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DNA Isolation Protocol from Dry Seeds of Cardamom (*Elettaria cardamomum* Maton) for Polymerase Chain Reaction Analysis

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ABSTRACT: *Small cardamom is the queen of all spices and is valued all over the world for its pungent and aromatic capsules. Nevertheless, Indian cardamom is often adulterated with seeds from small and large cardamoms from other countries. The present paper describes an efficient protocol for DNA isolation and PCR amplification of small cardamom dry seeds. The procedure would be useful to detect mixing of types by studying variability in molecular marker amplifications.Contrary to expectations, presence of proteins, polyphenols and polysaccharides have not limited DNA isolation from small cardamom dry seeds. A procedure as simple as slight modification of the DNA isolation protocol by Doyle and Doyle (1987) with phenol chloroform isoamyl alcohol mixture have worked well for isolation and purification of DNA, amenable for PCR amplifications.*

Keywords*: small cardamom, Elettaria cardamomum Maton, DNA isolation.*

INTRODUCTION

Small cardamom is a member of Zingiberaceae family and is valued all over the world for its pungent and aromatic capsules. It is popularly known as the 'queen of spices'. Small cardamom with a perennial, herbaceous and rhizomatous habit, is a shade loving plant and is cultivated in the evergreen forests of Western Ghats of South India. Apart from India, cardamom is cultivated in Guatemala, Tanzania, Sri Lanka, El Salvador, Vietnam, Laos, Thailand, Cambodia, Honduras, and Papua & New Guinea.

Cardamom seeds are often adulterated with seeds from lower grades and also from large cardamom (Korikanthimath, 2003)grape seeds, unroasted coffee grains and other aromatic seeds (Prakashan, 2009). To detect adulteration, DNA based molecular methods are considered more convenient and accurate when the adulterants are biological substances. However DNA isolation from dry material especially seeds have posed problems for researchers as contaminants such as proteins, polyphenols, and polysaccharides may interfere with enzymes such as restriction enzymes (in blotting techniques) and Taq polymerase in polymerase chain reaction (Ausubel *et al*., 1994). Several protocols have been described for isolation of DNA from polysaccharide and polyphenol rich plant materials (Porebski *et al*., 1997, Angeles *et al*., 2005). DNA isolation from seeds of several crops has also been documented (Chunwongse *et al*. 1993, Sharma *et al*. 2002, Li *et al*., 2007). But the chemical composition varies from plant to plant and tissues to tissues and hence variation in the DNA isolation protocols as well. Seeds being storage organs, will be rich in starch and lipids. Syamkumar *et al*. (2005) have reported that cardamom has so far proven recalcitrant to traditional DNA extraction methods and described a DNA extraction step, followed by a purification step to remove polysaccharides, proteins, and polyphenols, which are abundant in its storage tissues.

In the present study, a rapid and simple method for extraction of DNA from dry cardamom seeds was developed by modifying the existing protocols. The DNA was amenable to PCR techniques and was found suitable for detecting mixing of types as polymorphic amplification patterns were observed in different samples tested.

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MATERIALS AND METHODS

DNA Isolation

Dried / processed small cardamom capsules of released varietyICRI 1 and one mixed packed sample were collected from Indian Cardamom Research Institute. DNA was isolated using CTAB (Doyle & Doyle 1987) protocol comprising 3% CTAB, 20mM EDTA (pH 8), 100mM Tris (pH 8), 1.4M NaCl.1gm seeds were soaked in distilled water for 1hour and were surface sterilized using 70% ethanol for 1 minute. Seeds were then homogenized in50µl β mercaptoethanol in a pre-chilled mortar with a pestle to which 8ml pre-warmed CTAB extraction buffer (3%) was added. The homogenate was transferred to a 2ml centrifuge tube and after adding 10µl proteinase K the mixture was then incubated at 65 °C for one hour and 30 minutes. To this, equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) was added and thoroughly mixed and kept for 15 minutes at room temperature with intermittent mixing. After centrifugation at 10,000 rpm for 15 minutes at 4°C, supernatant transferred to a fresh tube and equal volume of chloroform- isoamyl alcohol mixture (24:1) was added, mixed carefully and further centrifuged at 10,000 rpm for 15 minute at 4 °C. About 1 ml ice cold isopropanol was addedto the supernatant and gently mixed, followed by a 3 hour incubation at-20°C to precipitate the DNA.After washing and centrifugation of the DNA pellet with 70% alcohol followed by 100% ethanol, the pellet was air dried and dissolved in 20µl sterile distilled water and stored at-20 °C for further analysis.

Qualitative and Quantitative Analysis of DNA

The qualitative analysis of the isolated DNA was done by agarose gel electrophoresis (0.8%) and DNA yield was measured in aUV spectrophotometer at 260 nm. DNA purity was determined by calculating the absorbance $A_{260/280}$ ratio.

DNA Amplification using ISSR PCR

ISSR analysis was performed in a 25µl volume of reaction mixture containing 1X Taq polymerase buffer, 2.5mM MgCl2, 1U of Taq DNA polymerase, 200µM dNTPs, 12 pmoles of universal ISSR primers UBC 807- AGA GAG AGA GAG AGA GT and UBC -840-GAG AGA GAG AGA GAG ACT (Genei, Bangalore) and 25ng of DNA. Amplifications were carried out using a thermal cycler (Biorad) with the following parameters: One cycle at 94 °C for 4min followed by 40 cycles of denaturation at 94 °C for 45sec, primer annealing at 49 °C for 1min and extension at 72 °C for 1min; and final extension at 72 °C for 10min. The products were size fractionated on 2% agarose gels and visualized under UV light after Ethidium Bromide staining.

RESULTS AND DISCUSSIONS

Exhaustive studies with respect to chemical composition of cardamom seeds have been documented (Chempakam and Sindhu 2008, Padma kumari *et al*. 2010) and it was found to contain volatile oils mostly terpinyl acetate, phenolics, starch and proteins. However, contrary to expectations and as per reports (Syamkumar *et al* 2005) that DNA isolation from cardamom seeds have limitations due to presence of volatiles, proteins, polyphenols and polysaccharides, the simple procedure outlined above could be efficiently utilized for isolating reasonably good levels of DNA. This protocol with modified usage of Proteinase K and Phenol – Chloroform - Isoamyl alcohol yielded good quality DNA which was free of protein and polyphenol contamination (Fig 1). DNA isolation protocols from both fresh and dry leaves of large cardamom, a related species (Mary *et al*. 2014) have posed problems due to polyphenol and polysaccharide interference and isolation was possible only after several trials. Similarly, seed tissues of several crops have exhibited such problems while extracting DNA (McDonald *et al.* 1994, Li *et. al.*, 2007). In the present study, qualitative and quantitative tests proved DNA to be of good quality with a 1.7-1.8 ratio in Spectrophotometer analysis and a yield of 5-6µg per gram seed tissue. PCR amplification using ISSR primers revealed polymorphisms as observed for leaf DNA samples of cardamom underlining the possibility of using seed DNA for differentiating Indian varieties of cardamom from traded mixed seed capsules.

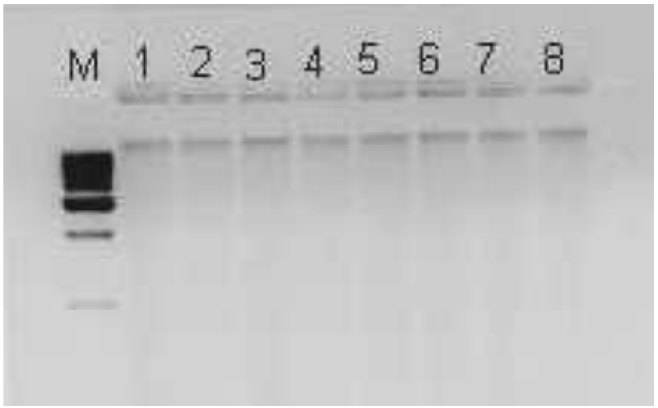

Figure 1: Gel picture showing DNA. M- 1kb ladder, Lanes 1-8 isolated DNA

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