An efficient method for isolation of RNA and DNA from plants containing polyphenolics

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Isolation of high quality RNA and DNA from plants especially cotton (*Gossypium hirsutum*; family *Malvaceae*) is notoriously difficult. This problem has been attributed to high content of phenolic terpenoids and tannins present in cotton cells (Katterman, and Shattuck, 1983). These compounds bind to RNA and DNA upon cell lysis and cannot be removed by conventional extraction procedures. Such RNA is not amenable to *in vitro* translation or cDNA cloning. We have encountered the same problem in many plants from family *Malvaceae* as well as from related family *Bombacaceae*. The methodology described here is a modified protocol of Chirgwin, *et al.*, (1979) to include higher buffering capacity, alkaline pH, and most importantly polyvinyl pyrrolidone, which through hydrogen bonding, complexes with polyphenolics, effectively removing them from the homogenate. The homogenate is then subjected to ultracentrifugation to isolate RNA or DNA. The isolated RNA is suitable for cDNA cloning, *in vitro* translation or polymerase chain reaction.

Plant tissues (leaf, fiber, root, stem, ovules or flowers) were quick frozen in liquid nitrogen. The frozen tissue was powdered in a mortar in liquid nitrogen and then homogenized in a buffer for 1.5 minutes using a polytron at full speed. The homogenization buffer was added at a ratio of 1:2 of tissue (weight) to buffer (volume). In some instances it was found that increasing the ratio to 1:4 enhances the yield. Homogenization buffer was: 5 M guanidine isothiocyanate, 0.2 M Tris-acetate (pH 8.5) 0.7% β-mercaptoethanol, 1% polyvinyl pyrrolidone (soluble PVP, MW 40 Kd), and 0.62% sodium lauroyl sarcosine). Both β-mercaptoethanol and PVP were added to the buffer just before homogenization. PVP should be prepared fresh as a 20% stock each time. The homogenate was filtered through Mira cloth and layered over a 1.5 ml pad of 5.7 M cesium chloride. The homogenate was then centrifuged for 18 hours at 36,000 rpm in a SW 50.1 rotor at 20°C. After centrifugation, the RNA was collected as described by Chirgwin, *et al.*, (1979) and then further purified by phenol:chloroform extractions and precipitations in the presence of ammonium acetate, as described for DNA by Crouse and Amorese (1987). Omitting the PVP in the homogenization buffer yielded RNA that could not be translated *in vitro* systems.

Genomic DNA was prepared according to Dellaporta, *et al.* (1984). Preparative DNA isolation was carried out by cesium chloride density gradient centrifugation as described in Current Protocols in Molecular Biology (1987). In both instances the homogenization buffer was modified to contain 1% PVP.

RESULTS

The RNA and DNA isolation procedures described were found to be effective for plants from family *Malvaceae, Bombacaceae* and *Moraceae*. The average yield of RNA from cotton leaf was 0.5 mg/gm of tissue. In addition the above protocol is also equally suitable for other plants (soybean, corn or tobacco). The isolated RNAs were used to prepare cDNA libraries from cotton fiber, ovule, leaf, root and pollen. Similarly genomic libraries have been constructed from cotton and kapok (*Ceiba pentandra*: family *Bombacaceae*). Figure 1 shows 3H-tyrosine labelled *in vitro* translation products from cotton fiber (lane 1) and root (lane 2) RNAs. Lanes 3, 4 and 5 are Northern blots containing RNAs from cotton flower leaf and fiber hybridized to cDNA clones identified from various cotton cDNA libraries. Lanes 6 and 7 are Southern blots containing EcoRI digested genomic DNAs of cotton and kapok hybridized to a cotton genomic clone, CKE6, and a kapok genomic clone, CPE6, respectively.

In summary the protocol described can be reliably used to isolate RNA and DNA suitable for enzymatic manipulations from plants that are recalcitrant to other methods due to the presence of polyphenolics.

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