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A DNA Extraction Protocol from Various Tissues in Woody Species

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Abstract. **A DNA extraction protocol was developed for tissues from woody species. DNA was extracted successfully from 11 species and five different types of tissues and was suitable for RAPD and restriction analysis. Spermine precipitation was used to further purify DNA. The protocol can be used for large-scale analysis and mini-preparations.**

Most protocols for DNA extraction from woody species require young leaf samples to obtain high-quality DNA for RAPD and RFLP analysis (Aitchitt et al., 1993; Collins et al., 1992; Howland et al., 1991; Li et al., 1994). For some studies, leaf samples must be collected from remote areas and are difficult to transport long distances. In other cases, identification of fruit scion and rootstock cultivars using molecular markers must be conducted in the winter, when leaf samples are not available. A protocol is needed that permits year-round extraction of DNA from woody species.

Woody species contain phenolics and polysaccharides that contaminate DNA preparations and interfere with subsequent analyses (Couch and Fritz, 1991; Luro and Laigret, 1995; Ziegenhagen et al., 1993). When plants are dormant, a high level of secondary substances may accumulate. We developed a mini-prep protocol, modified from that of Webb and Knapp (1990), for DNA extraction from various tissues. The major modifications include 1) increasing concentrations of 2-mercaptoethanol and soluble polyvinylpyrrolidone (PVP) in the extraction buffer to suppress polyphenol oxidation; 2) increasing hexadecyltrimethylammonium bromide (CTAB) and sodium chloride concentrations in the extraction buffer to reduce polysaccharide contamination in the early steps; and 3) using a low concentration of spermine to selectively precipitate and purify DNA in the final step.

Materials and Methods

Plant material. Plant samples tested include apple (*Malus ×domestica* Borkh.) bark (including cambial tissue), buds, and leaves, Austrian pine (*Pinus nigra* Arnold.) bark, barberry (*Berberis thunbergii* DC.) bark, buttonwood (*Platanus occidentalis* L. Eastern S.) bark, cherry [*Prunus avium* (L.) L.] bark, grape (*Vitis vinifera* L.) xylem, hazelnut (*Corylus avellana* L.) dormant buds and catkins, paper birch (*Betula papyrifera* Marsh.) bark, peach [*Prunus persica* (L.) Batsch] bark and buds, pear (*Pyrus communis* L.) bark, and white fir [*Abies concolor* (Gord.) Lindl.] bark. One- or 2-year-old shoots, dormant buds, and catkins were collected and carried to the laboratory. The samples were stored in a plastic bag at 4 °C for 1 week.

Solutions and buffers. The extraction buffer contained 2% CTAB, 1.5 M NaCl, 20 mM EDTA, 100 mM Tris, pH 8.0, 2% soluble polyvinylpyrrolidone (PVP-40T; Sigma, St. Louis), and 2% 2-mercaptoethanol. Spermine tetrahydrochloride (Sigma) was dissolved in water to make a 0.1-M spermine solution. Spermine pellet extraction buffer (10×, pH 8.0) was made with 3 M sodium acetate and 0.1 M magnesium acetate.

Extraction protocol. About 0.05 to 0.1 g of bark, buds, xylem, or catkins was ground in liquid nitrogen using a mortar and pestle. A 1-mL aliquot of extraction buffer was added and the homogenate was transferred to an Eppendorf microcentrifuge tube (1.5 mL). The tube was incubated for 30 min at 65 °C. Chloroform/octanol (24:1) was added to almost fill the tube and the tube was shaken vigorously to form a complete emulsion. The tube was centrifuged at $8000g_n$ for 5 min to separate phases and then the aqueous phase was transferred to a new 1.5-mL tube. The sample was mixed with 0.8 to 1.0 mL of cold 95% ethanol and set at –20 °C for 5 min. The tube was centrifuged at $8000g_n$ for 5 min. The supernatant was poured off and the tube was drained on a paper towel. About 600 µL of 1 M NaCl solution was added to the pellet and the tube was set at 65 °C for 10 min to dissolve DNA. The sample was gently mixed with 300 µL buffer-saturated phenol and centrifuged at $8000g_n$ for 3 min. The supernatant was transferred to a 1.5-mL tube and mixed with 500 µL chloroform. The tube was centrifuged at $8000g_n$ for 5 min and the supernatant was transferred to another 1.5-mL tube. The sample was mixed with 1.0 mL cold 95% ethanol and set at –20 °C for 30 min. The tube was centrifuged at $8000g_n$ for 10 min and drained. The pellet was dissolved in 600 µL water, mixed with 6 µL of 0.1 M spermine, and incubated on ice for 20 min. The tube was centrifuged for 10 min at $8000g_n$ and drained. In the presence of 75% ethanol, 1 x of spermine pellet extraction buffer was added to the pellet and the tube was incubated on ice for 1 h. The tube was drained and washed with 75% ethanol. The pellet was dried by air or vacuum and dissolved in Tris-EDTA buffer or water.

Results and Discussion

High-quality DNA was extracted from diverse tissues of woody species. DNA quality was measured by RAPD analysis and restriction digest, and A_{260} : A_{280} ratio quantified on a spectrophotometer. Banding patterns from RAPD analysis of various species and tissues were clear and the bands were sharp (Fig. 1). The genomic DNA was totally digested with several restriction enzymes, such as Eco RI and Hind III. The A_{260} : A_{280} ratio of DNA ranged from 1.8 to 2.0, indicating minimal protein contamination. Average yields ranged from 10 to 35 $\mu g \cdot g^{-1}$ of fresh tissue. These results demonstrated that the protocol we developed is suitable for extraction of high-quality DNA from diverse woody species and tissues for RAPD and restriction analysis. Twenty primers (OPK1 to 20; Operon Tech, Alameda, Calif.) were tested and no major difference was found in banding patterns for the RAPD products amplified from DNA of various tissues in apple, hazelnut, and peach (Fig. 1). Therefore, tissues such as bark and dormant buds could be used to extract DNA when leaf samples are not available. This protocol was also suitable for use in large-scale DNA extraction. Some components, such as the PVP and 2-mercaptoethanol in the DNA extraction buffer, are critical to suppress oxidation of phenolic compounds in the initial step. We found that concentrations of these compounds should be >1% in the solution to suppress bark phenolics in most species stud-

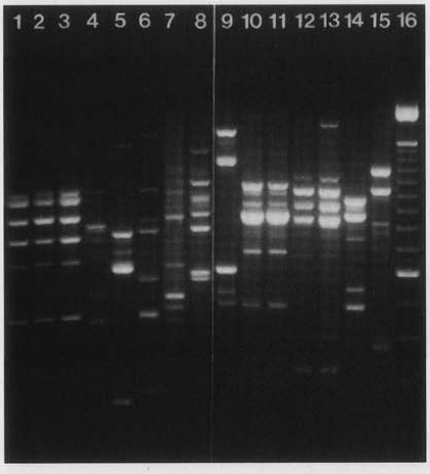

Fig. 1. Separation of amplification products generated by primer OPK8 (5'GAACACTGGG3') in various species and tissues. Sample order: 1) apple leaves, 2) apple bark, 3) apple buds, 4) pear bark, 5) Austrian pine bark, 6) buttonwood bark, 7) barberry bark, 8) white fir bark, 9) grape xylem, 10) peach buds, 11) peach bark, 12) hazelnut buds, 13) hazelnut catkins, 14) cherry bark, 15) paper birch bark, 16) DNA ladder.

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ied. In the last step, spermine, used to selectively precipitate nucleic acids, was added to further purify the DNA. Only low spermine concentrations should be used to get dissolvable pellets (Hoopes and McClure, 1981). Our restriction analysis results demonstrated that the addition of a spermine step was necessary to obtain DNA of sufficient quality for restriction digests (data not shown).

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