BM 2013, Vol.4, No.4, 19−22
http://bm.sophiapublisher.com

Research Report Open Access

Extraction of High-quality Intact DNA from Okra Leaves Despite Their High Content of Mucilaginous Acidic Polysaccharides

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Bioscience Methods 2013, Vol.4, No.4 doi: 10.5376/bm.2013.04.0004
Received: 29 Apr., 2013
Accepted: 28 May, 2013
Published: 04 Jun., 2013

Preferred citation for this article as:
Ahmed et al., 2013, Extraction of high-quality intact DNA from okra leaves despite their high content of mucilaginous acidic polysaccharides, Bioscience Methods, Vol.4, No.4 19-22 (doi: 10.5376/bm.2013.04.0004)

Abstract The presence of mucilaginous acidic polysaccharides in okra leaves interferes with the extraction of high-quality intact DNA for later restriction digestion and PCR amplification. We have developed a simple, efficient and reliable protocol to extract high-quality, intact DNA from the highly mucilaginous leaves of okra. The isolated DNA was free of contaminating agents like polysaccharides, protein and polyphenols and (A/260: A/280) ratio of 1.6~2.1 indicate minimal presence of other contaminating metabolites. The extracted DNA was digested with restriction enzyme AvaII and analyzed by PCR using random amplified polymorphic DNA primers. The extraction protocol is simple and does not require liquid nitrogen. The yield and quality of the resulting DNA are satisfactory, and the protocol can be scaled-up.

Keywords Okra, DNA extraction, Mucilaginous acidic polysaccharides, SDS

1 Background

Okra (*Abelmoschus esculentus* Moench) is an edible hibiscus of the Malvaceae family. It is often cross-pollinated and is a popular vegetable crop in the tropics. Okra is very useful against genito-urinary disorders, spermatorrhoea and chronic dysentery (Nandkarni, 1927). It has been reported to use as medicine in curing ulcers and relief from hemorrhoids (Adams, 1975). Previous studies have reported that 100 gm of okra endow with calcium (20%), iron (15%) and vitamin C (50%) of human dietary requirements (Grubben, 1977).

Leaves of okra have high mucilaginous acidic polysaccharides content forming pectin and its main component is polygalacturonic acid associated with minerals, which presents a major problem during the purification of okra DNA. When cells are lysed nucleic acids come into contact with these polysaccrides (Loomis, 1974). In the oxidised form these polyphenols bind covalently and irreversibly to proteins and nucleic acids (Guilletmaut and Marechal-Drouard 1992) resulting in a brown gelatinous material that reduces the yield and purity of the extracted DNA (Porebski et al., 1997; Aljanabi et al., 1999) and prevents it from redissolving completely. Furthermore, DNA which dissolves even in the presence of these polysaccharides inhibits the activity of different restriction enzymes (Sahu et al., 2012), PCR, or in vitro labeling.

The availability of high quality intact genomic DNA is a precondition for almost every molecular genetic analysis of crops. But when a plant tissue is rich in polysaccharide contaminants, Isolation of good quality DNA for PCR, gene mapping, diversity assessments and other molecular analyses is challenging, secondary metabolites and polyphenoles. Protocols for the isolation of DNA from tissues with high levels of polysaccharides and polyphenols have been reported (Dellaporta et al., 1983; Bernatzky and Taksley, 1986; Lodhi et al., 1994; Wang et al., 1996; Wang et al., 2011; Kim et al., 1997; Li et al., 2002; Geuna et al., 2004) but these did not give satisfactory results with okra.

Due to problems with DNA isolation, there have been few studies at the molecular level in okra. Appropriate

protocol for DNA Isolation from okra is necessary for such studies as PCR amplification (RAPD analysis) and restriction endonuclease. After following published protocols and failing to obtain high quality DNA that was not contaminated with polyphenolic compounds, we have developed an extraction system for okra genomic DNA. This protocol yields 8~10 μg high-quality intact genomic DNA from 100 mg okra mature and young leaves applicable to enzymatic digestion and PCR amplification in 1.5 mL tube without using liquid nitrogen.

2 Results and Discussion

We present a simple, efficient and reliable method to extract high-quality genomic DNA from okra without liquid nitrogen. We attempted to isolate DNA using a few traditional methods (Doyle and Doyle, 1990; Cheng et al., 2003; Couch and Fritz, 1990.) but failed to obtain high quality pure intact DNA from okra. The tissues of okra have high mucilaginous acidic polysaccharides content forming pectin and its main component is polygalacturonic acid associated with minerals. This presents a major contamination problem in the purification of okra DNA. Okra is undoubtedly the most difficult plant we have worked on but this method has enabled us to prepare the DNA with good spectral qualities.

The DNA prepared with this procedure was tested for yield, purity, susceptibility to restriction endonuclease digestion and ability to be amplified by PCR. We obtained approx. μg high-quality DNA 100 mg^{-1} fresh leaves, enough for 500 RAPD reactions. The A260/A280 of the extracted DNA ranged from 1.6~2.1 consistent with the ratio recommended for pure DNA preparations. The use of high concentration NaCl with isopropanol helps the release of DNA from polysaccharides. The polysaccharides settle in the bottom with NaCl and precipitated DNA remains suspended in the isopropanol in the upper layer. Undigested okra DNA showed a discrete band of unbreakable DNA (Figure 1).

DNA isolated by this protocol was accessible to a wide range of restriction enzymes. The restriction pattern of the isolated DNA from okra gave characteristics length distribution of digested chromosomal DNA. Repetitive bands were not visible (Figure 2).

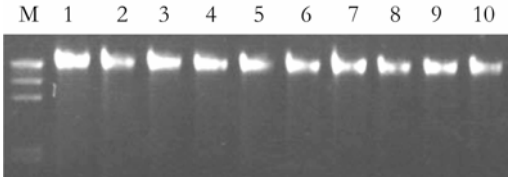

Figure 1 Agarose gel electrophoresis of unrestricted DNA prepared from 10 cultivars of okra

Note: DNA was resolved in a 0.8% (w/v) agarose gel; Samples are; M: DNA markers; 1~10: 300 ng DNA of 10 okra cultivars (Better Five, Marusaya Okra, Gokaku Okra, Blue Sky, Green Sword, Early Five, Lady Finger, Red Sun, Emarld and Benny, respectively)

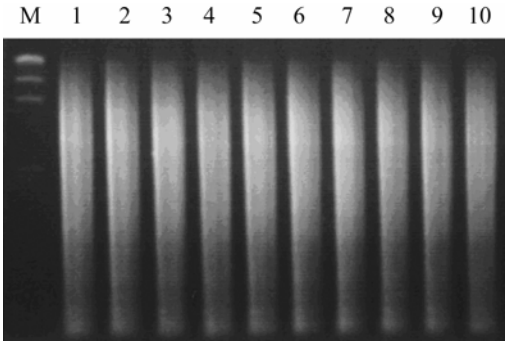

Figure 2 Agarose gel electrophoresis of total DNA (300 ng) digested with *Ava* II prepared from 10 cultivars of okra

Note: DNA was resolved on 0.8% agarose gel. Samples on gel are lane M: DNA marker; 1~10: Loaded with 300 ng DNA of okra cultivars (Better Five, Marusaya Okra, Gokaku Okra, Blue Sky, Green Sword, Early Five, Lady Finger, Red sun, Emarld and Benny, respectively)

To check the suitability of extracted DNA for further analysis, RAPD PCR was conducted. Reproducible amplification was observed in PCR reactions in several independent extractions and replications (Figure 3).

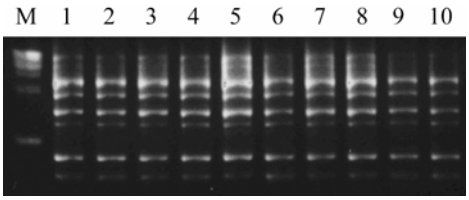

Figure 3 RAPD PCR amplification of okra leaf DNA

Note: Amplifications were performed using RAPDs 12-base oligonucleotide 5-GCCCCGTTAGCT-3 primer A04 (Operon); Samples on the gel are: M: DNA Marker; 1~10: Loaded with PCR amplified DNA of okra cultivars Better five, Marusaya Okra, Gokaku okra, Blue sky, Green sword, Early five, Lady finger, Red sun, Emarld and Benny, respectively

To prevent residual ribonucleosides from acting as primers during PCR amplification (Porebski et al., 1997), the DNA was made RNA free by using RNaseA.

Small amounts of okra leaf tissue are sufficient for successful DNA extraction, and many samples can be processed at the same time. The chances of contamination are low as reactions are carried out in screw-top Eppendorf tubes. No liquid nitrogen is required. The protocol was standardized for 100 mg fresh leaves, which can be handled in a 1.5 mL disposable Eppendorf tube. Similar conditions on a large scale, using 1~10 g samples, can be scaled up and fit into a 50 mL screw capped tube. The method may also be beneficial for other plant species tissues containing high levels of polysaccharides.

3 Materials and Methods

Extraction buffer: [Tris-HCl (pH maintained at 7.5 and 200 mmol/L conc.), Sodium chloride (250 mmmol/L), Ethylenediaminetetra acetic acid (25 mmmol/L), Sodium Dodecyl sulfate (0.5% w/v)]; Isopropanol; 5 mmol/L NaCl; Ethanol 75% (v/v); Tris EDTA: [10 mmmol/L Tris-HCL (pH 8.0), 0.1 mmmol/L Ethylenediaminetetra acetic acid (pH 8.0)]; 10 mg/ml, RNase A (Sigma); Phenol-chloroform; Chloroform-isoamylalcohol; Absolute ethanol; 3 mol/L sodium acetate (pH 5.2)

3.1 DNA extraction

Vacuum-dry [Eyela Freeze Dryer FDU 506 (Tokyo Rikakikai Co Ltd, Japan)] 100mg okra leaves in a 1.5 mL screw cap tube (Assist, Japan) then grind for 20 s at 3 000 r/min in multi-beads shocker (Yasui Kikai, Japan). Add 400 μL extraction buffer and mix by vortexing and leave the mixture for some time. Centrifuge at 10,000 xg. Take the supernatant; add 300 μL ice-cold isopropanol along with 100 μL 5 mol/L NaCl and invert gently to mix the ingredients. Incubate at -20℃ for approximately 1 h. Transfer the upper layer gently, without disturbing the polysaccharides to a new vial. Centrifuge at 10,000 xg for 10 min at room temperature. Wash the pellet with 75% (v/v) analytical grade ethanol. Dry the pellet and dissolve in 100 μL TE buffer. Add 1 μL (10 mg/mL) RNaseA (Promega) and incubate at 37℃. Add equal volume of phenol-chloroform (1:1, v/v) and centrifuge at 10,000 g for 5 min at room temperature. Transfer the supernatant to new tube and add one volume of chloroform-isoamylalcohol (24:1, v/v). Centrifuge at 10,000 xg for 5 min at room temperature and transfer the supernatant to a 1.5 mL eppendorf tube. Add 0.1 volume of 3 mol/L sodium acetate and 2.5 volumes absolute ethanol. Leave at -2℃ for 20 min. Centrifuge at 10,000 xg for 10 min at 4℃ and discard the supernatant then wash pellet with 75% (v/v) ethanol. Dry the pellet for 30 min and dissolve in 50 μL TE buffer.

3.2 DNA analysis

The quality of the extracted DNA was observed by electrophoresis, followed by staining with 0.5 mg^{-1} ethidium bromide and photographed using gel documentation system. DNA yield and quality was calculated using a NanoDrop ND-1000 spectrophotometer (USA) at 260 nm.

3.3 Digestion of DNA with restriction enzymes

DNA (300 ng) was digested for 3 h. at 37℃ with 1 U of *Ava* II in a total volume of 25 μL and electrophorased in agarose gel 0.8% (w/v) with undigested DNA as a control.

3.4 PCR amplification

RAPD PCR Amplification of okra leaf genomic DNA was carried out in a total reaction volume of 25 μL containing 20~25 ng genomic DNA, 1X Taq polymerase reaction buffer, 2 mmol/L $MgCl_2$, 0.1 mmol/L dNTP, 0.2 μmol/L 12-mer primer 5'-GCCCCGTTAGA T-3'; Operon A04 (Operon Technologies Inc., Alameda, Calif; USA) and 1.5 Units of polymerase (Taq DNA polymerase).

The amplifications were carried out using the icycler (BIO-RAD, USA) after 5 min at 94℃ for 1 cycle, followed by 35 cycles of 1 min at 94℃, 1 min at 35℃, and 3 min at 72℃, with a final 7 min extension of 72℃. Amplification products were separated in 3% (w/v) agarose-LE (nacalai tesque, Japan) TAE gels run at 100 V for 80 min.

Acknowledgements

We are thankful to Dr S. Niikura and N. Hashimoto for kindly providing okra seeds.

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