; BLAICH, R.; 1993: Identification of grapevine cultivars by DNA analyses: Pitfalls of random amplified polymorphic DNA techniques. *Vitis* **32**, 187-188.
- ; BACHMANN, O.; BLAICH, R.; 1994: On the origin of the grapevine variety Müller-Thurgau as investigated by the inheritance of random amplified polymorphic DNA (RAPD). *Vitis* **33**, 15-17.
- GALET, P.; 1990: *Cépages et Vignobles de France. Tome II: L'Ampélographie Française, 2ème Edition.* Imprimerie Charles Déhan, Montpellier.
- GOETHE, H.; 1887: *Handbuch der Ampelographie, 2. Aufl.* Verlag Paul Parey, Berlin.
- HILLEBRAND, W.; LOTT, H.; PFAFF, F.; 1993: *Taschenbuch der Rebsorten, 10. Aufl.* Fachverlag Dr. Fraund GmbH, Mainz.
- MÜLLER, K. (Ed.); 1930: *Weinbau Lexikon.* Verlagsbuchhandlung Paul Parey, Berlin.
- NÖBAUER, W.; TIMISCHL, W.; 1979: *Mathematische Modelle in der Biologie.* Vieweg Verlag, Braunschweig, Wiesbaden.
- THOMAS, M. R.; MATSUMOTO, S.; CAIN, P.; SCOTT, N. S.; 1993: Repetitive DNA of grapevine: classes present and sequences suitable for cultivar identification. *Theor. Appl. Genet.* **86**, 173-180.