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NOTE

An Effective Method for Isolating Genomic DNA from Leaves of Sesame and Perilla

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Introduction

Some problems are encountered when isolating genomic DNA from plant materials. These problems are generally derived from co-precipitation of polysaccharides and/or other substances in the genomic DNA preparations which have great influence on various plant molecular analyses.[1-4] Until recently, a number of workers have reported a variety of methods for effectively eliminating polysaccharides from plant genomic DNA extractions.[5-9] The general treatments for this involved using high-priced equipment, expensive chemicals or other uncommon materials.[10-13] Some plant materials, for example, yam tissues, grapevine or other wood plant tissues have their own unusual chemical components. Accordingly some appropriate procedures for isolating their genomic DNA were developed,[9,14-16] but those procedures were not applicable to the isolation of genomic DNA from leaves of sesame and perilla because mainly of precipitation of unknown substance by application of restriction buffers or unacceptable genomic DNA purity. As a result, for isolating functional genomic DNA from their leaf tissues, development of a new simple method was needed. Consequently we developed a simple, rapid method for isolating genomic DNA from leaf tissues of sesame (*Sesamum indicum* L.) and perilla (*Perilla frutescens*) both of which could promise as valuable resources for producing useful vegetable oils[17,18] or other commercially important substances.[19,20] In this report, therefore, the procedure that we have developed is described in detail and discussed.

Materials and Methods

Plant materials

Seeds of sesame and perilla were germinated and grown in a growth chamber. The leaf tissues cut from young and old leaves were randomly mixed, frozen in liquid nitrogen and stored in deep freezer.

Other methods and treatments

Agarose gel analysis, Southern blot and other molecular techniques were conducted by general procedures. The purity and quantification of the isolated DNA were measured spectrometrically using Hewlett Packard 8452A. All glassware and other materials were baked at 165℃ overnight, rinsed in chloroform or autoclaved.

Genomic DNA extraction

Leaves of sesame and perilla were powdered in a mortar containing liquid nitrogen and homogenized with an extraction buffer consisting of 200 mM boric acid, 200 mM Tris (pH 7.6), 1.5% SDS, 10 mM EDTA (pH 8.0), 250 mM NaCl and 0.5% 2-mercaptoethanol (the last one was freshly added just before homogenation). After the homogenate was transferred to a clean centrifuge tube (Teflon FEP, Nalgene), it was rapidly frozen at −80℃, then slowly thawed at 60℃ and centrifuged at 8,000×g for 10 min at 4℃. Following filtration of the supernatant through 3 layers of nylon meshes (200 mesh), 1~1.2 volumes of phenol mixture (phenol : chloroform : isoamyl alcohol, 25 : 24 : 1) was added to the filtrate and this mixture centrifuged at 20,000×g for 45~60 min at 20℃, then the aqueous extract phase was collected using wide-bored pipette tips. This phenol/chloroform extraction was repeated until a clear interface between the upper and the lower phenol phase appeared (usually 2~3 times of the extractions). After an equal volume of chloroform was mixed with the collected aqueous phase and then centrifuged at 20,000×g for 45~60 min at 20℃, 0.35 volumes of absolute ethanol was dropped little by little and mixed rapidly with the upper extracts. This mixture was then placed on ice for 15 min and centrifuged at 10,000×g for 6 min at 4℃ to precipitate polysaccharides. To the a-

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bove supernatant an equal volume of isopropanol was added, the mixture stood on ice for 15 min and then centrifuged at 5,000×g for 20 min at 4℃ to precipitate nucleic acids. The nucleic acid pellets recovered were washed with 75% ethanol, vacuum-dried and dissolved completely in water or TE buffer. [The following steps can be omitted but are highly recommeded because considerable amounts of RNA present in the genomic DNA preparations could be removed (data not shown). After dissolving the dried pellets in water, 12 M LiCl was added to make a final concentration of 3 M LiCl and then the solution left on ice for 30 min. Following centrifugation of this solution at 11,600×g for 10 min at 4℃,[21] the pellets recovered were washed, vacuum-dried and dissolved in water or TE buffer.]. The RNA present in the genomic DNA preparations was completely removed by treatment of DNase-free RNase [20~30 μl of RNace-It ribonuclease cocktail (Stratagene) for 200~300 μl of the nucleic acid solution dissolved in water or TE buffer] for 1~3 h or overnight depending on total amount of RNA or types of RNA contained in the DNA preparations. After incubation at 37℃ (optional: one or two times of phenol and one time of chloroform extraction may be applied.), 0.1 volumes of 3 M sodium acetate (pH 5.2) and an equal volume of isopropanol were added and then centrifuged at 5,000×g for 20 min to recover the genomic DNA. Finally the genomic DNA pellets were washed, vacuum-dried and dissolved in water as described above.

Results and Discussion

Quality and quantity of genomic DNA was evaluated by measuring O.D. at wavelengths of 260 nm and 280 nm. For DNA from sesame leaves the absorbance ratio at 260 nm to 280 nm and its yield were 1.70 and 18 μg/g fresh tissue on the average, respectively. In contrast, for DNA from perilla leaves, the ratio and the yield were 1.75 and 28 μg/g fresh leaves, respectively (the above values are the mean of at least 3 independent preparations). These spectrometric values indicate that the genomic DNAs isolated with this method could be quality DNA.[22,23]

Fig. 1 shows that by treatment of LiCl adjusted to 3 M LiCl, considerable amounts of RNA present in the genomic DNA preparations have been removed, but this treatment is not an essential step in our procedure (see Materials and Methods).

Fig. 2A and Fig. 3A show agarose gel analyses of the genomic DNA from the leaves of sesame (Fig. 2A) and perilla (Fig. 3A). According to these analyses, it was evident that no inhibitory influence on restriction reactions was observed. Fig. 2B and Fig. 3B show the Southern analyses of both genomic DNAs. These results also suggest that the genomic DNAs prepared by our method were quality DNAs. Based on the analyses of gel, Southern blotting and data of spectrophotometric measurements, we could draw a conclusion that our method

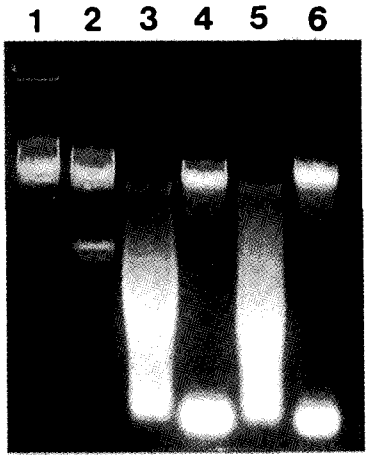

Fig. 1. Agarose gel analysis of pellets and supernatants obtained by centrifuging the nucleic acid mixture after treatment of LiCl. As described in Materials and Methods the dried nucleic acids were first dissolved in water completely and then 12 M LiCl added to make a final concentration of 3 M LiCl. After centrifugation at 11,600×g for 10 min, considerable amounts of RNA were pelleted while most DNA remained in supernatants. Lanes 3 (sesame) and 5 (perilla) show agarose gel analysis of the pellets, and Lane 4 (sesame) and Lane 6 (perilla) exhibit the gel analysis of the supernatants (Lane 1, undigested λDNA; Lane 2, λDNA digested with *Eco*RI and *Hin*dIII).

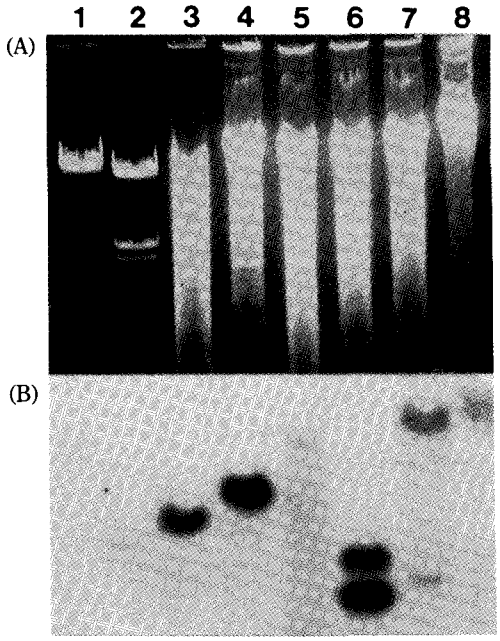

Fig. 2. Agarose gel and Southern blot analysis of genomic DNA from sesame leaves. A: About 6 μg of DNA was digested with *Eco*RI (Lane 3), *Bam*HI (Lane 4), *Sau*3AI (Lane 5), *Eco*RV (Lane 6), and *Xba*I (Lane 7) at 37℃ for 4 h except *Sau*3AI. Lane 1, undigested λDNA; Lane 2, λDNA digested with *Eco*RI and *Hin*dIII; Lane 8, undigested leaf DNA. B: The above gel was hybridized with a cDNA probe prepared from sesame seeds using random priming method.

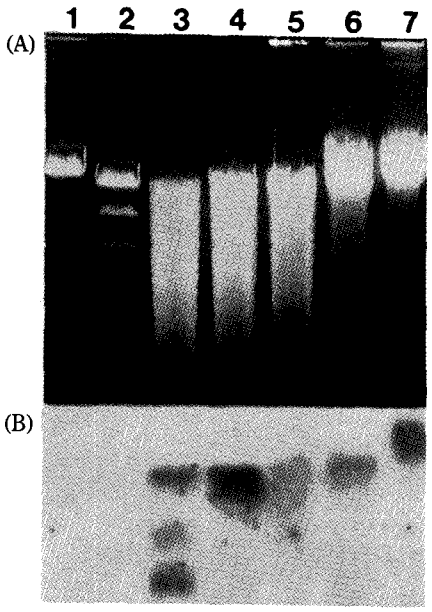

Fig. 3. Electrophoretic gel and Southern blot analysis of perilla leaf genomic DNA. A: The genomic DNA (6 μg) from perilla leaves was digested with *Eco*RI (Lane 3), *Eco*RV (Lane 4), *Sau*3AI (Lane 5) and *Pst*I (Lane 6) at 37℃ for 4 h except Sau3AI and then electrophoresed at 50 V for 2.5 h in 0.7% agarose gel. Lane 1, undigested λDNA; Lane 2, λDNA digested with *Eco*RI and *Hin*dIII; Lane 7, undigested DNA. B: A cDNA probe prepared from perilla seeds was randomly labeled with ^{32}P and hybridized with the above gel.

was very effective for isolating quality genomic DNA from leaves of sesame and perilla. Although from leaves of both sesame and perilla polysaccharides were differentially precipitated by addition of 0.35 volumes of ethanol,[7] the spectrophotometric values of DNA recovered were between 1.2~1.4, indicating those values are generally unacceptable DNA purity when other buffers[5-8,13-15,23] were used. Moreover, some pigments or other insoluble substances were observed in the final genomic DNA preparations (data not shown). However, our results showed that the above problems were eliminated by our method. It was difficult to precisely explain the reason why the problems were removed. Nonetheless it was not ruled out that the presence of boric acid in the buffer or their interaction between the buffer components might be responsible for the removal of those problems. One other finding was that our procedure was not suitable to seeds of either sesame or perilla, and other methods, therefore, should be applied or developed for the seeds. This observation indicates that the choice of buffer was one of the most important factors in isolating quality genomic DNA from plants. In particular, our results show that the buffer used in this method could be effective for isolating functional genomic DNA from other tenacious plant leaves as well as sesame and perilla. In addition, this isolation method is relatively simple and can be done within 8~9h depending on the amount of RNA present in DNA preparations.

Acknowledgments

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참깨 및 들깨잎으로부터 효율적인 게놈 DNA 분리

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찾는말 : genomic DNA, 참깨, 들깨
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