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**Research Note**

# The isolation and purification of DNA from *Vitis vinifera* L. plants and *in vitro* cultures

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K e y w o r d s : DNA, isolation, tissue culture, Vitis vinifera.

Grapevine is a woody perennial, yet despite its economic importance, the molecular biology of *Vitis* is not well developed for its biotechnological improvement (MULLINS 1990). Although molecular studies have been reported (THOMAS *et al.* 1993), this study compares several procedures for the isolation of DNA from glasshouse grown plants, and *in vitro* plantlets and callus cultures.

DNA was isolated from *V. vinifera* L. cv. Sultanina (Thompson Seedless). The *in vitro* plantlets were established (ROUBELAKIS-ANGELAKIS and ZIVANOVITIC 1991) and callus cultures were induced as described (KATSIRDAKIS and ROUBELAKIS-ANGELAKIS 1991).

The isolation of DNA from glasshouse grown grapevine plants was found to be problematic, and initial attempts at extracting total DNA using conventional procedures were unsuccessful. DNA preparations often became dark brown, presumably due to polyphenol oxidase activity from disrupted cell walls, vacuoles and organelles. Unknown compounds co-precipitated with the DNA to produce a viscous partially soluble product which reduced the yield of DNA; this complex strongly absorbed UV light in the 230-280 nm region resulting in misleading estimates of DNA. The isolated DNA complex could not be separated in a caesium chloride-ethidium bromide density gradient; the aggregated material inhibited restriction enzymes and prevented analysis by agarose gel electrophoresis. In view of these problems, a systematic study of DNA extraction procedures was performed.

The use of cetyltrimethyl ammonium bromide (CTAB, see method 1, Table) to extract total DNA from grapevine was performed according to the method described by ROGERS and BENDICH (1988). The initial yields of DNA from glasshouse plants were low due to an inability to precipitate DNA. This effect was attributed to *V. vinifera* leaf material having a relatively high mineral content and other unknown compounds (unpublished results), which prevented the precipitation of the CTAB-DNA complex. This problem was solved by increasing the volume of precipitation buffer 3–4 fold, however the isolated DNA invariably turned black and inhibited restriction enzyme digestion. The use of phenol (method 2) to extract DNA from homogenised tissues followed by ethanol precipitation as described by FLAVELL *et al.* (1988) produced a dark brown gelatinous complex compared to the modified CTAB procedure (method 3) reported by DOYLE and DOYLE (1990) and SDS-KAc precipitation procedure (method 4) by DELLAPORTA *et al.* (1983), which produced viscous DNA preparations without the formation of coloured complexes. The separation of nuclei (method 5) from the cytoplasmic components was essential for the isolation of DNA from glasshouse grown plants. DNA was successfully prepared from isolated nuclei after ultracentrifigation yielding 4.5 µg/g f.wt. of nuclear DNA. This DNA was of high molecular weight (30–50 kbp) and sufficient purity for restriction enzyme digestion (Figure) and molecular analysis by southern blotting. This procedure has been routinely used for the isolation of DNA from *V. vinifera* cv. Cabernet François No. 1, and a number of red and white Greek grapevines.

T a b l e

A comparison of *Vitis vinifera* L. DNA yields (µg/g f. wt.) derived from glasshouse plants, *in vitro* plantlets and callus cultures using different extraction procedures

| Extraction procedures | Glasshouse plants | In vitro plantlets | Callus cultures |
|---|---|---|---|
| CTAB | 18.5 | 40.8 | 9.3 |
| Phenol | 6.5 | 29.7 | 13.9 |
| Mod.CTAB | 9.7 | 7.1 | 7.2 |
| SDS-kAc | 6.9 | 26.0 | 4.5 |
| Nuclear | 4.5 | - | - |
| Average | 9.2 | 25.9 | 8.7 |

P r e p a r a t i o n o f n u c l e a r D N A from glasshouse plants: Prior to DNA extraction, 5 g of green healthy leaves were selected and washed in tap water, dis-


Figure: An ethidium promide stained agarose gel containing *Vitis vinifera* nuclear DNA isolated from glasshouse grown plants and purified by Caesium chloride ultra-centrifugation. Track 1 represents λ DNA digested with EcoRI/HindIII as a molecular weight marker; Track 2: intact nuclear DNA; Track 3: nuclear DNA digested with EcoRV.

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tilled water, 20 mM potassium chloride, and finally in 5 mM ethylene diamine tetra acetic acid (EDTA). The leaves were dried, and added to 50-100 ml of cold homogenisation (0.1 M sodium citrate, 0.35 M glucose, 5 mM EDTA, 0.1 M diethyl dithiocarbamic acid, 2 % polyvinyl pyrrolidone (PVP, M.wt. 40,000), and 1.0 % bovine serum albumin (BSA) adjusted to pH 6.0) buffer and homogenised in a Waring blender at high speed for 1 min. The homogenate was filtered through several layers of cheese cloth, collected in 30 ml Corex tubes and centrifuged (Sorvall SS34 rotor) at 1000 x *g* for 10 min at 4 °C. The pellet was re-suspended in 5-10 ml of lysis medium (1 g of ceasium chloride dissolved in 1 ml of 2 % (w/v) sodium lauryl sarcosin) and incubated at 4 °C for 10 min. The lysate was centrifuged at 14,000 rpm for 20 min (Sorvall SS34 rotor), and the supernatant transferred to a tube containing 500 µl of ethidium bromide (10 mg/ml). The density was adjusted to 1.58-1.60 g/ml and the resulting precipitate was removed by centrifuging the lysate at 14,000 rpm for 20 min. The gradient mixture was centrifuged in a vertical rotor (Vti 65) at 40,000 rpm for 12-16 h. The DNA was removed by the method of side puncture, and ethidium bromide extracted with isopropanol and the DNA was dialysed against TE buffer (10 mM Tris HCl, pH 8.0 and 1mM EDTA) as described by SAMBROOK *et al.* 1989).

The isolation of DNA from *in vitro* cultures was less problematic compared to glasshouse plants possibly reflecting a reduction in the levels of natural products. It was necessary to purify these preparations before use (HARDING and BENSON 1994). DNA isolated by the CTAB and phenol procedures resulted in the formation of coloured complexes and the same problems described for glasshouse plants. A comparison of DNA yields derived from glasshouse plants, *in vitro* plantlets and callus cultures using different extraction procedures is presented in the Table. The ability to extract DNA was variable in the glasshouse grown plants and *in vitro* cultures. The yields are expressed as µg/g f.wt. and the values represent the average of several experiments, these are comparable to those values reported by THOMAS *et al.* (1993). Average yields indicated 25.9 µg of DNA was isolated from *in vitro* grown plantlets. The modified CTAB procedure extracted 7.1 µg of DNA compared to the other procedures within this group; this value was attributed to variation between batches of plantlets. The lower yield of DNA isolated from callus reflects the relatively high water content of callus cultures.

There are likely to be many factors, which influence the yield of DNA from grapevine. Nonetheless, this study examines the variation in DNA yields between different sources of material and provides information to assist the further development of *Vitis* molecular biology and biotechnology.

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