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Research Note

Extraction and purification of DNA from grapevine leaves

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Introduction: Grapevine has been subjected to extensive genetic studies, *e.g.* DNA fingerprinting, gene mapping and genetic transformation.

The extraction of plant nucleic acids is a fundamental step for genome characterization and mapping techniques involving the use of molecular markers and for the identification and isolation of plant genes for genetic engineering.

Many simple, fast and high yielding procedures for the isolation of DNA from plant tissues including grapevine have been described (Doyle and Doyle 1990; Harding and Roubelakis-Angelakis 1994; Lodhi *et al*. 1994; Steenkamp *et al*. 1994). Some of them give good results for several types of analysis with molecular markers, *i.e.* RAPDs, RFLP (Bourquin *et al*. 1991) or SSRs, also known as microsatellites (Thomas *et al*. 1993). Meanwhile the latter are broadly used in grapevine genetic research to identify different cultivars and, as codominant markers, to analyse parentage (Meredith *et al*. 1997) and genetic similarities (Labra *et al*. 1999).

Vos *et al*. (1995) introduced a new tool in molecular analysis: Amplified Fragment Length Polymorphism (AFLP), which is widely used for genetic mapping, and for the analysis of genetic relationships among different samples (Labra *et al*. 1999) and the variability inside single cultivars. Contemporaneous use of both, AFLP and SSR markers can provide an improved insight in these matters. However, while microsatellite analysis is based on the use of specific primers and thus requires only a DNA suitable for PCR reactions, the production of AFLP markers is a much more complex process (digestion with specific restriction enzymes, ligation to specific adapters and two subsequent amplification reactions) and requires high quality DNA (*i.e.* high molecular weight molecules and absence of contaminating compounds) which is very difficult to obtain in grapevine, due to the presence of significant amounts of polysaccharides and/or polyphenolic compounds (Steenkamp *et al*. 1994). We developed a simple method to remove the majority of contaminants from grapevine leaf extracts.

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Material and Methods: - Extract DNA as in Doyle and Doyle (1990) up to the first resuspension in TE [10 mM Tris-Cl (pH 8.0), 1 mM EDTA (pH 8.0)] with the following modifications:

- Grind 0.5 g of fresh leaf tissue to a powder in liquid nitrogen and scrape it into 5 ml of preheated extraction buffer [2 % (w/v) CTAB (cetyltrimethylammonium bromide, Sigma, St. Louis, USA), 1.4 M NaCl, 0.1 % (v/v) β-mercaptoethanol, 20 mM EDTA (ethylenedinitrilo tetracetic disodium salt dihydrate), 1 % (w/v) polyvinylpyrrolidone, 100 mM Tris-HCl (pH 8.0)]

- After resuspension of the pellet in 1 ml TE [10 mM Tris-HCl (pH 8), 1 mM EDTA], transfer the sample to a clean 2 ml Eppendorf tube.

- Add α-amylase (Sigma) (150 $\mu g \cdot ml^{-1}$) and RNAase A (Sigma) (200 $\mu g \cdot ml^{-1}$) and incubate the samples at 50 °C for 30 min.

- Add 2 % (w/v) sodium dodecyl sarcosinate and 1 $mg \cdot ml^{-1}$ nuclease-free proteinase K (Boehringer Mannheim, Germany) and incubate overnight at 50 °C.

- Precipitate DNA with cold isopropanol (2/3 volumes), centrifuge at 7,500 rpm in a microfuge, rinse the pellets with 1 ml of wash buffer. Dry the samples and resuspend them in 1 ml TE.

- Add 100 µl 5 M NaCl and 100 µl 96 % EtOH, both of them drop by drop. Mix gently and place at -20 °C for 30 min; centrifuge in a microfuge at 6,000 rpm for 10 min. Transfer supernates to clean Eppendorf tubes.

- Repeat cold isopropanol precipitation and finally resuspend in 400 µl TE.

Results and Discussion: The yield for 0.5 g of young leaves was 30 µg of high quality DNA (Figure, A). DNA purified by the described method was used for microsatellite analysis with optimal results (data not shown). The purity of the samples was also evaluated by AFLP analysis (Labra *et al*. 1999). In Figure, B we compare three samples extracted from the cv. Marzemino with and without the purification steps. It can be noted that the samples purified with our procedure show an identical pattern, whereas in the lanes with non-purified samples some missing bands can be misleading, giving the impression of a polymorphic pattern. The phenomenon can be explained by a partial inactivation of restriction enzymes due to the presence of residual polysaccharides, which are almost completely eliminated by the method reported.

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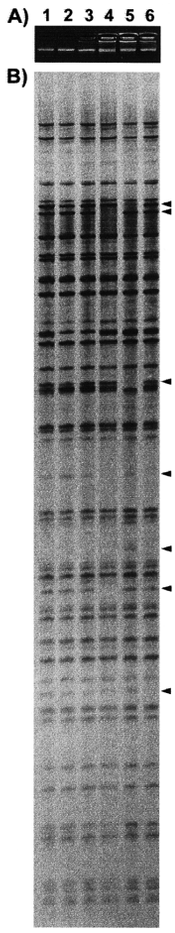

Figure: Comparison between different samples of genomic *Vitis* DNA (cv. Marzemino) with and without purification steps; lanes 1-3, samples processed by purification steps. Lanes 4-6, samples extracted by the standard procedure. **A**) Migration of uncut genomic DNA on a 0.7 % agarose gel, stained with ethidium bromide. **B**) AFLP analysis of the same samples. Arrows indicate the location of false polymorphisms.

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