Vitis 32, 187–188 (1993)

Research Note

Identification of grapevine cultivars by DNA analyses: Pitfalls of random amplified polymorphic DNA techniques using 10mer primers

NICOLE BÜSCHER, EVA ZYPRIAN and R. BLAICH

RAPD (random amplified polymorphic DNA) analysis is thought to be the method of choice for the identification of species and cultivars from crude extracts without previous knowledge of DNA sequences in the genome. Several PCR (polymerase chain reactions) are done with various primers until the electrophoretic separation of the reaction products yields suitable patterns which are not too complicated but still show enough variability to differentiate species or cultivars (for literature see CAETANO-ANNOLÉS *et al.* 1992).

However, even authors who claim a good reproducibility for their pattern admit that some interpretation work may be necessary which usually involves deliberate omission of "weak" or variable bands. At present PCR patterns show the same problems as isozyme patterns regarding the comparability of results of different laboratories. Systematic tests on some factors influencing the RAPD patterns in wheat and *Brassica* have been carried out by DEVOS and GALE (1992) and BOURY *et al.* (1992). On the way towards a standardized method for grapevines some experiences in our laboratory may be useful to colleagues planning to do similar work.

Materials and methods: *Grapevine DNA* was prepared from young leaves according to THOMAS *et al.* (1993); the DNA should contain no amplification inhibitors although Taq-DNA-polymerase is insensitive to many contaminations (ROSSEN *et al.* 1992). Primers: DNA dekamers were purchased from Operon Technologies, Alameda CA 94501, USA. *Standard samples* (50 µl) contained 10 mM Tris-HCl (pH 8.3); 50 mM KCl; 1.5 mM $MgCl_2$; 1 % gelatine (may be omitted); 100 µM dATP; 100 µM dCTP; 100 µM dGTP; 100 µM TTP; 30 ng primer; 20 ng genomic DNA; 1 U Taq-Polymerase; the surface is covered with oil (35 µl) which can be removed after the reaction by the same volume of chloroform/isoamyl alcohol (24/1) or with AmpliWax™ PCR Gem 100 (Perkin Elmer). *Amplification conditions*: 45 cycles 2 min at 94 °C (denaturation, 5 min for the first cycle), 2 min at 36 °C (primer annealing), 3 min at 72 °C (polymerisation). *Separation of amplification products*: Electrophoresis on 1.2-2 % agarose gel in TBE buffer (89 mM Tris, 89 mM borate, 2 mM EDTA) and staining with 1 µg/ml ethidium bromide.

Results and discussion: *Variation of DNA and primer concentration:* Lowering of the DNA concentration or rising of the primer concentration led to a better amplification of smaller products (Fig. A, solid arrow), longer DNA sections are diminished or diseappear (Fig. A, empty arrow). This effect may be explained by the different availability of primers for the binding sites on the DNA since the length of an amplification product is determined by the distance of two primers bound in opposite directions. If not all binding sites are occupied (due to a low primer concentration) the average distance is greater and hence the relative amount of long amplification products is higher.

Differences between Taq DNA polymerases: As a rule all Taq DNA polymerases are suitable for the RAPD technique. The results, however, may be different. In our example (Fig. B) the overall activity of polymerase a is lower than that of the polymerases b and c, where b prefers smaller amplification products and c amplifies even two additional bands at 940 and 1300 bp.

Differences between thermocyclers: Five different thermocyclers have been tested with identical samples in different holes. A typical (not the worst!) result is shown in Fig. C. Big

Bundesanstalt für Züchtungsforschung and Kulturpflanzen, Institut für Rebenzüchtung Geilweilerhof D-76833 Siebeldingen, Germany

differences were observed mainly in metal block thermocyclers even using well-fitting and thin walled reaction tubes and thermoconducting fluids. The temperature within the block may not always be absolutely homogenous and usually the block temperature is measured rather than the reaction temperature of the probe.

Phantom bands: Very often amplifications of DNA sequences occur in control mixtures without template DNA (Fig. D, k). This has already been mentioned by BOURY *et al.* (1992) and should be kept in mind although it seems to be no major problem since these phantom bands are suppressed if template DNA is present - even if no amplification occurs (Fig. D, c).

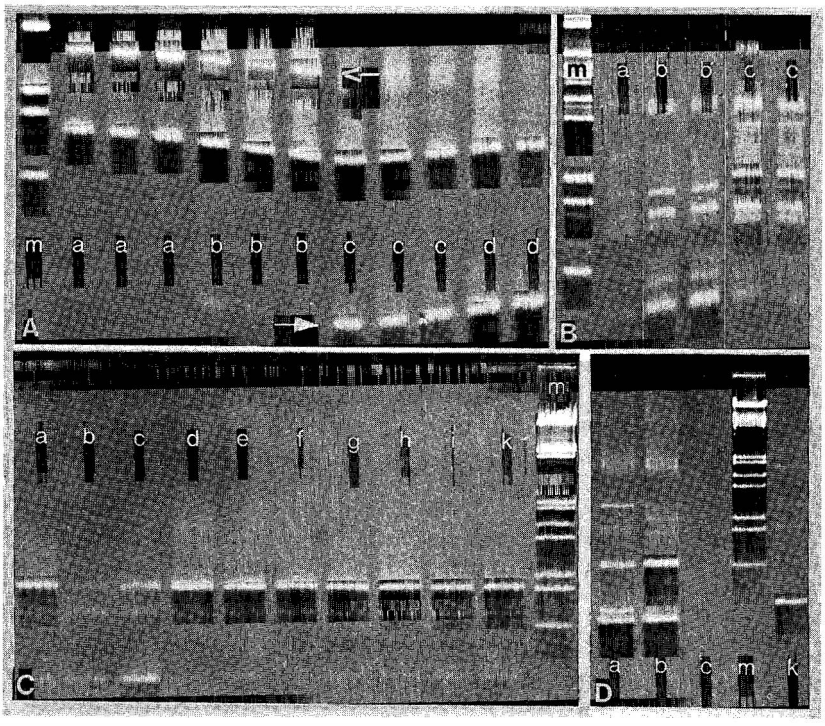

Figure: Electrophoretic separation of RAPD amplification products on agarose (1.5 %). Marker (m): lambda DNA cut with EcoRI and HindIII. **A:** 15 ng (a), 30 ng (b), 45 ng (c) and 60 ng of primer GAGACGCACA (OP-N06) with constant amount of DNA (20 ng) of cv. Bacchus. **B:** 30 ng primer GACCGACCCA (OP-N04) and 20 ng DNA of cv. Bacchus with 3 different Taq-polymerases (a, b, c). **C:** Ten identical samples (a-k) of Bacchus DNA amplified in different holes of a thermocycler (primer OP-F04 = GGTGATCAGG). **D:** (k) Standard sample without DNA, showing phantom bands, (c) standard sample with non-reactive DNA: no phantom band, (a, b) standard patterns of two fungus resistant grapevine cultivars Gf.Ga-48-12 and Gf.Ga-52-42 (primer OP-F06 = GGGAATTGGG).

BOURY, S.; LUTZ, I.; GAVALDA, M.-C.; SCHLESSNER, A.; 1992: Empreintes génétiques du chou-fleur par RAPD et vérification de la pureté hybride F_1 d'un lot de semences. Agronomie **12**, 669-681.

CAETANO-ANOLLÉS, G.; BASSAM, B. J.; GRESSHOFF, P. M.; 1992: DNA amplification fingerprinting with very short primers. In: Joint Plant Breed. Symp. Ser.; Proc. Symp. Appl. RAPD Technol. Plant Breed., 18-24.

DEVOS, K. M.; GALE, M. D.; 1992: The use of random amplified polymorphic DNA markers in wheat. Theoret. Appl. Genet. **84**, 567-572.

ROSSEN, L.; NORSKØV, P.; HOLMSTRØM, K.; RASMUSSEN, O. F.; 1992: Inhibition of PCR by components of food samples, microbial diagnostic assays and DNA-extraction solutions. Intern. J. Food Microbiol. **17**, 37-45.

THOMAS, M. R.; MATSUMOTO, S.; CAIN, P.; SCOTT, N. S.; 1993: Repetitive DNA of grapevine: classes present and sequences suitable for cultivar identification. Theoret. Appl. Genet. **86**, 173-180.