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Full Length Research Paper

A safe inexpensive method to isolate high quality plant and fungal DNA in an open laboratory environment

Chen Niu[1], Hirut Kebede[1, 2], Dick L. Auld[1], Jason E. Woodward[1, 3], Gloria Burow[4], and Robert J. Wright[1, 2]*

[1]Texas Tech University, Lubbock, TX, USA.
[2]Texas AgriLIFE Research, Lubbock, TX, USA.
[3]Texas AgriLIFE Extension, Lubbock, TX, USA.
[4]USDA ARS, Lubbock, TX, USA.

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The most commonly used plant DNA isolation methods use toxic and hazardous chemicals (phenol, chloroform), which require special equipment to minimize exposure and may limit their use in certain environments. Commercial DNA extraction kits are convenient and usually safe, but their availability to certain developing countries and high cost can be limiting, especially when handing a large number of samples and considering experiments with limited financial resources. Current reports on non-phenol/chloroform protocols have not thoroughly examined the quality and suitability of the DNA for studies that require high precision. A simple, economical and rapid method is presented to isolate high quality DNA from plant and fungal species. This method uses potassium acetate to remove proteins and polysaccharides in an SDS extraction buffer. Further DNA purification is achieved using a low salt CTAB treatment. This SDS/CTAB protocol was used to isolate high quality genomic DNA subject to restriction endonuclease digestion and AFLP analysis from both plant and fungi with minimum cost and health concerns.

Key words: CTAB, SDS, DNA isolation, phenol/chloroform free.

INTRODUCTION

An essential task in any molecular genetics laboratory is the isolation of genomic DNA (gDNA). Numerous DNA isolation protocols use phenol and chloroform to separate cellular molecules and debris from the DNA. Such organic reagents are toxic, hazardous, expensive, and require special containment facilities to maximize personnel safety and minimize environmental concerns. The disposal of phenol/chloroform waste also requires special equipment and care to avoid human and environmental exposure. The lack of well-equipped laboratories, particularly those in many developing countries, may prohibit the use of harmful chemicals (Mahuku, 2004).

Ideally, DNA could be isolated from a variety of plant species and tissues using a single protocol, a method applicable to an open laboratory environment with minimal equipment requirements and waste output. Commercial DNA extraction kits that do not use hazardous reagents have these ideal properties; however, their convenience and safety may be cost prohibitive when considering experiments with limited financial resources. Furthermore, in some instances, commercial kits have produced low DNA yields and variable quality (Sharma et al., 2000; Li et al., 2001; Buldewo and Jaufeerally-Fakim, 2002; Keb-Llanes et al., 2002; Horne et al., 2004).

Contaminants such as tannins, polysaccharides, and pigments can inhibit the annealing of DNA or the enzymatic activity of restriction endonucleases (Pandey et al.,

*Corresponding author. E-mail: robert.wright@ttu.edu. Fax: +1 806 742 0775. Tel: +1 806 742 4764.

Abbreviations: AFLP, amplified fragment length polymorphism; **CTAB**, cetyl-trimethyl ammonium bromide; **SDS**, sodium dodecyl sulfate.

1996; Rogstad, 2003). This is a problem for many technical applications including PCR and Southern analysis. Several non-toxic extraction methods use high salt concentration buffers, proteinase K treatments, or DEAE-cellulose to obtain a higher purity of DNA (Aljanabi and Martinez, 1997; de la Cruz et al., 1997; Sharma et al., 2000; Buldewo and Jaufeerally-Fakim, 2002; Hosaka, 2004; Angeles et al., 2005). DNA isolated from these methods is suitable for PCR applications but further purification is required for techniques that involve the enzymatic digestion of DNA. These methods are also less effective in plants rich with polysaccharide and polyphenolic compounds.

The objective of this study was to develop a simple method to isolate DNA in an open laboratory environment, a method that eliminates the need to use phenol or chloroform to purify the DNA. The resulting SDS/CTAB protocol was used to isolate high quality genomic DNA subject to restriction endonuclease digestion and PCR analysis from both plant and fungi with reduced cost and health concerns.

MATERIALS AND METHODS

Plant materials and fungal isolates

DNA was isolated from three plant and two fungal species. Two cultivars ('TTU-BG-Exp-1' and 'Top gun') of buffalograss (***Buchloe dactyloides***) and two ***Gossypium*** species (***G. herbaceum*** and ***G. arboreum***) represent both monocot and dicot taxa. Eight ***Alternaria tenuissima*** isolates and four ***Fusarium oxysporum*** f. sp. ***vasinfectum*** (***Fov***) isolates were also examined.

Solutions and reagents

The "Extraction Buffer" consisted of 100 mM Tris-HCl, 50 mM EDTA, and 500 mM NaCl adjusted to a pH of 8.0. Just prior to use adjust the solution to 0.2% (v/v) 2-mercaptoethanol and 2% (w/v) PVP-40,000 (optional for tissues rich in polysaccharides and polyphenols). The "Clean-up" solution consisted of 2% CTAB, 50 mM Tris-HCl, 50 mM EDTA, 0.35 M NaCl, 0.02% 1,10-phenanthroline adjusted to a pH of 8.0. Additional solutions include 20% SDS, 2 M potassium acetate (pH 5.5), 3 M sodium acetate, TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), isopropanol, and 70 and 95% ethanol.

DNA extraction protocol

For isolating plant gDNA, we used fresh young leaves less than one week old. Plant leaf tissues or fungal mycelia (50 - 100 mg) were ground to a fine powder using liquid nitrogen. Each sample was transferred to a 2.0 ml tube. Each sample received 800 µl of extraction buffer and 50 µg RNase A. The contents were mixed by a brief inversion of each tube. After mixing 100 µl of 20% SDS was added and mixed by inversion. Each tube was incubated at 65^{o}C for 15 min to lysis the cells. After incubation, 225 µl of 2 M potassium acetate was added and each sample gently mixed by inversion. Samples were incubate on ice for 15 min, and then centrifuged at 12,000 g for 10 min at room temperature (RT). The supernatant was transferred to a clean tube followed by an equal volume of isopropanol. The precipitated DNA was centrifuged at 12,000 g for 10 min at RT and the pellet was washed with 0.5 ml of 70% ethanol. The DNA was re-suspended in 300 µl of sterile water or TE buffer (DNA samples can be used directly at this stage for some PCR reaction that does not require high purity). To purify the DNA, 0.5 ml of the Clean-up solution was added and shaken at 100 rpm for 30 - 60 min at RT. After shaking impurities were removed by centrifuging at 8,000 g for 5 min to pellet the DNA. The pelleted DNA was re-dissolved in 90 µl of sterile water. Once dissolved 10 µl of 3 M sodium acetate and 400 µl of 95% ethanol were added. Samples were mixed by inversion and put on ice for 15 min. DNA was pelleted by centrifuging at 10,000 g for 10 min at RT and washed with 0.5 ml of 70% ethanol. The DNA pellet was air- or vacuum dried and dissolved in 50 - 200 µl of TE buffer.

DNA analysis

Total gDNA was isolated from each plant and fungal species described above. As a point of comparison plant gDNA was also isolated using the Qiagen DNeasy plant mini kit (Qiagen 69104, Qiagen Sciences, Maryland 20874. USA). Fungal gDNA was compared with gDNA isolated using a traditional CTAB-chloroform based method (Saghai-Maroof et al., 1984). DNA yields were measured using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE USA) and the quality assessed by agarose gel electrophoresis, restriction enzyme digestion, and AFLP analysis.

Plant (500 ng) and fungal (500 ng for ***Alternaria*** isolates and 1000 ng for ***Fusarium*** isolates) DNA was digested using 10 units of ***EcoRI*** (Gibco BRL Life Technologies, Gaithersburg, MD, USA) at 37^{o}C for 2 h. Undigested and digested (***EcoR I***) total gDNA was separated on a 1% agarose gels in TAE buffer. AFLP analysis was performed using fluorescent labeled primer pairs following the Li-COR AFLP kit instruction (Li-COR Biotechnology, Lincoln, Nebraska USA). Six fluorescent labeled primer pairs were used to amplify AFLP target loci from DNA isolated from each method and species. Amplified PCR products were separated on 6% polyacrylamide gel and scanned with the Li-COR 4300 DNA Analysis System (Li-COR Biotechnology, Lincoln, Nebraska USA).

RESULTS

The physical characteristics of the final DNA pellet were white with no visible discoloration. The A_{260}/A_{280} ratio of the DNA ranged from 1.75 to 2.21 indicating the isolated DNA is free from protein contamination. The SDS/CTAB method consistently resulted in higher DNA yields compared to the Qiagen Kit for buffalograss (274.5 vs 29.0 µg/g tissue) and cotton (49.0 vs 18.1 µg/g tissue) (Table 1). When compared with a traditional CTAB-chloroform method, lower fungal DNA yields were noticed for ***Fusarium*** (30.8 vs 52.0 µg/g tissue) and ***Alternaria*** isolates (1123.5 vs 1726.3 µg/g tissue) (Table 1). However, the DNA yield from 100 mg of fungal mycelia was enough for multiple restriction digestions or more than a hundred PCR reactions. Isolated gDNA examined by gel electrophoresis (1.0% agarose in TAE buffer) showed no visible DNA degradation or RNA contamination (Figure 1A). Plant and fungal gDNA were subjected to restriction en-

Table 1. DNA purity and yield isolated from plant leaves and fungal mycelia using different isolation methods.

Source	Method	A260/A280	Yield (µg/g tissue)
Buffalograss			
TTU-BG-Exp-1	SDS/CTAB	1.97	276.0
TTU-BG-Exp-1	Qiagen	1.87	35.9
Top gun	SDS/CTAB	2.04	273.0
Top gun	Qiagen	1.92	22.1
Cotton			
G. arboreum	SDS/CTAB	1.88	48.5
G. arboreum	Qiagen	1.86	18.1
G. herbaceum	SDS/CTAB	2.17	52.1
G. herbaceum	Qiagen	2.00	18.1
Fusarium			
Isolate 1	SDS/CTAB	2.05	20.7
Isolate 1	CTAB-Chloroform	1.75	53.8
Isolate 2	SDS/CTAB	1.78	40.8
Isolate 2	CTAB-Chloroform	1.83	50.1
A. tenuissima			
Isolate 1	SDS/CTAB	2.17	1150.8
Isolate 1	CTAB-Chloroform	2.21	1569.3
Isolate 2	SDS/CTAB	2.17	1096.2
Isolate 2	CTAB-Chloroform	2.19	1883.2

donuclease digestion and in both cases, the gDNA was completely digested (Figure 1B). These results indicated that the extracted DNA quality was suitable for applications involving restriction digestion.

Cotton AFLP fingerprints (sized fragments) were indistinguishable using either DNA isolation method (Figure 1C). An identical AFLP banding pattern and obvious polymorphisms among different ***A. tenuissima*** isolates were consistently identified using either DNA isolation method (Figure 1D). These results were consistent among all AFLP primer pairs. The AFLP analysis for buffalograss and ***Fusarium*** were also indistinguishable between methods (data not shown). These results demonstrate the utility of the SDS/CTAB method to isolate DNA for a number of applications.

DISCUSSION

The isolation of high quality DNA is an important step in the field of plant molecular biology. Herein, a simple, inexpensive method to isolate gDNA is described. This method eliminates the need to used phenol or chloroform to obtain high quality gDNA from plants and fungi. During the SDS lyses phase, proteins and polysaccharides become trapped in large complexes that are coated with dodecyl sulfate. These complexes are precipitated when sodium ions are replaced by potassium ions (Dellaporta et al., 1983; Ish-Horowicz and Burke 1981). The residual proteins and other cellular contaminants are usually removed or separated from the DNA using phenol/chloroform. The protocol proposed here first precipitates the DNA using isopropanol and then purifies the DNA using a treatment of low salt CTAB buffer, thus, eliminating the need for the phenol/chloroform purification.

At lower NaCl concentrations (< 0.4 M), DNA and CTAB form an insoluble complex (Murray and Thompson, 1980) which is separated from the residual soluble proteins, polysaccharides and other molecules by centrifugation. Similar low salt/CTAB strategies have been used to collect DNA in mung bean (***Vigna radiata***) (Murray and Thompson, 1980), orpine (***Sedum telephium***) (Barnwell et al., 1998), and cotton (***Gossypium*** spp.) (Zhang and Stewart, 2000). However, chloroform was needed to remove major protein, phenolic, and cell debris contaminates (Murray and Thompson, 1980; Barnwell et al., 1998; Zhang and Stewart, 2000). It was noticed that some contaminants that inhibit PCR could not be removed with chloroform extraction (Horne et al., 2004). The SDS/CTAB method consistently recovers high quality DNA for precise downstream analysis indicated that the Clear-up treatment is very efficient in the purification of DNA.

In developing countries, particularly in remote laboratories or plant breeding stations a major limitation in the application of molecular DNA technologies is the high

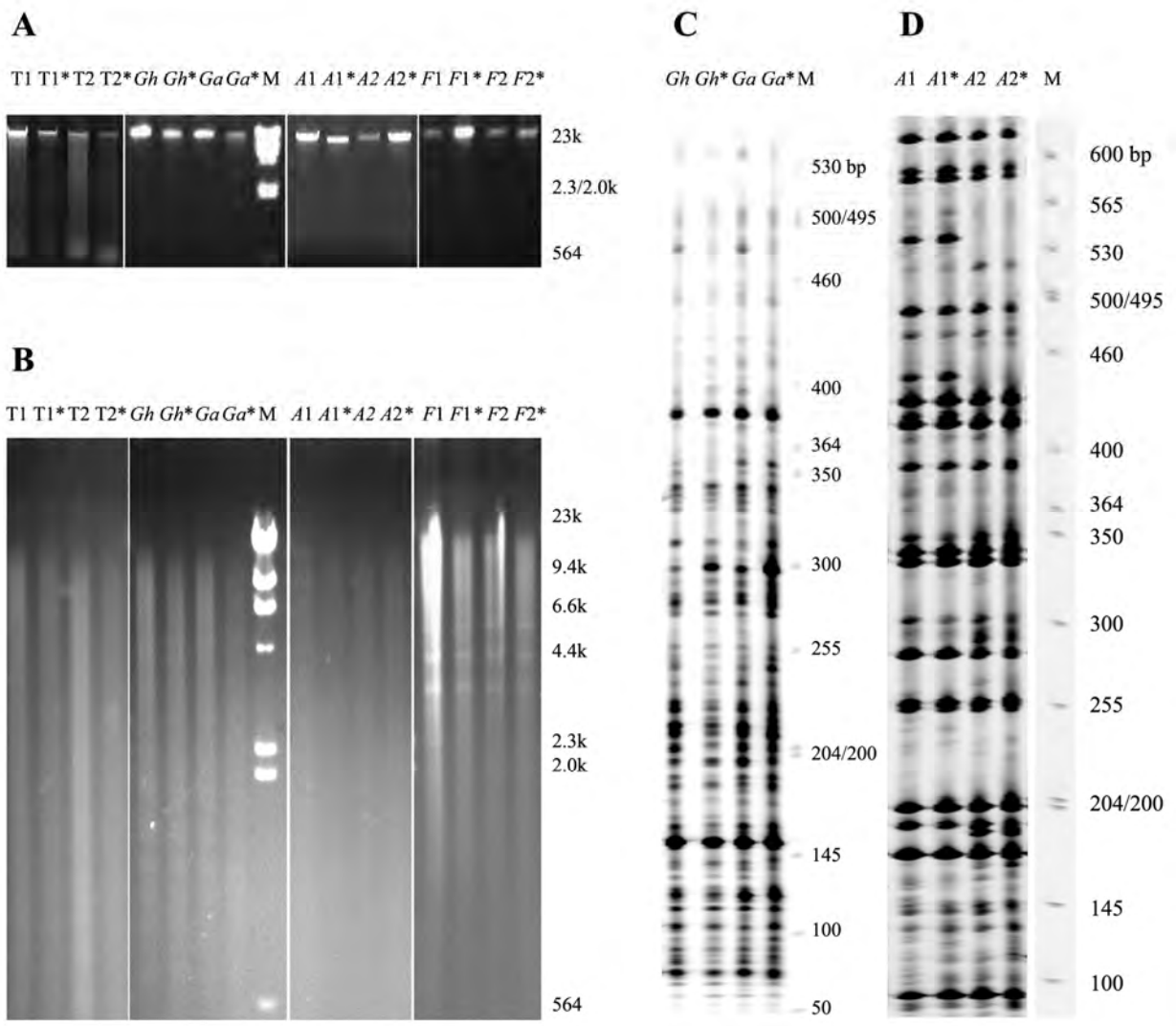

Figure 1. Analysis of isolated DNA. Undigested (A) and ***EcoRI*** digested (B) total genomic DNA from TTU-BG-Exp-1 (T1), Top Gun (T2), ***G. herbaceum*** (***Gh***), ***G. arboreum*** (***Ga***), ***Alternaria*** isolates 1 and 2 (***A***1 and ***A***2), and ***Fusarium*** isolates 1 and 2 (***F***1 and ***F***2). (C) AFLP (E-AAC + M-CAC) fragments of cotton DNA isolated using the SDS/CTAB and Qiagen methods. (D) AFLP (E-AAC + M-CAA) fragments of ***Alternaria*** DNA isolated using the SDS/CTAB and chloroform methods. *Denotes DNA samples extracted with SDS/CTAB method. Standard DNA size ladders are denoted by "M".

cost and safety to personnel. The SDS/CTAB method provides both an inexpensive and safe alternative to these limitations and to the high cost of commercial isolation kits. Compared with the high cost of $3.04 per sample to use the commercial kit (Qiagen DNeasy Plant Mini Kit $760 for 250 samples), the cost of chemicals plus consumable supplies is approximately $0.33 per sample using the SDS/CTAB method. Expensive safety equipment or the waste disposal for toxic chemical such as phenol and chloroform is greatly reduced or eliminated with the SDS/CTAB method.

In summary, here we describe a simple, safe, and cost efficient SDS/CTAB DNA isolation method that provides high quality DNA from plant and fungi, including recalcitrant plant species that contain elevated concentrations of polysaccharide and polyphenolic compounds.

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