

MOLECULAR BIOLOGY

Economical and Rapid Method for Extracting Cotton Genomic DNA

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INTERPRETIVE SUMMARY

In cotton breeding and genetic studies hundreds, even thousands, of accessions or individual plants need to be evaluated at the DNA level using molecular markers. A fast, simple, and especially reliable DNA extraction method is a prerequisite. However, only about 28 samples a day can be processed by one person with the traditional cetyltrimethylammonium bromide (cTAB)-based macro-prep method. It is a time-consuming, tedious procedure that requires some 5 g of plant tissue and large amounts (ca. 90 mL) of chemical solutions.

Even though many modified methods have been suggested, in reality no easy method for preparing DNA has been proposed and utilized in the cotton research community. A rapid DNA extraction method should be not only fast and simple, but also should produce high quality and quantity DNA and use small amounts of tissue and extraction solutions.

With these criteria in mind we have made substantial modifications on the traditional cTAB method. Although our mini-prep DNA extraction method is cTAB based, the entire process is carried out in 1.5 mL tubes. With this modified method, only one to three folded or nearly unfolded young leaves are harvested into a 1.5 mL tube for storage in a freezer or immediately ground in the traditional cTAB buffer with an electric drill. Our method eliminates the use of liquid N, mortar and pestle, and the need for powder transfer.

During the extraction process, our micro-prep method uses only 0.5 mL each of extraction buffer, chloroform/isoamyl alcohol (24:1), and isopropanol, instead of the 15 mL required by the macro-prep method. Because the extraction buffer to tissue ratio (v/w) is higher, no re-extraction is necessary. Also,

no tube balancing is required before each centrifugation. The DNA precipitated by isopropanol is centrifuged into a pellet, rather than spooled out by a glass hook. The dissolved DNA in Tris-EDTA buffer undergoes a single cleanup procedure to remove polysaccharides, polyphenols, and other colored materials. One person easily can process 200 samples in a day. Compared with the traditional method that produced 50 μg of DNA when 5 g of leaf tissue was used, the DNA yield obtained by this small scale method was as high as 60 μg per 50 to 100 mg of fresh leaf tissue, sufficient for 3000 to 6000 polymerase chain reactions.

ABSTRACT

A fast, simple, and reliable mini-prep method for the extraction of DNA from *Gossypium* species and cultivars has been developed. This small-scale method is cetyltrimethylammonium bromide (cTAB)-based, and it extracts DNA from one to three folded or nearly unfolded young leaves processed in a 1.5 mL tube with 0.5 mL of extraction buffer and homogenized by an electric drill. Compared with the macro-prep cTAB method, the improved mini-prep method is highly efficient and much cheaper in terms of time, chemical use, and labor input. Easily 200 samples per day can be processed by a single person. The DNA yield is greater (60 μg per 50-100 mg of fresh leaf tissue) than that obtained from the macro-prep method (50 μg from 5 g of fresh leaf tissue) and it provides DNA for 3000 to 6000 polymerase chain reactions (PCRs). The DNA quality is sufficient for PCR-based and endonuclease restriction marker analysis.

With the development of polymerase chain reaction (PCR) technology, molecular markers

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Abbreviations: PCR, polymerase chain reaction; RAPD, random amplified polymorphic DNA; SSR, simple sequence repeat; AFLP, amplified fragment length polymorphism; RFLP, restriction fragment length polymorphism; cTAB, cetyltrimethylammonium bromide.

based on PCR soon found vast application in plant genetics and breeding (Lee, 1995). To accommodate the need for PCR-based markers, a rapid, simple, and reliable DNA preparation method is required to provide high quality and quantity DNA for the analyses. Although numerous DNA extraction methods for plants have been reported in the literature, the cTAB extraction method is used most often.

The traditional macro-preparation of DNA usually requires from 0.5 to several grams of plant tissue, making it impractical to analyze individual plants during early seedling stage. Also, the methods are time consuming and laborious due to their multi-step procedures. Furthermore, large amounts of hazardous chemical solvents are required.

Modifications have been made for plant species such as cotton that are high in polysaccharides and polyphenols. The compounds form a sticky, brown gelatinous matrix during DNA preparation that interferes with DNA digestion and PCRs. These modified methods usually employ high salt concentrations to remove polysaccharides, and polyvinylpyrrolidone to bind polyphenols (Lodhi et al., 1994; Porebski et al., 1997). Ascorbic acid, β -mercaptoethanol, and activated charcoal were found to improve extracted DNA quality (Paterson et al., 1993; Bi et al., 1996).

For PCR-based DNA markers used in marker-assisted selection, a fast DNA extraction method is needed. A reliable method should meet the following criteria: (i) require only a small amount of tissue; (ii) involve simple procedures; (iii) use minimal number and amount of chemicals; (iv) yield high-quality DNA; and (v) yield large quantities of DNA. Many mini-prep methods for obtaining DNA have been developed that have included such modifications as no grinding, no centrifugation, and/or no liquid transfer.

One-tube method

Commercial kits are available from many biotech companies, but they are expensive when hundreds or thousands of samples are extracted for DNA. For example, the PhytoPure system (Nudeon Bioscience, Cambridge, UK) can produce as high as 23 μ g DNA from 0.1 g of fresh cotton tissue, but the cost is \$3 to \$5 (USA) per sample (Lee and

Nicholson, 1997). Following extraction with sodium dodecyl sulfate and potassium acetate, chloroform and silica particles are added. DNA is recovered by precipitation with isopropanol.

In the ROSE method (Steiner et al., 1995), 5 to 10 mg of plant tissue is placed in 1.1 mL tube to lyophilize with glass beads, or glass rods in liquid N, and then extracted with Tris-EDTA buffer with sodium lauryl sarkosyl and polyvinylpyrrolidone. At a cost of \$ 0.48 (USA) per sample, 0.8 to 1.2 μ g of DNA can be obtained at a rate of up to 6000 samples per day.

The method of Benito et al. (1993) also eliminates the use of cTAB; 30 mg of leaf tissue is extracted with Tris-EDTA, NaCl and sodium dodecyl sulfate that produces 30 μ L of extractant for 30 PCRs. However, different band patterns were found for leaf and endosperm tissues in the same cultivar. The protocol reported by Williams and Ronald (1994) utilizes potassium ethyl xanthogenate to release DNA without normal tissue homogenization. This method yields enough DNA for 20 PCRs from 0.33 cm² of tissue, but it gives low DNA yield and involves many steps (water bath, vacuum, vortex, and centrifugation). A single person can process 100 to 200 samples per day.

The extraction buffer also is eliminated in the method of Wang et al. (1993b), in which DNA yield can accommodate up to 500 PCRs after extraction by NaOH from a few milligrams of tissue in a 1.5 mL tube. However, this method yielded a predominance of low molecular weight DNA that did not reliably produce PCR amplification products of > 600 bp in length.

Polymerase chain reaction can be done directly on plant tissue with or without pretreatment. Alkali pretreatment (including boiling and neutralizing) of tomato (*Lycopersicon esculentum* Mill.) and barley (*Hordeum vulgare* L.) tissue as a DNA source for PCR was reported (Klimyuk et al., 1993; Clancy et al., 1996). Forty samples could be prepared for PCR in ca. 2 h for less than \$0.50 (USA). However, this method is less efficient with products of > 1 kb, and it did not work with root, embryo, and endosperm tissues. A small piece of leaf or root tissue, or even a single pollen grain can be used directly in PCR buffer, but only DNA sequences present in multiple copies could be amplified (Berthomieu and Meyer, 1991; Peterson et al., 1996).

Additional one tube methods have been reported. Dayteg et al. (1998) extracted DNA in a microtitre dish with boiling NaOH treatment with a yield of 8000 samples per day per person. In the method of Langridge et al. (1991), plant tissue was squashed onto a membrane and rinsed with NaCl, Tris, and EDTA solutions. Several hundred samples could be done in a day. Guidet (1994) used lyophilized tissue and extracted with 100 μ L of buffer, treated with RNase A and boiled. The diluted solution could be used directly for 6000 PCRs. This method could handle 2000 samples a day at a cost of \$0.67 (USA) per sample. Brunel (1992) proposed that small amounts of plant tissue could be crushed in a 96-well microtitre plate with 100 μ L of lysis buffer to yield 50 μ L of lysate for up to 50 PCRs.

Multiple-step methods

Wang et al. (1993a) reported a mini-prep method to extract 200 DNA samples a day from cotton seedling cotyledons. This method requires phenol, Sephadex and spin columns, which makes it expensive and unfeasible for some poorly equipped laboratories.

Many inexpensive and high-yielding procedures that involve liquid transfer have been described for the isolation of DNA from small quantities of plant tissue (Steenkamp et al., 1994; Rogers et al., 1996). In the method of Aljanabi and Martinez (1997), isopropanol is added to the supernatant to precipitate DNA after 50 to 100 mg of fresh tissue is extracted by Tris-EDTA buffer with NaCl and sodium dodecyl sulfate, and treatment with proteinase K. This method produced 500 to 800 ng DNA per mg fresh tissue that could be used for > 3000 PCRs.

Edward et al. (1991) proposed a similar method except that leaf disks are used. Even though hundreds of samples could be done per day with this method, only 40 PCRs could be made from the DNA extracted.

In cotton, the cTAB method of Paterson et al. (1993) has often been used to extract DNA for RFLP analysis (Reinisch et al., 1994; Jiang et al., 1998; Wright et al., 1998). However, in working, we found that this method is extremely time consuming and laborious. A single person can process only about 28 samples a day.

The present study reports a rapid, simple, and inexpensive procedure for extraction of cotton DNA on a small scale. This mini-prep method produces a high yield of clean DNA that can be used to facilitate PCR-based marker analysis.

MATERIALS AND METHODS

Plant Materials

Leaf tissues for DNA extraction included: (i) 14 cultivars or genetic lines of *G. hirsutum* and *G. barbadense*; (ii) representative accessions of wild cotton species in the A, B, C, D, F, G and K genomes; and (iii) five segregating populations involving intra-specific hybrids in upland cotton and an interspecific hybrid between upland cotton and a synthetic tetraploid (A_2D_8).

DNA Extractions

Macro-prep Method

This method was described by Altaf et al. (1997). Briefly, young expanded leaves were collected from each plant and kept in the dark overnight at room temperature to metabolize starch. Approximately 5 g of leaf tissue from each plant of the segregating population ($D8R \times T - 586$) $F_1 \times$ ARK8518 were ground in liquid N, mixed with 15 mL of extraction buffer [0.1 M Tris-HCl, pH 8.0; 1.0 M NaCl; 0.02 M EDTA, pH 8.0; 2% (w/v) cTAB; 2% (w/v) polyvinylpyrrolidone-40; 1 mM 1,10-phenanthroline; 0.2% (v/v) β -mercaptoethanol], and incubated for 1 h at 70°C. The solution was then twice extracted with equal volumes of chloroform:isoamyl alcohol (CIA, 24:1, v/v) and centrifuged at 15 000 \times g, 4°C for 10 min. The aqueous phase was transferred to a fresh tube, and the DNA was precipitated with an equal volume of isopropanol and kept at -80°C for 1 h or at -20°C overnight. The DNA was then spooled out and washed with 80% ethanol + 15 mM ammonium acetate, and 100% ethanol sequentially, each for 20 min with gentle shaking. The DNA pellet was air dried and dissolved in 5 mL of high salt Tris-EDTA buffer (10 mM Tris, pH 8.0; 1 mM EDTA, pH 8.0; 1 M NaCl) by incubating at 60°C for 1 to 2 h. The DNA was then subjected to an additional cleaning

procedure. Briefly, the Tris-EDTA DNA solution was mixed with 1 volume of the extraction buffer without β -mercaptoethanol and subjected to a minimum of 2 h of shaking. The DNA was then precipitated with isopropanol, spooled out and washed with ethanol as described before. The cleaned DNA pellet was air dried and resuspended in 50 to 250 μL of low-salt Tris-EDTA buffer (i.e. without NaCl), containing RNase A (20 μg per 100 μL of DNA solution). The DNA concentration was quantified by a Hoefer TKO 100 DNA Fluorimeter (Hoefer Scientific Instruments, San Francisco, CA).

Mini-prep Method

One to three folded or newly unfolded young leaves from each plant were collected in a 1.5 mL microcentrifuge tube and stored at -80°C , or were used immediately. After 0.5 mL of the extraction buffer (described previously for the macro-prep method) was added in the tube, the contents were ground with an electric drill fitted with a bit to which was molded a plastic head with the shape and size of the interior base of a 1.5 mL centrifuge tube. Following tissue maceration, the tubes were capped and contents mixed by a few inversions. The bit head was thoroughly rinsed with distilled water between each sample. After incubation at 65°C for 15 min to 1 h, an equal volume of chloroform:isoamyl alcohol (CIA, 24:1, v/v) was added and the tube centrifuged at $12\,000 \times g$, 4°C for 10 min. The supernatant was transferred to a fresh 1.5 mL tube, mixed with 0.5 mL of isopropanol, and kept at -20°C for 1 h for precipitation of DNA (this step was optional). The tubes were centrifuged at $12\,000 \times g$, 4°C for 10 min and the liquid phase discarded. After sequentially washing with 70% and 100% ethanol, the DNA pellet was air or vacuum dried and suspended in 300 μL of low salt Tris-EDTA buffer. The extracted DNA was then subjected to an additional cleaning procedure. Briefly, the preliminary DNA solution was mixed with 0.5 mL of cleaning solution [0.05 M Tris-HCl, pH 8.0; 0.05 M EDTA, pH 8.0; 2% (w/v) cTAB; 2.05% (w/v) NaCl; 0.02% (w/v) 1,10-phenanthroline] and shaken for 1 to 2 h at room temperature. The solution was then centrifuged at 4°C or room temperature for 5 min. After the liquid phase was discarded, the DNA pellet was washed with 0.5 mL of 80% ethanol + 15 mM

ammonium acetate and 0.5 mL of 100% ethanol, sequentially. The cleaned DNA pellet was air or vacuum dried, and resuspended in 300 μL of low-salt Tris-EDTA buffer with incubation in a water bath at 65°C if necessary. DNA concentration was determined as described in the previous section. The DNA yield and quality were estimated by absorbance spectra between 220 and 320 nm and $\text{A}_{260}/\text{A}_{280}$ ratio with a Hitachi 2000 spectrophotometer. To determine digestibility of DNA with endonucleases, 2.5 μg of the cleaned DNA was digested with RNase A and then incubated overnight with 5 units of *EcoRI* and *HindIII*. The digested DNA was electrophoresed on 1.5% agarose gels.

PCR Analysis

The DNA from a series of plants was adjusted to 10 ng L^{-1} . The amplification reactions were in a total volume of 20 μL consisting of 10 ng DNA, 2.5 μL of 10X buffer II, 3.75 μL of 10 mM MgCl_2 , 0.5 μL of 10 mM deoxynucleotide triphosphates, 0.33 μL of 50 pM primer, 0.1 unit (μL) of *Taq* polymerase and 16.82 μL of distilled deionized H_2O . The 10X buffer II, 10 mM MgCl_2 and *Taq* polymerase were all purchased from Perkin-Elmer (Foster City, CA). Amplification was performed in a Hybaid Omnigene thermocycler (Hybaid Omn-E-02HL) for 45 cycles after initial denaturation at 94°C for 2 min. Each cycle consisted of 15 s at 94°C , 30 s at 40°C , and 90 s at 72°C . A 5 min final extension at 72°C followed the end of the cycling program. The amplification aliquots were resolved by gel electrophoresis in 1.4% or 1.0% agarose gels with 0.5 X Tris-borate-EDTA buffer (45 mM Tris-borate, 1.0 mM EDTA, pH 8.0), and were stained with 1 g L^{-1} of ethidium bromide. In some cases, ethidium bromide was added directly to the gels during preparation. The size of the amplified DNA fragments was estimated based on 100 bp DNA ladder from MBI (Amherst, NY). The RAPD bands were visualized under ultraviolet light.

RESULTS AND DISCUSSION

Procedures

In the present study, a number of changes were made to simplify extraction of cotton DNA that did

Table 1. Comparison of macro-prep and mini-prep DNA extraction methods.

Steps involved	Macro-prep method	Mini-prep method
1. Sample collection/prep	Minutes/sample	Seconds/sample
2. Extraction buffer	15 mL	0.5 mL
3. Sample grinding		
# samples/person	28/day	200/day
Time needed	2-3 h	0.5-1 h
Facilities	Mortar and pestle, liquid N ₂ , transfer	Electric drill, directly grind in tube
4. Incubation	70°C, 1 h	65°C, 15 min-1 h
5. CIA [†] (24:1)	15 mL	0.5 mL
6. Centrifuge	16 000 × g, 10 min	12 000 × g, 10 min
7. Supernatant transfer	Multiple pipetting	One pipetting
8. Re-extraction	Required	None
9. Isopropanol	15 mL	0.5 mL
10. Storage	-80°C, 1 h	-20°C, 1 h (optional)
11. DNA precipitation	Spool out one at a time by glass hook, more time needed for many samples	Centrifuge at 12 000 × g, 10 min
12. DNA wash	80% ethanol, NH ₄ OAc, 5 mL 100% ethanol, 5 mL	70% ethanol, 0.5 mL 100% ethanol, 0.5 mL
13. DNA suspension	High salt Tris-EDTA, 5 mL	Low salt Tris-EDTA, 300 mL

† Chloroform:isoamyl alcohol (24:1, v/v).

not have negative effects. First, ascorbic acid and diethyldithiocarbamic acid were not used in the extraction buffer. Second, one to three very young (folded or nearly unfolded) true leaves were collected in a 1.5-mL tube that is ready for grinding in-tube with an electric drill without pre-incubation. Samples also can be stored in a minimum of space in a freezer for later use. The electric drill can be secured to a holder so that tissue crushing requires only manual movements of the tube on the rotating mold. Tissue weighing and tube balancing are not required. Third, traditional chloroform-isoamyl alcohol (24:1, v/v) was used instead of phenol-chloroform (1:1). Fourth, a cleaning procedure was used instead of using Sephadex and spin columns (Wang et al., 1993a) or other chemicals (Lee and Nicholson, 1997) to produce high-quality DNA.

From the comparison between our modified method and the routine macro-prep method (Table 1), the advantages of the mini-prep method are evident. Much smaller volumes of the chemical solutions are required (ca. 3 vs. 90 mL), thus, the expense and chemical hazards are greatly reduced. In the macroprep method, it takes considerable time to harvest several grams of leaf samples and transfer the samples into a mortar and pestle for grinding. Also, grinding in liquid N is tedious and time consuming. Re-extraction is required and the time for supernatant transfer and DNA separation from the liquid is lengthy.

We could process only about 28 samples per day per person with the macro-prep method, a rate not

suitable when working with a large number of tissue samples. On the contrary, 200 samples per day could easily be extracted by the mini-prep method. The comparison shows that the mini-prep method is much faster, simpler, and less expensive.

DNA Yield

We obtained very high DNA yields for cotton using the cTAB-based mini-prep procedure. In the preliminary preparations before cleaning the apparent DNA concentration ranged from 370 to 1550 ng μL^{-1} , with an average of 990 ng μL^{-1} in a total volume of 300 μL . The average yield was approximately 3000 to 6000 μg of DNA per g of fresh tissue. After the cleaning procedure, the DNA concentration obtained from 50 to 100 mg of leaf tissue averaged 200 ng μL^{-1} in a total of 300 μL of DNA solution. The total DNA yield was 60 μg of DNA per 50 to 100 mg of leaf sample, i.e., 600 to 1200 μg per g of fresh leaf tissue. Surprisingly, total yield was 23.4% greater than the total obtained with the macro-prep method using ca. 5 g of newly expanded leaf tissue (Table 2). The macro-prep method produced a total yield of 49.0 μg of DNA (i.e., 10 μg of DNA per g of fresh leaf tissue).

The DNA yield from the mini-prep method is comparable to that (500-1000 μg per g) obtained from 0.5 g of fresh leaf tissues from *Vitis* cultivars and species (Lodhi et al., 1994). It is much greater than that (232 μg DNA per g of cotton leaf tissue) extracted by the PhytoPure method (Lee and

Table 2. Comparison of DNA yield between macro-prep and mini-prep DNA extraction methods.

Lot	Date	Samples no.	Yield [†]			S.D.	CV %
			Min	Max	Mean		
			ng μL^{-1}				
Macro-prep method							
1	22 Jan. 1997	26	35	1207	389.9	298.1	76.4
2	27 Jan. 1997	28	7	357	123.7	76.0	61.5
3	2 Feb. 1997	26	4	257	64.5	62.1	96.3
4	5 Feb. 1997	12	15	243	72.3	6.02	83.2
Average					126.6	124.1	79.3
Mini-prep method							
1	4 Sept. 1997	20	137	583	301.1	118.4	39.3
2	4 Sept. 1997	89	5	662	228.2	131.4	57.6
3	7 Sept. 1997	58	18	667	153.4	139.4	90.9
4	17 Sept. 1997	65	10	550	169.2	110.1	65.1
5	1 Oct. 1997	41	13	401	151.3	90.1	59.5
Average					200.6	117.9	62.5

[†] All were adjusted to a total volume of 300 μL .

Nicholson, 1997). In their test, even lower DNA yield (7.3-98.2 μg DNA per g of cotton leaf tissue) was obtained by other methods using cTAB, polyvinylpyrrolidone, KCl, or sodium dodecyl sulfate.

Although many one-tube methods have been suggested, most of them only apply to marker-assisted selection using a specific primer, since only a limited number of PCRs can be made from the DNA yielded. Assuming that 10 to 20 ng of DNA is needed per reaction for PCR-based RAPD analysis, the cleaned DNA by the mini-prep method can provide enough template for 3000 to 6000 PCRs or 10 southern blot analyses. The DNA yield obtained by the method is sufficient for various marker analyses, including genotyping, gene mapping, and quantitative trait loci analysis. Thus, the method is also suitable for extraction of the high quantity of DNA required for RFLP analyses.

The high yield of DNA can be attributed in part to the proper choice of leaf tissues for extraction. The rationale for selecting very young leaves was to maximize the ratio of DNA to tissue weight. After differentiation, but before expansion, young leaves contain essentially the same amount of nuclear DNA as a fully expanded leaf. Also, the youngest leaf tissue is much easier to crush to release DNA. Thus, the ratio of DNA to other cell constituents, such as polyphenols and polysaccharides that might compromise extraction or purity, is very high.

In essence, the method allows the extraction of the total nuclear DNA from the equivalent of fully expanded leaves using only 0.5 mL of extraction

buffer. The fraction percentage of chloroplast and mitochondrial DNA will be less if using very young leaves, compared with fully expanded tissues. Furthermore, the buffer volume to tissue weight ratio (v/w) could make a difference.

With the mini-prep method, 50 to 100 mg of leaf tissue were extracted in 0.5 mL of buffer, i. e. 100 to 200 mg of leaf tissue per mL of buffer, while ca. 5,000 mg of leaf tissue was in 15 mL of buffer (333 mg of leaf tissue per mL of buffer) for the macro-prep method. Thus, in the mini-prep method, more extraction buffer was used per unit weight of leaf tissue, and that could result in more DNA being solubilized while contaminating polysaccharides and polyphenols are more diluted.

Another advantage of our mini-prep method is that, leaf samples can be collected any time during the growing season, as long as leaf buds are available, even in late season when cotton leaves turn yellow or plants are infested with diseases or insects. Usually, the unfolded leaf buds are clean and without pest damage.

DNA Quality

Although the preliminary DNA preparations often were sufficient for PCR, in general the cleaning procedure was necessary. Before cleaning, the DNA was brownish and obviously contained contaminants including polysaccharides, polyphenols, and other secondary compounds. In the cleaning procedure, these were removed by NaCl, polyvinylpyrrolidone and 1,10-phenanthroline. This last compound is a

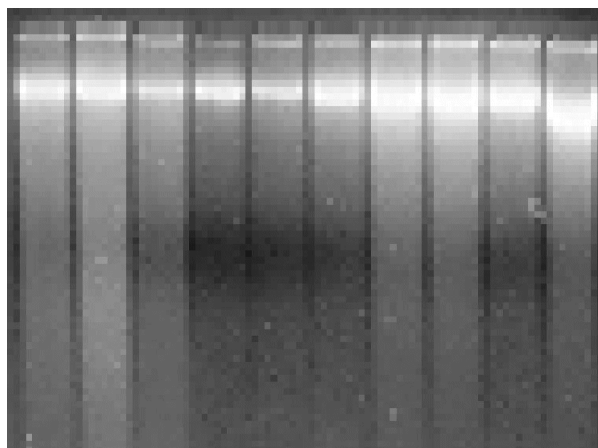


Fig. 1. Agarose gel electrophoresis of the undigested DNA extracted by the mini-prep method.

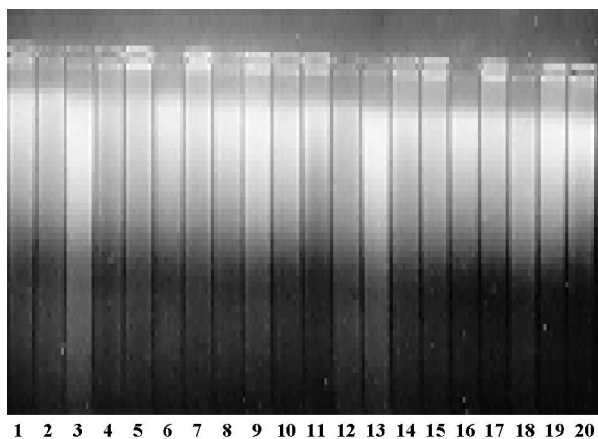


Fig. 2. Agarose gel electrophoresis of the DNA restricted with *EcoRI* and *HindIII*. Lane 1 to 10: 10 different DNA samples restricted with *EcoRI*. Lane 11 to 20: same DNA samples restricted with *HindIII*.

chaotrophic and metal chelating agent that helps reduce intermolecular binding in the extraction and cleaning buffer. Studies showed that this chemical inhibits the degradation of DNA and protects DNA from damaging effects in the presence of Fe ions (Wajahatallah et al., 1997).

The DNA purity was judged by the $\text{Å}260/\text{Å}280$ ratio which ranged from 1.96 to 2.12, indicating that ultraviolet absorbing contaminants were not a problem. Figure 1 shows that the DNA prepared by the mini-prep method was not degraded, and Figure 2 shows that the DNA was completely digested with restriction enzymes *EcoRI* and *HindIII*. Therefore, DNA produced by the mini-prep method is of sufficient quality for use in RFLP analysis.

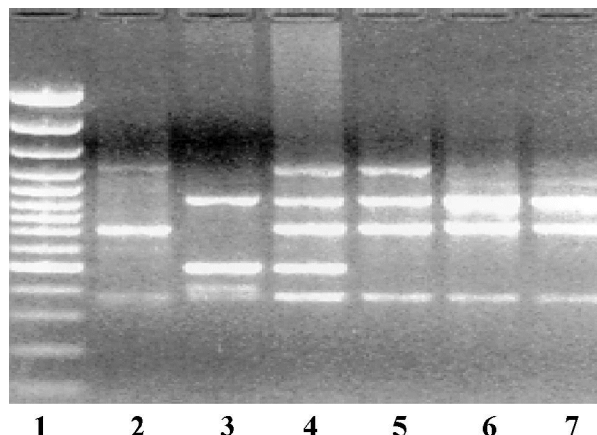


Fig. 3. Single primer PCR amplification of mini-prep DNA from different cotton species and genotypes. Lane 1: 100-bp DNA marker. Lane 2: *Gossypium arboreum* (A_2). Lane 3: *G. trilobum* (D_8). Lane 4: ($D8R \times TM - 1$) F_1 (*G. hirsutum*, AD_1). Lane 5: Pima S-1 (*G. barbadense*, AD_2). Lane 6: 57-4 (*G. barbadense*, AD_2). Lane 7: ARK8518 (*G. hirsutum*, AD_1).

PCR

Originally, an initial mini-prep method was introduced and used to prepare soybean DNA from leaf disks directly for SSR analysis without DNA quantification (C. Sneller, personal communication, 1997). Following modification of the procedure for cotton in 1997, we have successfully used DNA extracted by this method for RAPD analysis to map genes responsible for restoration of cytoplasmic male sterility, red-anther, and semigamy, and to genotype *Gossypium* species and cultivars.

All the DNA templates produced clear, sharp and reproducible PCR banding patterns. Figure 3 shows typical PCR results with template DNA prepared by the mini-prep method from six genotypes including four *Gossypium* species. This method has been distributed to several cotton research groups in the USA, China, and Brazil, and it has been successfully used by others in SSR and RAPD analyses (R.G. Cantrell, personal communication, 1999; R. Kloth, personal communication, 2000).

The DNA extracted by a macro-prep cTAB method, as described by Altaf et al. (1997), was used successfully for amplified fragment length polymorphism (AFLP) analysis. Since the mini-prep method is essentially an adaptation of that method, the DNA prepared by the mini-prep procedure

should have similar reliability for AFLP analysis. This is supported by the observation that the DNA is amenable to digestion with restriction enzymes.

CONCLUSIONS

A cTAB-based mini-prep method for DNA extraction from *Gossypium* species and cultivars has been established that requires only one to three folded or nearly unfolded leaves collected in a 1.5-mL tube for tissue homogenization with an electrical drill. The extraction process is undertaken in 1.5-mL tubes with 0.5 mL of the respective solutions. The DNA is separated by centrifugation. The improved, efficient, small-scale method is time and labor saving, and much simpler than conventionally used procedures. Two hundred leaf samples can be processed per person a day. Contaminants such as polysaccharides and polyphenols are removed by a cleanup buffer containing NaCl, polyvinylpyrrolidone and 1, 10-phenanthroline. Very high DNA yield and quality are obtained that can be used for PCR- and endonuclease-based marker analysis.

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