Short Communication

A simple and rapid leaf genomic DNA extraction method for polymerase chain reaction analysis

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Abstract
In plants, secondary metabolites and polysaccharides interfere with genomic isolation procedures and downstream reactions such as restriction enzyme analysis and gene amplification. The removal of such contaminants needs complicated and time-consuming protocols. In this study, a simple, rapid and efficient method for leaf DNA extraction was optimized. This method use small amount of plant material to reduce inhibitory agents (alkaloids, phenolic). The procedure involves homogenization of the plant leaf in extraction buffer, incubation at 60°C, extraction by chloroform: iso-amyl alcohol and finally DNA precipitation by cold isopropanol. The results showed that the extracted DNA could be used directly for PCR.
Keywords: DNA extraction; Leaf tissue; Polysaccharide removal

Plant species often produce secondary metabolites, i.e. alkaloids, flavonoids, phenolic compounds, gummy polysaccharides, terpenes and quinine, which interfere with successful DNA isolation and follow-up reactions such as DNA digestion, amplification and cloning. Isolation and purification of genomic DNA from plant species are faced with problems that include: 1) degradation of DNA due to endonucleases 2) co-isolation of highly viscous polysaccharides and 3) co-isolation of inhibitor compounds like polyphenols and other secondary metabolites which directly or indirectly interfere with enzymatic reactions (Khanuja *et al*., 1999; Loomis, 1974). Polyphenols as powerful oxidizing agents can reduce the yield and purity of extracted DNA (Porebski *et al*., 1997).

The polymerase chain reaction (PCR) procedure used for crop analyses, especially during Genetically Modified (GM) screening requires high quality DNA to ensure successful amplification with reproducible results. Several DNA extraction procedures for isolating genomic DNA from various plant sources have been described. The Cetyl Trimethyl Ammonium Bromide (CTAB) method and its modifications (Huang *et al*., 2000; Doyle *et al*., 1987) were extensively used in different laboratories, but these methods are time consuming (Cheng *et al*., 2003). Other conventional DNA extraction protocols, which can remove some contaminants (Jobes *et al*., 1995), require large amounts of plant tissue to be grounded. On the other hand, these methods require long periods for plant growth and are not efficient for screening and analyzing transgenic plants. Other methods (Sharma *et al*., 2002) use liquid nitrogen, which is not considered to be safe. There are also a number of protocols which require small quantities of tissues, but these methods have limitations, such as the use of specialized apparatus (e.g. the matrix mill) (Hill-Ambroz *et al*., 2002). Today, there are numerous DNA isolation kits, but the main problem with these commercially available kits, is their high cost per sample (Ahmed *et al*., 2009; Kang *et al*., 2004).

Therefore, the need for a simple and efficient plant

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DNA extraction procedure is crucial when hundreds of samples need to be analyzed rapidly, for purposes such as mutant screening, GM analyses and marker assisted selection (MAS) programs. Furthermore, high purity DNA is required for PCR and other PCR-based techniques, such as random amplified polymorphic DNA (RAPD), micro- and macrosatellite analyses, restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) used for genome mapping and DNA fingerprinting (Khanuja *et al*., 1999).

In this study, a method used for the rapid extraction of genomic DNA from small amounts of plant material was modified for the purpose of PCR analysis. The method is applicable to a variety of plant species, and has many advantages, such as dispensing with the use of hazardous chemicals like phenol. Thus, it is fast and complete DNA extraction can be achieved within 2 h. This method requires small amount of plant tissue to reduce inhibitor agents and could extract optimal amount of DNA.

In the present study, samples for PCR analysis (usually leaf tissue) were collected into a sterile eppendorf tube. The tissue was softened for 15 s at room temperature, using a sterile tip as grinder, without any buffer, 500 µl of extraction buffer (200 mM Tris-HCl (pH 7.5), 250 mM NaCl, 25 mM EDTA, 0.5% SDS) (Edwards *et al*., 1991) was added to the tube and mixed for 5 s. In order to disrupt plant cells completely, the tube was placed in a water bath at 60ºC for 30 min. Subsequently, an equal volume of chloroform: iso-amyl alcohol (24:1) was added to the sample, which was then mixed and centrifuged at 15,000 g for 5 min, at 4ºC. The aqueous supernatant was transferred to a new eppendorf tube, to which an equal volume of isopropanol was added, mixed and incubated at -20ºC for 30 min. Following centrifugation at 15,000 g for 5 min, the pellet was dried and dissolved in (100 µl) TE buffer. Finally, for precipitation of starch and other insoluble polysaccharides, the tube was placed on ice for 5 min and centrifuged at 15000 g for 2 min; the resulting white pellet was mostly starch (Deshmukh *et al*., 2007). The supernatant containing the DNA was stable at 4ºC (Fig. 1). The purity of the extracted genomic DNA was confirmed through its A_{260}/A_{280} ratio (1.8), having a concentration of 10 µg/ml. For a standard PCR reaction, 2-10 µl of DNA solution will work well.

In order to demonstrate the efficiency of the method, genomic DNA was prepared from the leaves of 50 transgenic canola (*Brassica napus* L.) and tobacco (*Nicotiana tobaccum* L.) plants transformed with the mutated EPSPS (5-enol pyrovyl shikimate 3-phosphate synthase) gene (Kahrizi *et al*., 2007) and the antigenic synthetic gene from *Escherichia coli* O157:H7 (Amani *et al*., 2009). PCR was carried out with specific primers, using the following conditions: 95ºC; 1 min, 58ºC; 1 min, 72ºC; 1 min, for 35 cycles. The PCR products (10-15 µl) were resolved on an agarose gel (1%), which was subsequently stained with ethidium bromide and visualized under UV light (Fig. 2). By using this simple and rapid protocol, it was possible to isolate DNA and perform PCR for a large number of samples in a single working day.

The efficiency and the speed of this method together with the use of inexpensive facilities and the absence of toxic chemicals make the present method an attractive alternative for the extraction of plant DNA. These results show that the DNA produced by this simple, low cost, fast and safe protocol can be

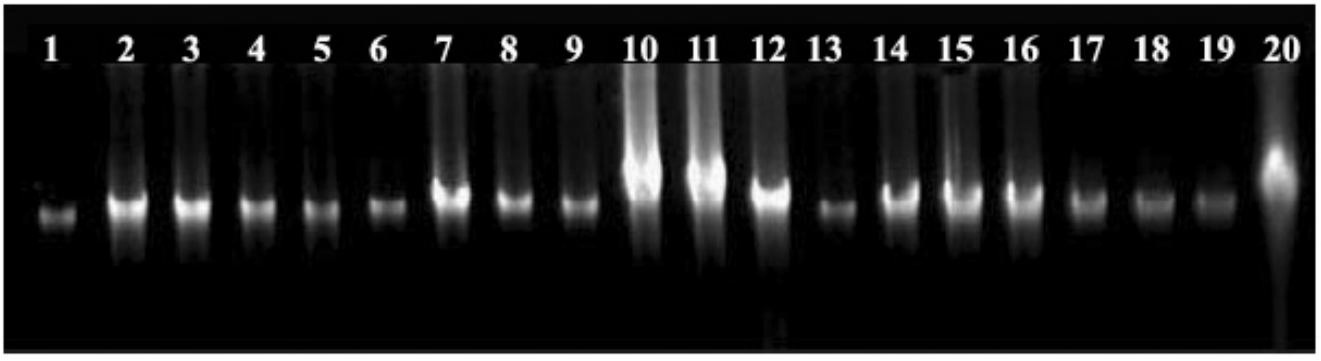

Figure 1. Electrophoresis of genomic DNA extracted by this protocol on 1% agarose gel in TBE 1X buffer: lanes 1-10, transgenic canola containing mutated EPSPS; and lanes 11-20 represent transgenic tobacco containing the antigenic gene from *E. coli* O157:H7 for screening of transgenic plants.

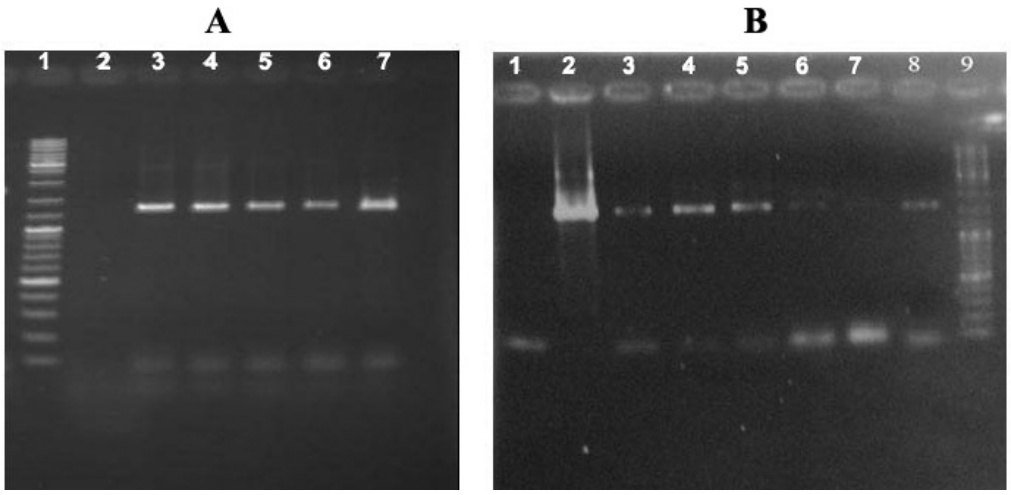

Figure 2. Electrophoresis of PCR products from genomic DNA on 1% agrose gel. A: PCR amplifications of DNA extracted from transgenic canola containing the mutated EPSPS gene; lane 1: DNA molecular weight marker (DNA ladder mix, Fermentas, Lithuania); lane 2: wild type canola as negative control; lane 3: plasmid containing the mutated EPSPS gene as positive control; lanes 4-7: transgenic canola. B: PCR amplifications of DNA extracted from transgenic tobacco plant containing the *E.coli* O157:H7 chimeric antigenic gene; lane 1: wild type tobacco as negative control; lane 2: amplification of plasmid containing antigenic genes as positive control; lanes 3-8: transgenic tobacco; lanes 9: DNA molecular weight marker (DNA ladder mix, Fermentas).

used in PCR-based techniques on a wide range of organisms, and in laboratories lacking state-of-the-art equipments and technology.

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