Optimised DNA isolation from *Acacia karroo* (Fabaceae)

Sandra du Plessis*, M.H. Buys[1] and Marna Nel

*Division of Botany, School for Environmental Sciences and Development and [1]A P. Goossens Herbarium, Potchefstroom University for CHE, Private Bag X6001, Potchefstroom, 2520 Republic of South Africa

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The isolation of high quality DNA from Acacia karroo Hayne is impeded by the release of secondary metabolites. The isolation method of Bousquet *et al.* (1990) was modified to include numerous CTAB extractions coupled with an increased buffer to tissue ratio and the addition of 5 M NaCl, yielding high quality DNA.

Keywords: *Acacia karroo*, DNA isolation, secondary metabolites.

*To whom correspondence should be addressed.
(E-mail: plbsdp@puknet.puk.ac.za)

Acacia karroo Hayne is a widespread polymorphic species with about six recognised forms that occur in different localities in South Africa (Swartz 1982). To fully exploit the natural products produced by this species, a sound systematic knowledge is required. It is, therefore, necessary to clearly distinguish between the different forms of *A. karroo*. Since no intraspecific categories can be confidently identified based on morphological characteristics, DNA data will be utilised to establish phylogenetic relationships within the *A. karroo* species complex. However, the isolation of high quality DNA from plants is impeded by the release of secondary metabolites, such as polyphenolics, polysaccharides, terpenoids and tannins, during cell lysis. These brown coloured and sticky substances irreversibly adhere to DNA, often inhibiting digestion and/or PCR amplification.

To this end, a study was undertaken to optimise DNA isolation techniques for the successful extraction of high quality DNA from different *Acacia* Mill. species for use during DNA fingerprinting, utilising the AFLP technique.

Three methods of plant DNA isolation were investigated to isolate high quality DNA from *Acacia* leaf tissue. The quality of genomic DNA, isolated from freeze dried material, was evaluated by determination of the 260:280 nm absorbance ratio as well as by gel electrophoresis of undigested and digested DNA.

The most commonly used method for isolation of plant DNA removes proteins and polysaccharides by complexing it with Sodiumdodecyl Sulphate (SDS) and potassium acetate to form an insoluble precipitate (Dellaporta *et al.* 1983). Pich and Schubert (1993) advocate the addition of Polyvinylpolypyrrolidone (PVP) to the extraction mix to remove polyphenolic compounds, the latter causing a brown matrix to form around the DNA. Both these methods did not, however, alleviate the problem of high viscosity and low yield caused by the polysaccharides present.

Table 1 Comparison of the results obtained from three DNA isolation methods used in *Acacia karroo*

	Dellaporta *et al.* (1983)	Pich & Schubert (1993)	Modified Bousquet *et al.* (1990)
Solubility	difficult	difficult	easy
Colour	brown	brown	clear
Digestion	no	no	yes
A260/280	1.3–1.4	1.6	1.8
Yield	1.3 µg/gdw	6.7 µg/gdw	112.5 µg/gdw

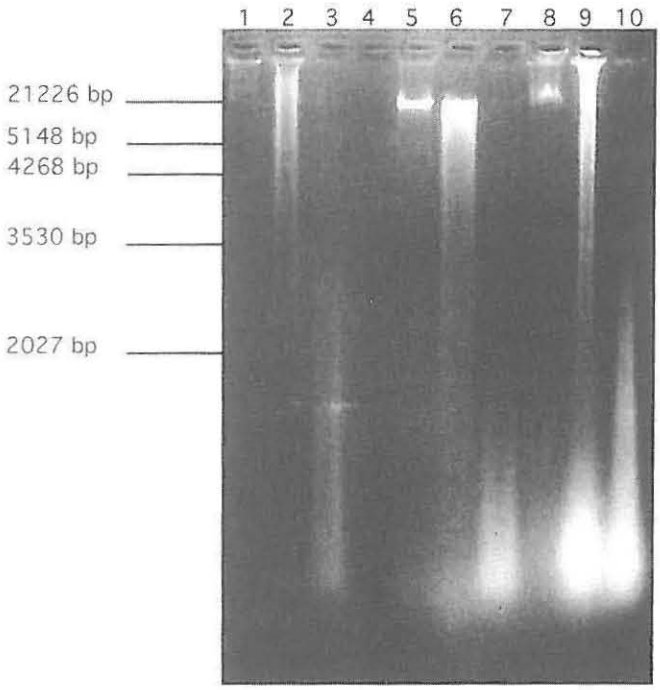

Figure 1 Gel electrophoresis of total genomic DNA of *A. karroo*. Five micrograms (quantified spectophotometrically) of DNA was loaded in each lane. Lanes 1–3 isolated according to the Dellaporta *et al.* (1983) method; Lanes 5–7 isolated according to the modified Bousquet *et al.* (1990) method; Lanes 8–10 according to the Pich and Schubert (1993) method. The DNA was digested with Eco RI (lanes 2, 6, 9) and Mse I (lanes 3, 7, 10) respectively.

The polisaccharides also influenced the quantification of the samples. Figure 1 indicates that lanes 1–3 carry less DNA than lanes 5–7, although spectophotometric quantification indicated otherwise. The method of Bousquet *et al.* (1990) also did not provide satisfactory results. However, the further introduction of several Hexadecyl Trimethyl Amonium Bromide (CTAB) extractions and an increased buffer to tissue ratio (4 ml:100 mg) improved the quality and yield of DNA. The quality of the DNA was further improved with the addition of 5 M NaCl during precipitation, as suggested by Jobes *et al.* (1995). Polysaccharides are more soluble in ethanol in the presence of a high concentration of NaCl. This effectively decreases the co-precipitation of polysaccharides with the DNA. Our procedure yielded high quality DNA which was digested successfully with Eco RI and Mse I (Figure 1). A comparison of the isolation methods by Dellaporta *et al.* (1983), Pich and Schubert (1993) and our modified Bousquet *et al.* method is presented in Table 1.

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