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Update section

Description of novel methods

A method for isolation of cDNA-quality mRNA from immature seeds of a gymnosperm rich in polyphenolics

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Gymnosperms accumulate many secondary metabolites including bisflavonoids, mono- and diterpenes, etheric oils, resins, cyclitols, shikimic acid and lignins [2, 3]. These compounds make it generally difficult to prepare mRNA from gymnosperms which is suitable for cDNA synthesis. In the past few years we have successfully prepared mRNA and cDNA from many different angiosperms, yet our current efforts to construct cDNA libraries from various gymnosperm tissues reveal that methods for preparation of mRNA from angiosperms are, in many cases, not satisfactory. After several failures with methods suitable for most plants, we were finally able to prepare quite pure poly(A)$^+$ mRNA from whole seeds of *Taxus baccata* (English yew). This tissue is partially lignified and contains very high levels of phenolic compounds. The cDNA synthesis directed by this mRNA was very satisfactory. For the benefit of those working with similarly 'difficult' plant tissues, we describe here the method of mRNA isolation which we improvised through modification of a previously published procedure [6].

Whole immature seeds of *Taxus baccata* were ground in liquid nitrogen and suspended in 10 ml per g fresh weight of the recommended buffer [6]. A 0.5 g portion of insoluble polyvinylpyrrolidone (M_r 40 000) per gram fresh weight was added. The suspension was gently mixed for 2 min and 1/10 vol of 25% (v/v) Triton X-100 was added. The mixture was vigorously mixed and incubated on ice for 15 min. 0.9 vol of 3 M sodium acetate (pH 6.0) was added and the mixture incubated on ice for 15 min. The mixture was cleared by a 45 min centrifugation at $10\,000 \times g$. Nucleic acids and polysaccharides in the supernatant were precipitated for 5 min on ice with 0.8 vol of isopropanol and pelleted by a 45 min centrifugation at $10\,000 \times g$. The total RNA from this solution was then purified with Quiagen (Düsseldorf, FRG) Tips-500 according to the manufacturer's protocol [6]. RNA from 2.5 g fresh weight was applied per tip. Poly(A)$^+$ mRNA was purified from the total RNA through two rounds of oligo-dT cellulose chromatography as described [1] except that all binding, wash and elution buffers contained 0.01% (w/v) Proteinase K. The eluate from the second oligo-dT cellulose column was phenol-extracted and precipitated with ethanol. We obtained about 30 μg of poly(A)$^+$ mRNA from 10 g of immature whole *Taxus* seeds. OD_{230}:OD_{260}:OD_{280} values of roughly 1:2:1 indicated that most phenolics and polysaccharides had been removed.

The purity and intactness of the mRNA was

assayed by cDNA cloning as described [5]. We obtained 500 ng of agarose-gel size fractionated (> 500 bp) cDNA from 5 μg of *Taxus* mRNA. We screened 100000 recombinants as described [8] with a cDNA insert encoding glycolytic glyceraldehyde-3-phosphate dehydrogenase (*GapC*) from *Magnolia liliiflora* [4]. All of ten *GapC*-hybridizing *Taxus* cDNA clones picked at random had cDNA inserts > 1 kb in length. Three of these *Taxus baccata* cDNAs were subcloned into pBS plasmids (SK +, Stratagene) and their terminal sequences determined [7]. They all contained the entire *GapC*-coding region and thus were roughly full-size, indicating that the mRNA prepared by this procedure was satisfactory.

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