Conservation Genet Resour (2011) 3:409–411
DOI 10.1007/s12686-010-9367-2

TECHNICAL NOTE

Ethanol pretreatment increases DNA yields from dried tree foliage

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Received: 16 September 2010 / Accepted: 6 December 2010 / Published online: 21 December 2010

Abstract DNA isolated from silica gel dried leaves are frequently low in yield and quality due to accumulation of phenolic compounds, which interfere with the quality of the isolated DNA. In this work, we attempted to improve DNA quality of silica gel dried leaves. Hence, leaves of *Picea schrenkiana* were collected and soaked in different concentrations of ethanol (70, 80, 90, 95, 100%) for different periods of time (24, 36, 48 h). Thereafter, leaves were dried and stored for about 8 days in a cellophane bag containing silica gel. Afterwards, DNA was isolated from the leaf samples using Cetyltrimethyl-Ammoniumbromide (CTAB) protocol. The result shows that soaking *P. schrenkiana* leaves in ethanol before preserving them in silica gel improved the DNA yield. This result indicates that, soaking leaf samples in ethanol prior to silica gel desiccation can increase DNA yield. Ethanol may have acted in disrupting the foliage cell wall, deactivating DNases in the foliage, and extracting certain carbohydrates from the foliage prior to the drying process, and thus, increase the DNA yield.

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Keywords CTAB · DNA isolation · DNA yield · Foliage preservation · Silica gel

Drying plant foliage in silica gel appears to be the routine method for preserving plant tissues collected from distant locations for DNA isolation (Chase and Hills 1991; Adams et al. 1999; Weising et al. 2005). Though drying leaf tissues in silica gel seem to be convenient; there appear to be a tradeoff in that, metabolic and cellular responses of plant tissues to slow drying are similar to those during senescence (Savolainen et al. 1995). Thus, water stress in connection with wounding induces the accumulation of phenolic compounds, which may interfere severely with the quality and yield of the isolated DNA (Weising et al. 2005; Ribeiro and Lovato 2007). Apart from this, low amount of DNA in dried leaf samples could also result from active presence of DNases, which is activated by rehydration of leaf tissues stored in resealable plastic bags (Adams et al. 1999). This is usually indicated by change in the colour of silica gel crystals.

Although soaking leaf tissues in ethanol had been used to preserve leaf tissues before subsequent DNA isolation (Flournoy et al. 1996; Murray and Pitas 1996; Linke et al. 2010), previous application of this method was only applied to fresh tissues, and was viewed as an alternative to silica gel drying of leaf tissues. To our knowledge, attempt has not been made to improve DNA yield of leaf tissues dried in silica gel. Hence, the aim of the current study was to improve DNA yield of leaf tissues dried in silica gel by soaking collected leaf tissues in ethanol before drying them in silica gel. Our idea to soak leaf tissues in ethanol before drying in silica gel was based on the fact that ethanol has cell wall disruption capability (York et al. 1985; Murray and Pitas 1996; Linke et al. 2010), ability to irreversibly

deactivate DNases in leaf tissues (Adams et al. 1999; Flournoy et al. 1996) and hyrolysis of carbohydrate (especially sucrose) at room temperature (Streeter and Strimbu 1998). Hence, the test procedure is as thus: old and new leaves of *Picea schrenkiana* Fisch. & Mey. were collected and soaked in different concentrations of ethanol (70%, 80%, 90%, 95%, 100%) for different length of time (24, 36 and 48 h). After each period of soaking, ethanol were drained from the leaves and thereafter, the leaves were sealed together with silica gel granules in a cellophane bag, and were then stored for about 8 days. Thereafter, genomic DNA was isolated from the leaves using CTAB protocol (Clarke 2009). As a control for the experiment, leaf samples collected from the same stem were not soaked in ethanol but only dried with silica gel. Also for comparison, fresh leaf samples from the same stem were collected for direct DNA isolation. Each of these treatments was replicated thrice.

The quantity and quality of DNA was measured using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, USA). Additionally, DNA quality was visually checked on 0.8% agarose gel after staining with ethidium bromide. Furthermore, PCR amplification was carried out in a volume of 9.2 µl consisting of 1 µl of template DNA (15 ng/µl), 1 µl of 2.5 mM dNTPs, 0.7 µl of 5 pM RAPD-S381 primer (GGCATGACCT [5′–3′]), 0.3 µl of Taq DNA polymerase (Promega, Madison, USA), 1 µl of 10× buffer. The PCR was performed in a BIO-RAD Thermocycler (Bio-Rad Laboratories, Hercules, California, USA) under the following conditions: 94°C for 5 min; 40 cycles of 94°C for 30 s, 45°C for 60 s, 72°C for 90 s; 72°C for 10 min. PCR products were then stored at 4°C. The amplification products were analyzed by electrophoresis on 1.8% agarose gels in 1× TAE (Tris–acetate-EDTA) buffer, and stained with ethidium bromide. After running for approximately 40 min at 80 V, the gel was photographed by a Gel Documentation System (WD-9413B) (Beijing Liuyi Instrument Factory, Beijing).

The results show that DNA yield of leaf samples pretreated with ethanol prior to silica gel preservation were not significantly different from DNA yield of fresh samples, but were significantly higher than DNA yield of samples only preserved with silica gel (Fig. 1). Agarose gel visualization of the DNA (see Fig. 2a) further confirmed this, in that, DNA bands of all samples without ethanol pretreatment prior to silica gel preservation are either very weak or not visible. RAPD banding pattern of samples dried with silica gel but without ethanol pretreatment were also weaker (Fig. 2b). In terms of DNA purity, the 260/280 ratio of all samples ranged between 1.8 and 2.0, indicating that DNA of all samples were pure (Henry 1997). As expected, DNA yield from new leaves was significantly higher than old leaves (compare new and old leaves in Fig. 2a). This has been attributed to the fact that new leaves tend to contain less phenolic compounds than old leaves (Sytsma et al. 1993). With regard to the effect of duration of soaking, DNA quality appeared to be independent of the length of the soaking period (Fig. 2a).

The study indicates that soaking leaf samples in ethanol before drying with silica gel can increase DNA yields. Ethanol may have acted in disrupting the foliage cell wall (York et al. 1985; Murray and Pitas 1996; Linke et al. 2010), permanently deactivating DNases in the foliage prior to the drying process (York et al. 1985), extraction of certain carbohydrate from the foliage (Streeter and Strimbu 1998) and reducing the production of phenolic compounds

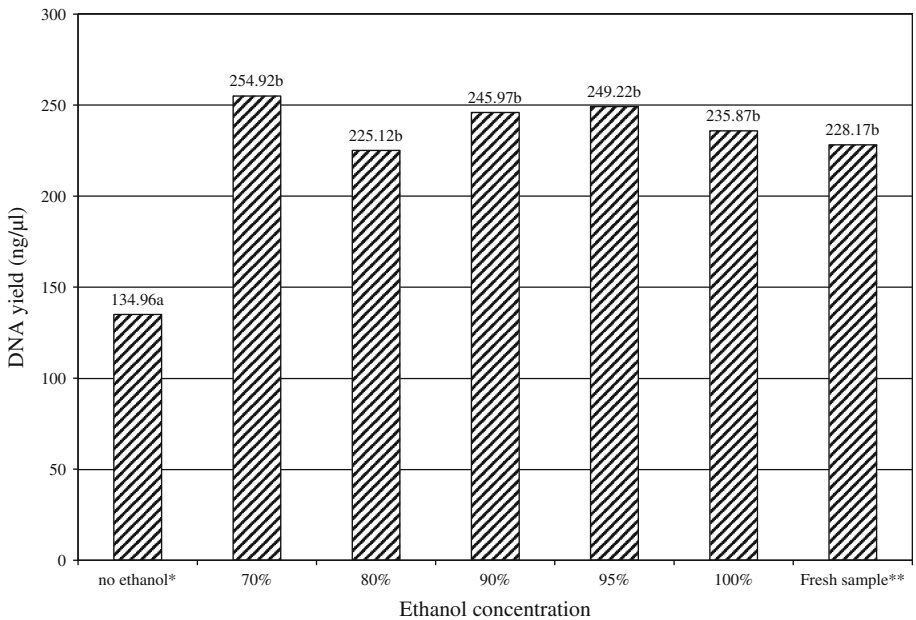

Fig. 1 Effect of ethanol pretreatment on DNA yield. *Samples desiccated with silica gel but without ethanol pretreatment; **fresh samples with neither silica gel desiccation nor ethanol pretreatment; Note: values are means and means with the *same letter* are not significantly different from each other and vice versa at 0.05 level of significance

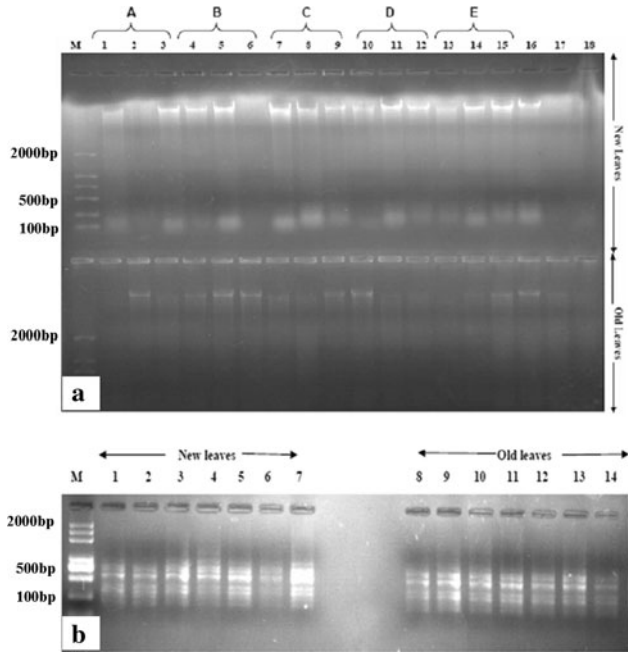

Fig. 2 **a** Genomic DNA isolated from *Picea schrenkiana* resolved on 0.8% agarose gel. *M* MK DL2000 λDNA, *A* samples soaked in 70% ethanol, *B* samples soaked in 80% ethanol, *C* samples soaked in 90% ethanol, *D* samples soaked in 95% ethanol, *E* samples soaked in 100% ethanol, samples 1, 4, 7, 10 and 13 were soaked ethanol for 12 h; samples 2, 5, 8, 11 and 14 were soaked ethanol for 24 h; samples 3, 6, 9, 12 and 15 were soaked ethanol for 36 h; *16* fresh sample with neither ethanol pretreatment nor silica gel drying, *17* and *18* samples dried with silica gel without ethanol pretreatment. **b** RAPD profiles generated for *Picea schrenkiana*. *1* and *8* samples soaked in soaked in 70% ethanol; *2* and *9* samples soaked in 80% ethanol; *3* and *10* samples soaked in 90% ethanol; *4* and *11* samples soaked in 95% ethanol, *5* and *12* samples soaked in 100% ethanol, *6* and *14* samples dried with Silica Gel without ethanol pretreatment, *7* and *13* fresh samples

during the drying process. It is possible the ethanol pretreatment approach will also be useful for increasing DNA yields from other tissue types and from tissues of other species of plants. So, it is recommended that the minimum soak length experimented in this study (24 h) can be used.

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