Detection of Plant Genes Using a Rapid, Nonorganic DNA Purification Method

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ABSTRACT

We have developed a simple procedure for the preparation of plant genomic DNA using FTA paper. Plant leaves were crushed against FTA paper, and the genomic DNA was purified using simple, nonorganic reagents. The 18S rRNA gene and the gene encoding the ribulose-1, 5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) from the chloroplast genome were detected by PCR amplification of DNA on FTA paper. DNA amplification was successful using extracts from 16 dicot and monocot plants. Studies of specific plant extracts revealed that extracts of leaf samples could be collected and stored at room temperature on FTA paper without a decrease in the DNA amplification success rate for more than a month. Both the 18S rRNA gene and the *rbcL* gene were detected in the genomic DNA isolated from various soybean cultivars stored in this manner. Furthermore, by modestly increasing the number of cycles of DNA amplification, we were able to detect the *uidA* gene in transgenic tobacco and rice leaves as well as a single copy gene linked to the resistance gene of cyst nematode race 3 using genomic DNA isolated on FTA paper. These results demonstrate that genomic DNA isolated using FTA paper can be used for the detection of plant genes, from a wide range of plants with either high or low gene copy number and of either nuclear or cytoplasmic origin.

INTRODUCTION

PCR is a common technique used in plant molecular research. Although PCR is a rapid method, isolation of plant genomic DNA is a critical but often laborious and time-consuming step for studying plants at the molecular level. Conventional techniques use organic solvents such as phenol or chloroform to inactivate enzymes that degrade genomic DNA and to denature proteins to release genomic DNA. In addition to the time and materials required for these techniques, the sol-

vents pose a potential health hazard and a considerable expense in their safe disposal. PCR DNA amplification can detect single copy genes in small amounts of plant tissue. Recently, a simple nonorganic genomic DNA purification procedure was developed for human blood and bacteria using FTA paper (1,8). After applying a small amount of a biological sample to FTA paper, genomic DNA within the paper was made accessible to PCR by washing the samples with nonorganic reagents. Furthermore, FTA paper can be used to collect DNA samples for long-term storage at room temperature. The possibility of high-throughput automation for genomic DNA purification using FTA paper has been described (8). These advantages make FTA paper attractive for plant genomic DNA purification.

In this paper, we developed the application of a new DNA purification procedure for plant tissues that is fast, simple and does not involve the use of organic chemicals. We collected leaf extracts from more than 16 different plant tissues on FTA paper. DNA was used as a template in PCR amplification to detect a high copy number nuclear gene (18S rRNA) and an abundant organelle gene ribulose-1, 5-bisphosphate carboxylase/oxygenase large subunit (*rbcL* gene), a low copy number gene (*uidA* gene) in transgenic plants and a single copy gene linked to the resistance gene of soybean cyst nematode race 3.

MATERIALS AND METHODS

Materials

Arabidopsis thaliana (L.) Heynh. Columbia, marijuana (*Cannabis sativa*), cassava (*Manihot esculenta*), coca (*Erythroxylum coca*), corn (*Zea mays*), orchid (*Arachins Aranda* Christine), papaya, (*Carica papaya*), Petunia, (*Petunia* spp.), opium poppy (*Papaver somniferum*), potato (*Solanum tuberosum*), rice (*Orzya sativa*), soybean (*Glycine max* L.), sugar-

Table 1. Detection of Plant Genes in Genomic DNA Isolated from Different Plants using FTA Paper

Plant	Numbers of positive assays/total PCR assays ^a		
	18S rRNA gene	<i>rbcl</i> gene	<i>uidA</i> gene
<i>Arabidopsis</i>	12/12	19/20	-
Cannabis	3/4	4/4	-
Cassava	4/4	4/4	-
Coca	7/8	6/8	-
Corn	17/18	16/18	-
Orchid	8/8	8/8	-
Papaya	3/4	3/4	-
Petunia	-	6/8	-
Poppy	3/4	8/9	-
Potato	8/8	8/8	-
Rice	37/37	22/23	49/49
Soybean	45/46	49/51	-
Sugarbeet	4/4	11/12	-
Sugarcane	-	7/8	-
Tobacco	22/22	17/18	15/16
Tomato	6/12	4/14	-

^aNumber of positive DNA bands based on the gel electrophoresis/number of total PCR assays. Genomic DNA was purified and amplified on FTA paper as described in Materials and Methods.

beet (*Beta vulgaris* L.), sugarcane (*Saccharum officinarum* L.), tobacco (*Nicotiana tabacum* L.) and tomato (*Lycopersicon esculentum*) plants were grown under standard greenhouse conditions at the USDA in Beltsville, MD. Transgenic plants containing the *uidA* gene encoding β -glucuronidase (GUS) were established by *Agrobacterium*-mediated transformation for tobacco (5) and by bombardment for rice (4). FTA paper, FTA purification reagent, Plant DNAzol[®], PCR SUPERMIX reagents were purchased from Life Technologies (Rockville, MD, USA).

Collection of Plant Tissue on FTA Paper

Newly developed leaves from *Arabidopsis thaliana*, *Cannabis sativa*, cassava, coca, corn, orchid, papaya, petunia, opium poppy, potato, rice, soybean, sugarbeet, sugarcane, tobacco and tomato were excised from the plants. Depending on the size of the leaves, either a whole leaf or approximately 1 g of a portion of the leaf was crushed on FTA paper. Mechanical disruption of leaves was performed by crushing the leaf tissues against the FTA paper using a hard object. After air drying the leaf extracts on FTA paper at room temperature for 1 h, one to two 2 mm circles of FTA paper containing leaf tissue were collected using a Harris Micro-Punch[™] (Life Technologies) and transferred into a thin-wall microcentrifuge tube.

Preparation of Plant Genomic DNA

The purification of plant genomic DNA using FTA paper was performed as recommended by the manufacturer. Briefly, 200 μ L of FTA purification reagent were added to a micro-

centrifuge tube containing two 2 mm circles of FTA paper impregnated with direct plant extracts of leaf tissue. The microcentrifuge tube was vortex mixed for 1–2 s at low speed. The tube was incubated at room temperature for 5 min, and the wash solution was discarded. The washes were repeated with FTA reagents two more times, followed by two more washes with 1 $\times$ TE buffer (10 mmol/L Tris, 0.1 mmol/L EDTA, pH 8.0). The FTA paper was air dried at room temperature for 1 h. The paper punches were then placed directly into PCR reagents for analysis. Isolation of plant genomic DNA from tobacco, rice and opium poppy using the cetyltrimethylammonium bromide (CTAB) method (3) or Plant DNAzol reagent (6) was performed as described previously.

Detection of Plant Genes

The PCR primers for the 18S rRNA gene were 5'-AACG-GCTACCACATCCAAGG-3' and 5'-CCGAAGGCCAACA-CAATAGG-3'. The PCR primers used for the *rbcl* gene were 5'-TTGGCAGCATTCGAGTAAC-3' and 5'-AAGTCCAC-CGCGAAGACATT-3'. The PCR mixtures contained 0.5 μ mol/L of each PCR primer in 50 μ L of PCR SUPERMIX. DNA amplification was performed at 94°C for 1 min, 55°C for 30 s, 72°C for 3 min for 30 cycles followed by a 10 min incubation at 72°C. For detection of the *uidA* gene from transgenic plants, the *uidA* primers (5'-AAACGGCAAGAAAAAGC-AGTC-3' and 5'-AGCGTCGCAGAACATTACATT-3') were added to 50 μ L of PCR SUPERMIX, and the same PCR con-

ditions were used for 40 cycles instead of 30 cycles. A similar PCR condition was performed for the detection of a single copy gene linked to the resistance gene of soybean cyst nematode race 3 using (5'-GCAGATATCAACAGTTGGGAC-3' and 5'-GGAATGGACAGCTCGAGAG-3') as the PCR primers. Approximately 10–25 μ L of PCR products were loaded onto a 0.8% (wt/vol) agarose electrophoretic gel and separated by gel electrophoresis. However, for the detection of the gene linked to the resistance of soybean cyst nematode race 3, a 2.0% (wt/vol) agarose gel was used. Gels were stained with ethidium bromide and examined using shortwave UV light.

RESULTS AND DISCUSSION

Detection of Plant High Copy Number Genes Using FTA Paper

The use of PCR-based molecular markers to construct mol-

ecular linkage maps and to study genomic diversity requires the isolation of DNA from different species and from a large number of plants of the same species. To examine the reproducibility of PCR amplification from DNA template purified by FTA paper, we prepared DNA from different species such as *Arabidopsis thaliana*, potato, cassava, soybean, orchid, tobacco, corn and rice as well as from nine different varieties of soybeans. The 18S rRNA nuclear PCR fragment and the *rbcL* chloroplast PCR product were seen in all different species (Figure 1). Similar results were obtained as we extended the FTA application into a total of 16 different species from a variety of both monocot and dicot plants (Table 1). Moreover, the genomic DNA purified by FTA paper from nine different varieties of soybean, after storage for one month at room temperature, was shown to have both the 18S rRNA nuclear PCR fragment and *rbcL* chloroplast PCR product after amplification (data not shown). These results demonstrate that FTA paper can be used to purify DNA for PCR amplification from various collections of plants with a high degree of reproducibility.

Figure 1. PCR products of different plant genomic DNA. Genomic DNA from different plants was isolated using FTA paper. PCR primers were designed to detect the *rbcL* gene (A) and 18S rRNA gene (B). PCR products from: *Arabidopsis*, lane 1; potato, lane 2; cassava, lane 3; soybean, lane 4; orchid, lane 5; tobacco, lane 6; corn, lane 7; and rice, lane 8. Each lane contains 25 μ L PCR products.

Figure 2. PCR products of transgenic rice genomic DNA. Genomic DNA was isolated from five different transgenic rice plants (1, 2, 3, 4 and 5) using Plant DNAzol reagent (A) and FTA paper (B). PCR primers were designed to detect an 872 bp DNA fragment of the *uidA* gene. For controls, plasmid DNA pBI121(P) or no genomic DNA (C) was used in the PCR amplification. Each lane contains 25 μ L PCR products.

The detection of plant genes by PCR amplification of FTA paper may, on rare occasions, miss some positive samples because of the variable DNA content on the paper matrix. However, in no case was a false positive sample produced. This minor variation of genomic DNA purification using FTA paper may be dependent on the existence of plant inhibitors such as polyphenolic compounds and polysaccharides. Because the amounts of the plant materials on a 2 mm circle of FTA paper is limited, it is difficult to accurately measure either the amounts of genomic DNA purified by FTA paper or the amounts of plant inhibitors on FTA paper. Screening samples in duplicate should assure the identification of >95% of positive genotypes in most plant systems tested (Table 1). In addition, the full-length 18S rRNA gene was detectable from rice genomic DNA purified by FTA papers. However, the PCR primers for the 18S rRNA gene or the *rbcL* gene were chosen for this study because of their highly conservative sequences among a wide range of plants.

Detection of Plant Low Copy Number or Single Copy Number Genes Using FTA Paper

In transgenic plants, only a few copies of foreign genes at most are normally integrated into the plant genome. DNA from transgenic tobacco and rice plants, containing the *uidA* gene was purified from plant leaf extracts and PCR amplified using FTA paper. The genomic DNA isolated by the Plant DNAzol method, which is described as similar to the conven-

tional CTAB method (6), was used for the comparison with those genomic DNA purified by FTA paper. The genomic DNA on the FTA paper served as a template for PCR amplification of an 872 bp fragment of the *uidA* gene. After 40 cycles of PCR amplification, the *uidA* gene was detected by gel electrophoresis in all the transgenic rice and tobacco plants tested, which varied from low expression to high expression of GUS (Table 1). Figure 2 shows an example of paired DNA extractions from transgenic rice using the Plant DNAzol method (lanes A) and the FTA method (lanes B) in which the 872 bp *uidA* gene fragment was amplified and run on an agarose electrophoretic gel. The amplified DNA products representing the 872 bp *uidA* gene fragment were the same size whether DNA was purified from FTA paper or isolated using Plant DNAzol reagents for template preparation. Similar results were observed using DNA from tobacco transgenic plants. These results demonstrate that DNA purified by FTA paper is useful for the detection of low copy genes in plant DNA and that the FTA technology is useful for screening putative transgenic plants for transformants.

To further examine the quality of DNA purified from FTA paper, DNA from soybean parental lines and F2 siblings was purified on FTA paper and PCR amplified. Separation of the amplified products by gel electrophoresis revealed the presence or absence of a low copy number molecular marker encoding aspartokinase-homoserine dehydrogenase (Figure 3). The gene that codes for this enzyme resides near the *Rhg4* locus that confers resistance to soybean cyst nematode race 3

(7,10). The assay for resistant soybean plants, derived from the cultivar Forrest, is characterized by two large amplified DNA products seen in the DNA isolated from Forrest. Susceptible plants were indicated by faster-moving, smaller DNA amplification products as seen in the DNA isolated from Essex (Figure 3). Banding patterns on agarose electrophoretic gels were clear and robust, with over 80% of the reactions providing scorable bands on a single PCR screen. In no case was a false positive indicated. However, a true positive could occasionally be missed. Duplicate screening assays are recommended for the scoring of a population of plants, while looking for low copy number genes.

FTA paper should be useful for scoring breeding populations using marker-assisted selection. For example, one assay used in this paper was to monitor an important soybean locus *Rhg4* that confers resistance to race 3 of the soybean cyst nematode. The PCR-based assay has been used successfully to characterize multiple soybean lines (2,7), and therefore has broad applicability. FTA paper can be used to collect and store DNA samples as template for marker assisted selection in breeding programs using PCR-based assay systems to monitor the inheritance of selected genes.

Freezing plant tissues in liquid nitrogen, followed by mechanical disruption, is a widely used procedure to homogenize plant tissues as an initial step in traditional DNA isolation procedures. Homogenization of plant tissue with liquid nitrogen is often a tedious mechanical process that includes some risk of dealing with potentially hazardous freezing solutions. The FTA DNA purification procedures described in this study are markedly easier than solvent extraction DNA isolation procedures. FTA only requires that the fresh plant tissue be crushed on the paper with simple physical pressure. It does not require the use of liquid nitrogen. Although a protocol for single-step preparation of plant samples for PCR analysis was described (9), it requires a standardized heating procedure and the optimization for both the preparation buffer and the PCR buffer such as salts, EDTA and pH before one can apply it in different plant samples. In contrast, only one

preparation procedure was applied in the plant DNA purification for all the different plant samples using FTA paper, and all the preparations were performed at the same room temperature. Moreover, one or two 2 mm diameter punches of FTA paper and a few steps of washing with nonorganic reagents can be used to isolate enough plant genomic DNA for several PCR-based gene detections. These results suggest that the automation of high-throughput plant DNA isolation is feasible. Also, FTA DNA storage should be useful to plant geneticists and breeders who wish to collect DNA samples from plants in the field and store the samples at room temperature. FTA paper represents a simple technique for obtaining, storing and isolating plant DNA.

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Figure 3. PCR products of a molecular marker close to a soybean gene conferring resistance to soybean cyst nematode race 3. Genomic DNA was isolated using CTAB (lane 4) or FTA paper (lanes 2, 3, 5 and 6). Soybean cultivar Forrest (lane 6) is resistant to soybean cyst nematode race 3, while Essex (lanes 4 and 5) and the F2 siblings (lanes 2 and 3) are sensitive. The negative control contained no DNA template (lane 1). The arrow indicates two polymorphic DNA fragments existing in the cultivar Forrest. A 1 kb DNA ladder is used as markers (lane M). Each lane contains 25 μ L PCR products.