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Publish by Abstract

Isolation and PCR Amplification of Genomic DNA from Dried Capsules of Cardamom (*Elettaria cardamomum* M.)

S SYAMKUMAR, MRUDULA JOSE and B SASIKUMAR*
Division of Crop Improvement and Biotechnology, Indian Institute of Spices Research, Calicut, Kerala-673012, India

Abstract. Cardamom is an important spice, condiment and medicine, and international commodity. DNA-based molecular profiling will be aid in protecting the intellectual property rights of those who trade cardamom on the world market. Commercial cardamom has so far proven recalcitrant to traditional DNA extraction methods. In this paper we report a protocol for the isolation of amplifiable genomic DNA from traded cardamom. The method involves a modified CTAB (hexadecyltrimethylammonium bromide) extraction step, followed by a purification step to remove polysaccharides, proteins, and polyphenols, which are abundant in storage tissue such as cardamom capsules. The yield of DNA was 6-7 $\mu g\ g^{-1}$ tissue. Spectrophotometric and electrophoretic analysis indicated that the isolated DNA was highly pure and of high molecular weight. The isolated DNA could be amplified using different random decamer primers. The protocol has trade implications as it will help in the PCR-based characterisation of traded cardamom. This protocol can be further extended to develop Sequence Characterised Amplified Regions (SCAR) markers for profiling cardamoms.

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

Key words: *Elettaria cardamomum,* cardamom, DNA isolation, RAPD PCR

Abbreviations: AEGB, Alleppey Extra Green Bold; EDTA, ethylenediaminetetraacetic acid; GI, Geographical Indication of Goods Act; PEG, polyethylene glycol; RAPD, Random Amplification of Polymorphic DNA; WTO, World Trade Organization.

Introduction

Cardamom [*Elettaria cardamomum* (L.) Maton, Family-Zingiberaceae], considered by many as the “Queen of Spices” because of its pleasant mild aroma and taste, is used as a spice, condiment and medicine. The cardamom of commerce is

*Author for correspondence. e-mail: bhaskaransasikumar@yahoo.com; ph: 091-495-2731410.
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the dried ripe fruit (capsule) of the cardamom plant. The Geographical Indication of Goods Act (GI), envisaged in the WTO agreement (source: http://www.wipo.int.about-ip/en/geographical_ind.html; Anonymous, 2005), offers protection to reputed commodities that are linked with specific geographical regions of all countries, such as "Alleppey cardamom" (AEGB) of India. In addition to India, the other major cardamom exporting nations are Guatemala, Tanzania, Sri Lanka, El Salvador, Vietnam, Laos, Cambodia, and Papua New Guinea. Apart from accurate physical and chemical descriptions of the commodity, which are based on tangible and intangible features of the produce, DNA-based profiling methods will help to protect these commodities through GI by restricting unfair trade practices. We have developed an efficient protocol for isolating high molecular weight PCR-amplifiable DNA from dry capsules of Indian, Sri Lankan and Guatemalan cardamoms, which will help towards the development of protocols for the molecular profiling of traded cardamom.

Materials and Methods

Whole dried cardamom capsules were ground to a fine powder using a Cyclotech 1093 sample mill and used for DNA extraction by the following protocol:

- Homogenize 2 g of the powdered sample with 3 % CTAB buffer (8 ml g^{-1} tissue) containing 1 M Tris (pH 8), 0.5 M EDTA (pH 8), 5 M NaCl and 0.3 % β-mercaptoethanol and transferred to a 50 ml Oakridge tube.
- Incubate homogenate at 65°C for 3 h with intermittent shaking.
- Add an equal volume of phenol : chloroform : isoamyl alcohol (25:24:1) and mix by inversion for 15 min.
- Centrifuge at 8,000 *g* for 15 min at 4°C using a Superspin R –V/FA tube (Plastocrafts India Ltd.).
- Transfer the aqueous phase to a fresh Oakridge tube and add 0.6 volume of ice cold isopropanol.
- Precipitate the DNA by incubating overnight at -20°C.
- Centrifuge at 8,000 *g* for 15 min at 4°C.
- Dry the DNA pellets and dissolve in a minimal volume of sterile double distilled water.
- Add an equal volume of phenol : chloroform : isoamyl alcohol (25:24:1), mix for 5 min and centrifuge at 8,000 *g* for 15 min at 4°C.
- Add the aqueous phase to an equal volume of chloroform : isoamyl alcohol (24:1).
- Centrifuge at 8,000 *g* for 15 min at 4°C.
- Add the aqueous phase to an equal volume of 100 % ice cold ethanol. Incubate at -20°C for 2 h to precipitate the DNA.
- Centrifuge at 8,000 *g* for 15 min at 4°C.
- Dry DNA pellet under vacuum and dissolve in a minimal volume of nuclease-free water.
- Add an equal volume of 30 % PEG 8000 to the DNA solution, mix and incubate on ice for 10 min to precipitate the DNA.
- Centrifuge to pellet the DNA and dissolved in nuclease free water.
- Store frozen DNA samples at -20°C.

RAPD analysis

PCR amplification of the isolated DNA was done using random decamer primers obtained from Operon Technologies, Alameda, USA. The RAPD reaction was performed in a 25 µl reaction volume with 20 ng genomic DNA, 0.15 mM dNTPs 10 picomols primer, 2 mM $MgCl_2$ and 1U Taq DNA polymerase using a PTC-100 Programmable Thermal Controller (M.J. Research Inc., USA) according to the procedure of Williams et al. (1990). The amplified products were separated on a 2 % agarose gel, visualized by staining the gel in 0.5 µg ml^{-1} ethidium bromide, and documented with a gel documentation system (Alpha Imager, 2220, USA).

Results and Discussion

Cardamon capsules are storage organs and hence rich in proteins, polysaccharides, alkaloids and other secondary metabolites. These compounds can interfere with DNA isolation and amplification. Polyphenols and polysaccharides, for example, bind to nucleic acids during DNA isolation and interfere with subsequent reactions (Prittila et al., 2001). A suitable method for extracting DNA from recalcitrant tissue can only be determined by trial and error. In this study we report a method for the extraction and amplification of DNA from dried cardamom capsules, as we previously reported for DNA extraction from turmeric powder and fresh rhizomes of ginger and turmeric (Remya et al., 2004; Syamkumar et al., 2003).

It has been observed that increasing CTAB and NaCl concentrations improves the DNA yield from many plant species (Smith et al., 1991). CTAB is generally used to remove polysaccharides. Similarly, NaCl concentrations greater than 1.5 M remove polysaccharides (Fang et al., 1992). PEG 8000 may improve the quality and yield of DNA by removing certain proteins. Substituting ethanol for isopropanol has been shown to increase the yield and purity of DNA (Bult et al., 1992; Rogers and Bendich, 1985). DNA contaminated with secondary compounds and carbohydrates is especially difficult to amplify by PCR. The precipitation of DNA samples with polyethylene glycol (PEG 8000) yields highly purified homogenous DNA preparations devoid of proteins and polysaccharides (Agudo et al., 1995).

We have successfully isolated DNA from dried, powdered capsules of cardamom by following CTAB method of Doyle and Doyle (1987) after its modification (Figure 1a). In addition to the original Doyle and Doyle protocol, we also used the modified protocols of Singh et al. (1999) to isolate DNA from market samples of dry tea, and of Cheng et al. (1997) for dried rhizomes of *Coptis.* All of these methods failed to yield amplifiable DNA. Hence we modified the original Doyle and Doyle protocol by precipitating the DNA in ethanol instead of isopropanol, increasing the concentrations of CTAB, NaCI, and β-mercaptoethanol, adding a phenol : chloroform : isoamyl alcohol extraction step, and precipitating the DNA with PEG 8000.

Spectrophotometric measurements of DNA samples at 260 nm and 280 nm gave an absorbance ratio (A_{260}/A_{280}) of 1.82-1.91 indicating high purity. The quality of the DNA was also checked by agarose gel electrophoresis. We observed conspicuous bands of high molecular weight DNA about 22,000 bp with little

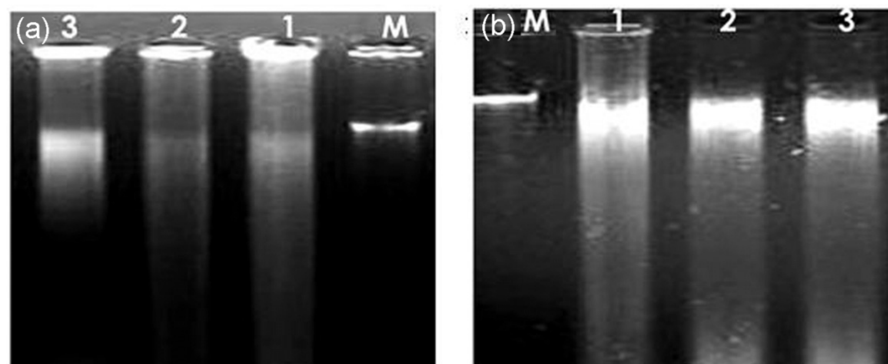

Figure 1. DNA isolated from different cardamoms. (a) DNA isolated using Doyle and Doyle method. (b) DNA isolated using the modified CTAB method described in this paper. M: Marker (Human Genomic DNA); Lane 1: Indian cardamom; Lane 2: Guatemalan cardamom; Lane 3: Sri Lankan cardamom.

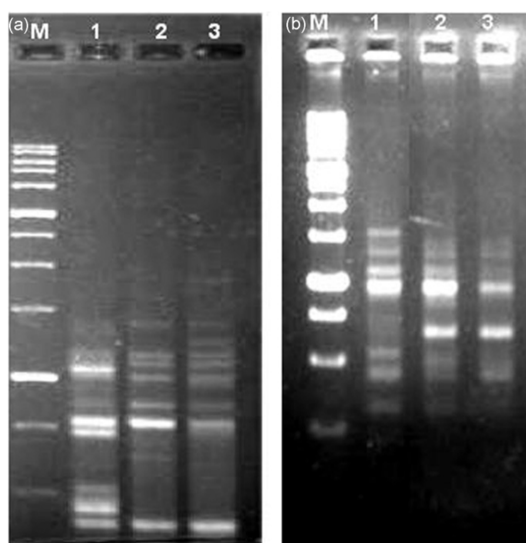

Figure 2. RAPD profile of DNA isolated from powdered capsules of Indian, Guatemalan and Sri Lankan cardamoms with: (a) primer OPA-10 (5′GTGATCGCAG3′); and (b) OPB-11 (5′GTAGAC CCGT3′). M: Marker (1 kb DNA ladder); Lane 1: Indian cardamom; Lane 2: Guatemalan cardamom; Lane 3: Sri Lankan cardamom.

shearing (Figure 1b). The yield of DNA isolated from the samples ranged from 6 to 7 μg g^{-1}.

The DNA isolated with this method was successfully amplified by RAPD PCR (Figure 2). This protocol will be aid in molecularly profiling traded cardamom, an important food commodity used worldwide.

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