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Isolation and Amplification of DNA From Rhizomes of Turmeric and Ginger

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Abstract. Turmeric and ginger are used as spices and in alternative systems of medicine. They are rich in polysaccharides, polyphenols, and alkaloids. A simple and rapid method for isolating good quality DNA with fairly good yields from mature rhizome tissues of turmeric and ginger has been perfected. Isolated DNA was amenable to restriction digestion and PCR amplification.

Full text†: This manuscript, in detail, is available only in the electronic version of the *Plant Molecular Biology Reporter.*

Key words: *Curcuma,* DNA isolation, ginger, RAPD, rhizomes, turmeric, *Zingiber*

Abbreviations: CTAB, hexadecyltrimethylammonium bromide; EDTA, ethylenediaminetetraacetic acid; EtBr, ethidium bromide, PVP, polyvinyl pyrrolidone.

Introduction

Turmeric (*Curcuma longa* L) and ginger (*Zingiber officinale* Rosc) are rhizomatous spices that are also used worldwide in alternative systems of medicine. The whole plant is aromatic, but it is the underground rhizomes, fresh or processed, that are the valuable commodities. Rhizomes are the planting material in both species. Molecular marker technology is important in spices and medicinal and aromatic plants for identifying varieties/accessions, germplasm organization, identifying market samples, and gene cloning. Thus, isolating pure DNA of high molecular weight is essential. DNA extraction and amplification from ginger and turmeric leaf tissue has been described (Rout et al., 1998). However, a protocol to isolate and amplify DNA from recalcitrant tissue, like rhizomes, is needed because preserving leaf material for a long time is difficult and the leaf itself is not available during the off-season. In recalcitrant tissues, the presence of polysaccharides, polyphenols, alkaloids, and other secondary metabolites can hamper DNA isolation, amplification, restriction digestion, and cloning (Porebski et al., 1997;

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Weishing et al., 1995; Demeke and Adams, 1992; Do and Adams, 1991; Couch and Fritz, 1990; Webb and Knapp, 1990; Adams, 1990).

Here we describe a method for isolating and amplifying DNA from mature rhizomes of turmeric and ginger.

Materials and Methods

Reagents and chemicals (chemicals from Sigma Chemical Co., USA)

- 1 M Tris-Cl (pH 8)
- 0.5 M EDTA (pH 8)
- 5 M NaCl
- Chloroform-isoamylalcohol (24:1 [v/v])
- β-Mercaptoethanol
- N-cetyl N,N,N-trimethylammonium bromide (CTAB)
- Phenol-chloroform-isoamylalcohol (25:24:1 [v/v])
- Polyvinylpyrrolidone (PVP)
- *Taq* DNA polymerase (2 U/μL) (Finzymes)
- *Eco*R I and *Hin*d III (Bangalore Genei, India)
- *Taq* DNA polymerase buffer (Finzymes)
- Restriction endonuclease buffer (Bangalore Genei, India)
- dNTPs (10 mM) (Finzymes)
- Random decamer primers (Operon Technologies, Inc., USA)
- Extraction buffer: 100 mM Tris-Cl (pH 8), 20 mM EDTA, 2 M NaCl, 4% CTAB, 0.3% β-mercaptoethanol (added immediately before use), 1% PVP (w/v) (added immediately before use)

Plant samples for DNA isolation

Plant material samples were collected from fresh mature rhizomes of turmeric (30 accessions/varieties) and ginger (10 accessions/varieties). Rhizomes were washed in sterile distilled water, scraped, and sliced into thin pieces.

DNA isolation protocol

- Grind sliced rhizome samples (6 g fresh tissue) in liquid nitrogen.
- Transfer to a 50-mL polypropylene tube. Add 3 mL of freshly prepared pre-heated extraction buffer per gram of tissue.
- Incubate at 60°C in a water bath for 45-60 min, with occasional mixing.
- Add an equal volume of chloroform-isoamylalcohol (24:1). Mix by inversion for 15 min.
- Spin at 8410 *g* at 25-30°C for 10 min. Carefully transfer the clear upper aqueous layer to another 50-mL polypropylene tube.
- Add 0.6 volume of ice-cold ethanol.
- Incubate at -20°C for 2 h. Careful mixing will produce floating fibrous DNA, which can be separated by centrifugation at 13,141 *g* at 25-30°C for 10 min.
- Discard the supernatant and wash the pellet with 80% ethanol.
- Dry the pellet in a vacuum for 15 min. Dissolve in sterile double distilled water.
- Add 10 μg/mL of RNase A and incubate at 37°C for 30 min.

- Extract with an equal volume of phenol-chloroform-isoamylalcohol (25:24:1) and mix by inversion for 15 min. Centrifuge at 18,922 *g* at 25-30°C for 15 min.
- Transfer the aqueous layer. Add an equal volume of chloroform-isoamylalcohol (24:1) and mix by inversion for 15 min. Centrifuge at 18,922 *g* at 25-30°C for 15 min.
- Transfer the aqueous layer to a fresh 2-mL Eppendorf tube. Add an equal volume of ice-cold 100% ethanol to precipitate the DNA.
- Spin at 8410 *g* at 25-30°C for 5 min.
- Discard the supernatant and wash the pellet with 80% ethanol.
- Dry the pellet in a vacuum and dissolve in nuclease-free water.
- DNA concentration was measured by obtaining the $A_{260/280}$ ratio with a spectrophotometer. An aliquot (4 µL) of DNA from each sample was loaded on a 1% agarose gel to check DNA quality and concentration.

Restriction digestion

According to the supplier's instructions, 2 µg of DNA was restricted with *Eco*R I and *Hin*d III and visualized on 1% agarose gel.

PCR amplification

PCR amplification was performed in 25-µL reaction volumes (25 ng of DNA, 1 U *Taq* DNA polymerase, 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 5 pmol of decanucleotide primers) using a DNA Thermal Cycler (Gene Cycler, Bio Rad, USA). Amplified products were loaded in a 1.5% agarose gel containing 0.5 µg mL^{-1} of EtBr and documented by a gel documentation system (Bio Rad 1000, USA).

Results and Discussion

The presence of essential oils, polysaccharides, polyphenols, alkaloids, and other secondary metabolites in ginger and turmeric rhizomes can interfere with DNA isolation, PCR amplification, and gene cloning. Increased CTAB and NaCl concentrations have increased the yield of total cellular DNA in many monocots (Smith et al., 1991). A NaCl concentration greater than 1.5 M removes polysaccharides and permits subsequent manipulation of DNA (Fang et al., 1992.). Similarly, substituting ethanol for isopropanol in the last step of DNA precipitation has been shown to increase DNA yield and purity (Bult et al., 1992; Rogers and Bendich, 1985). We have successfully isolated DNA from mature rhizome samples of turmeric and ginger by following a modified CTAB method of Doyle and Doyle (1987). The original protocol was modified by precipitating in ethanol instead of isopropanol and by increasing concentrations to 4% CTAB, 2 M NaCl, 0.3% β-mercaptoethanol, and 1% PVP. The modified protocol yielded more than 14 µg of DNA per gram of fresh mature tissue. The increased concentrations of CTAB, PVP, β-mercaptoethanol, and NaCl helped to remove polyphenols, polysaccharides, and alkaloids from the sample. DNA yields ranged from 10.83-14.44 µg per gram of fresh mature tissue for turmeric and 10.13-13.38 µg per gram of fresh tissue for ginger. The average molecular weight of a single gel band was 220 kb for ginger and 200 kb for turmeric.

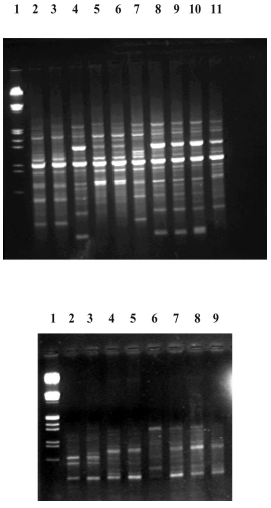

Figure 1. (*a*) RAPD profile of the DNA samples from turmeric amplified with primer OPA 7-(5'-GAAACGGGTG-3'). Lane 1, marker (*Eco*R I / *Hin*d III double digest); lane 2, IISR Prabha; lane 3, IISR Prathibha; lane 4, IISR Suvarna; lane 5, IISR Suguna; lane 6, IISR Sudarsana; lane 7, Alleppey; lane 8, Amalapuram; lane 9, Amrithapani; lane 10, Armoor; lane 11, Jabedi. (*b*) RAPD profile of the DNA samples from ginger amplified with primer OPE 7-(5'-AGATGCAGCC-3'). Lane 1, marker (*Eco*R I / *Hin*d III double digest); lane 2, Himachal; lane 3, Maran; lane 4, Rejatha; lane 5, Sabarimala; lane 6, Wyanad local; lane 7, Varada; lane 8, Ellakkallan; lane 9, Mahima.

DNA purity is a concern in extraction procedures. Restriction endonuclease digestion requires fairly clean and large quantities of DNA (Dowling et al., 1990; Doyle and Dikson, 1987). Random amplified polymorphic DNA and related techniques require less DNA, but purity is necessary to ensure repeatability and confidence (Welsh and McClellard, 1990; Williams et al., 1990). We successfully digested 2 µg of isolated DNA with 8 U of *Eco*R I and 10 U of *Hin*d III. The *Eco*R I digestion was more complete. DNA purity was determined from the $A_{260/280}$ ratio, which averaged 1.83-1.87 in all samples.

RAPD analysis of genomic DNA was performed according to Williams et al. (1990) using 25 ng of genomic DNA, 0.2 mM dNTP, 5 pmol of primer, 2.5 mM $MgCl_2$, and 1 U *Taq* DNA polymerase in a 25-µL reaction volume. Good amplification was obtained with various random decamer primers (Figures 1a-b). This protocol is suitable for isolating a good yield of good quality DNA from recalcitrant tissues such as rhizomes.

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