Commentary

An Improved Method for the Isolation of Total RNA from Spruce Tissues

Key Words: mRNA purification, Picea abies, spruce tissues

Abstract: After many unsuccessful attempts to obtain biologically active mRNAs from spruce (Picea abies) tissues using available protocols, we have adapted a procedure for the isolation of RNAs from needles, shoots, and callus of Picea species. Our modifications permit the recovery of an average of 300 µg RNA per g of needles that is suitable for translation in vitro, northern hybridizations, and the construction of cDNA libraries.

Despite the availability of numerous protocols devoted to improved nucleic acid extraction methods, many plants remain recalcitrant and among them especially conifers. The main problem encountered in the isolation of active mRNAs, which are needed inter alia for the construction of cDNA libraries, is the presence in conifers of water-soluble products, such as polysaccharides, and large amounts of polyphenols. These products typically bind to RNAs during extraction with the formation of either a viscous, insoluble jelly leading to large losses of nucleic acids, or of brownish adducts completely devoid of biological activity.

RNAs are usually isolated by using detergents, such as sodium dodecyl sulfate, and organic denaturing agents, such as phenol or guanidinium isothiocyanate, which inhibit ribonucleases and remove most non-nucleic acid components (Chirwig et al., 1979; Ragueh et al., 1989). These methods are very efficient for RNAs extraction from many plant species, but when used for RNA extractions from needles, shoots or calli of spruce we were unable to obtain any biologically active material. Methods have been described to overcome these problems and to obtain intact RNAs that retain biological activity from conifers; these technics have been successfully used to extract nucleic acids from various species of Pinus species (Baker et al., 1990; Graham, 1993). Although these methods are efficient for the isolation of RNAs from Pinus, they yielded very low yields of biologically inactive material in our hands when applied to spruce (Picea species).
The method reported here is based on an observation made by Guillemaut and Maréchal-Drouard (Guillemaut and Maréchal-Drouard, 1992), who employed an extraction medium of low pH (5.5) to avoid ionization and the subsequent oxidation of phenolics compounds and to precipitate large amounts of non-nucleic acids.

**Materials and Methods**

*Extraction medium*: 100 mM sodium acetate, 50 mM EDTA pH 8.0, 500 mM sodium chloride, 2% soluble PVP (MW 10,000, Sigma). Adjust the pH to 5.5, then add SDS to 1.4%. Add 10 mM cysteine immediately before use.

*Binding buffer*: 10 mM Tris-HCl pH 7.5, 0.5 M NaCl, 1 mM EDTA, 0.5% SDS.

*Loading buffer*: 2x binding buffer.

*Washing buffer*: 10 mM Tris-HCl pH 7.5, 0.1 M NaCl, 1 mM EDTA

*Elution buffer*: 10 mM Tris-HCl pH 7.5, 1 mM EDTA

**Protocol**

**RNA isolation**

- Needles or young shoots of spruce are harvested, immediately frozen in liquid nitrogen, and used immediately or kept at -80°C.
- 1 g of frozen tissue is transferred to a mortar and ground to a fine powder in the presence of liquid nitrogen.
- Transfer the frozen powder into 10 mL of extraction buffer and homogenize carefully.
- Incubate the extract in a water bath at 65°C for 10 min with occasional shaking.
- Centrifuge for 10 min at 10,000 g at room temperature.
- To the supernatant add 1/3 volume of 5 M potassium acetate, pH 4.8. Mix gently and cool on ice for 30 min.
- Centrifuge at 10,000 g for 10 min at 4°C, collect the supernatant, and centrifuge again to obtain an extract free of particles.
- Precipitate the nucleic acids with 2 volumes of ethanol for 2 h at 0°C.
- Centrifuge at 10,000 g for 10 min at 4°C, discard the supernatant, and add to the pellet 7 mL of cold 3 M sodium acetate, pH 6. Mix by vortexing to disperse the pellet. Keep on ice for 5 min and centrifuge for 20 min at 10,000 g at 4°C.
- Repeat the washing step once.
- Redissolve the pellet in 1 mL of sterile, RNase-free water, add 100 μL 3 M sodium acetate and 2 mL ethanol, and allow RNA to precipitate at -20°C for 30 min.