EXTRACTION OF HIGH-QUALITY RNA AND CONSTRUCTION OF A CDNA LIBRARY FROM FRUITS OF Lycium barbarum LINNAEUS (FRUCTUS LYCI)
Biotechnol. & Biotechnol. eq. 24/2010/1

et al. (12). Sixteen clones were randomly selected and PCR conservation of the cDNA library were according to Sambroock dntP, 1.5 mM MgCl2, 1U of was added to the top aqueous phase, vortexed for 2 min and centrifuged as before. 1/2 vol of isopropyl alcohol and 1/2 vol high salt solutions (0.8M sodium citrate + 1.2M sodium chloride) were added to the collected supernatant, mixed by gentle inversion, and incubated at room temperature for 10 min. After centrifugation at 12 000 g for 10 min at 4°C, the pellet was washed 2-3 times with 75% (v/v) ethanol. After 2-5 min air-dry of the RNA precipitator in a superclean bench, the pellet was dissolved with 50-100 µl of DEPC treated water. Five micro liters of RNA solution was diluted to be qualitatively assayed with a Shimadzu UV-1601 spectrophotometer and tested on a 1.1% formaldehyde denaturant agarose gel.

Reverse transcription–polymerase chain reaction (RT-PCR) analysis

The first strand cDNA was prepared using the RevertAid™M-MuLV KIT (MBI, Lithuania) according to the manufacturer’s instructions. RT-PCR was performed by primers that were designed and based on the conserved sequences for β-carotene hydroxylase (BCH) deposited in GenBank (accession No: ABi23730, BAi47580, ABA43903, ACF21782, ABB49053 and CAA70888): forward primer 5’-GGGTGGAGAAGTGCCTTTTT -3’, reverse primer 5’-CAGTCCATCGTGAACGAACA -3’. PCR was carried out in a PTC-200 thermocycler (MJ Research, USA). PCR reaction contained 1 µl of reverse transcriptase mix, 0.2 mM of each primer, and ddH2O added to a final volume of 20 µl. Cycles were programmed as follows: one cycle for initial denaturation at 94°C for 3 min, 40 cycles of denaturation 1 min at 94°C; annealing 45 s at 53°C; and elongation 1 min at 72°C; and one final cycle of 5 min at 72°C followed by storage at 4°C. The amplification product from fruits of Lycium barbarum was cloned into pMD18-T vector using the corresponding M13-: 5’-AACAGCTATGACCATGTTCA-3’. About 100 clones were randomly picked and incubated in LB broth containing 30µg/ml chloramphenicol for 16-24 h. After incubation, all these clones were used to determine the recombination rate.

Results and Discussion

RNA isolation procedure is usually evaluated by the RNA quantity, quality and the integrity of the obtained RNA (2). Using the RNAiso™ for Polysaccharide-rich Plant Tissue solutions, in this study, 86.5 µg/g of RNA per fresh weight was yielded. The A260/280 ratios were observed greater than 1.91, and the A260/230 ratios were always between 1.95 and 2.01. The RNA, as indicated in Fig. 1, was intact, with distinct 28S and 18S rRNA bands without any degradation, which indicated that the isolated RNA was pure, without any contaminants, and amenable to use for other downstream applications (Table 1). The following results confirmed that.

Absorbancy ratios and yield of total RNA isolated from Fructus Lycii

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<th>A260/A280</th>
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<th>Yield (µg g⁻¹ fresh weight)</th>
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<tr>
<td>1.91 ± 0.14</td>
<td>1.95 ± 0.063</td>
<td>86.5 ± 8.6</td>
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Values are means ± SD (n = 5)

With the use of the first strand cDNA, part of the β-carotene hydroxylase (BCH) gene was successfully cloned (Fig. 2). The results from the electrophoresis of the dscDNAs (Fig. 3) revealed that most mRNA had a size ranging from 400 bp to 4 kb. Typical abundance of mRNA, exhibiting on bright bands, occurred in two positions, one in 800 bp, and the other in 1 kb. Electrophoresis results of 16 randomly selected clones indicated that the size of cDNAs ranged from 400 bp to 2 kb, with an average size of 1 kb (Fig. 4), suggesting that the insertion fragments harbored most of the mRNAs (12). Tittering results showed that the cDNA library incorporated about 6.0×10⁶ clones in total. Results of 100 independent clones showed a high recombination efficiency of 97%. Taken

BIOTECHNOL. & BIOTECHNOL. EQ. 24/2010/1
these together, it could be inferred that the constructed cDNA library was successful.

In our previous experiments, we encountered difficulties from the stage of RNA separation in the supernatant and subsequent reactions. The major problem was low yield, and sometimes degradation. Several standard and modified methods for RNA isolation, including SDS (14), CTAB (2, 8), GT (15), commercially available RNA kits (RNeasy Plant Mini Kit, Qiagen), were applied to Fructus Lycii, but gave poor results or failed to yield usable RNA for further investigations. The average RNA yield was observed to be only 10-15 µg RNA per gram fresh weights (data not shown), although these methods could produce 50-70 µg/g RNA per fresh weights in other fruits.

The routinely used methods such as SDS (14) and CTAB (2, 8) for RNA isolation from tissues rich in polysaccharides were based on precipitation with LiCl. LiCl methods were simpler but also more time-consuming because they included an overnight precipitation. Some authors have also reported that LiCl precipitation may not be effective enough, mainly in the amplification of rare transcripts, when an increasing number of cycles or a large amount of template RNA has to be used (5). It has also been reported that LiCl must be avoided for RT, since chloride ions suppress or reduce the activity of RNA dependent DNA polymerase (6). These properties hindered the utilization of LiCl methods. Our present protocol might be a good choice for isolating high-quality RNA from polysaccharides-rich plant tissues. The key step in this protocol was complete homogenization of the tissue in the extraction solution. The RNA was selectively released from Fructus Lycii and remained in the solution, whereas most of the protein and polysaccharide were kept in the tissues. Together with the presence of high concentration of NaCl and sodium citrate, most of the polysaccharides formed a jelly-like precipitate.

Conclusions
In conclusion, the method reported here allowed for the isolation of RNA from polysaccharides-rich Fructus Lycii, for which other methods failed to deliver RNA suitable for cDNA library construction. The method described here was, therefore, simple and efficient for the isolation of RNA from plants that possessed a wide range of properties that could interfere with RNA extractions and analysis. Furthermore, this method was not complicated and did not require long ultracentrifugation but was straightforward. This method has been routinely used in our lab for isolation of RNA from different tissues rich in polysaccharides. The constructed cDNA library is currently put in use for isolated genes that are involved in carotenoid biosynthesis during fruit ripening of Fructus Lycii.

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REFERENCES