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ORIGINAL ARTICLE

Molecular fingerprinting of *Helicanthus elastica* (Desr.) Danser growing on five different hosts by RAPD

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Abstract Mistletoes are hemiparasitic plants growing on aerial parts of other host trees. Many of the mistletoes are reported to be medicinally important. The hemiparasitic nature of these plants makes their chemical composition dependent on the host on which it grows. They are shown to exhibit morphological dissimilarities also when growing on different hosts. *Helicanthus elastica* (Desr.) Danser (mango mistletoe) is one such less explored medicinal mistletoe found on almost every mango tree in India. Traditionally, the leaves of this plant are used for checking abortion and for removing stones in the kidney and urinary bladder while significant antioxidant and antimicrobial properties are also attributed to this species of mistletoe. The current study was undertaken to evaluate molecular differences in the genomic DNA of the plant while growing on five different host trees using four random markers employing random amplified polymorphic DNA (RAPD) followed by similarity matrix by Jaccard's coefficient and distance matrix by hierarchal clustering analysis. Similarity and distance matrix data employing just 4 random markers, separately and the pooled data as well, revealed significant difference in the genomic DNA of *H. elastica* growing on five different hosts. Pooled data of similarity from all the 4 primers cumulatively showed similarity between 0.256 and 0.311. Distance matrix ranged from of 0.256 to 0.281 on pooling the data

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from all the four primers. The result employing a minimum number of primers could conclude that genomic DNA of *H. elastica* differs depending upon the host on which it grows, hence the host must be considered while studying or utilizing this mistletoe for medicinal purposes.

1. Introduction

Helicanthus elastica (Desr.) Danser (syn. *Loranthus elasticus* Desr. and *Dendrophthoe elasticus* (Desr.) Danser) belonging to family Loranthaceae is a less known underutilized medicinally important species occurring in India. The members of the Loranthaceae family, generally known as mistletoes and mostly distributed in the tropics, are semiparasitic shrubs attached to the hosts by modified root, generally called as haustoria. The leaves of this plant are used for checking abortion and for removing stones in the kidney and urinary bladder (Shanavaskhan et al., 2012). This aerial parasitic plant is grown on a wide range of hosts. Shinde et al. (2007) reported that the chemical composition of a plant species depends upon the genetic identity which gets modified as part of modifications in the physiology of the plant due to the environmental conditions in which the plant grows. To explore the genetic diversity of a plant species, molecular markers independent of environment have been successfully used. Different types of molecular based DNA fingerprinting techniques are in practice for plant materials (Powell et al., 1996). random amplified polymorphic DNA (RAPD) is a polymerase chain reaction (PCR)-based method where arbitrary short primers are used which anneal to complementary DNA sequences. If two such sites are closely situated on the DNA strain, amplification of the interjacent nucleotides are carried out in a PCR reaction (Williams et al., 1990). It is convenient in performance and does not require any information about the DNA sequence to be amplified (Weder, 2002). Ahmed et al., 2006 found RAPD as one of the best tools for detecting species variation among plants.

2. Materials and methods

2.1. Plant material

Tender shoots of *H. elastica* growing on *Croton oblongifolius* Sieber ex Spreng. – Euphorbiaceae (CO), *Mangifera indica* L. – Anacardiaceae (MI), *Samanea saman* (Jacq.) Merr. – Mimosaceae (SS), *Terminalia chebula* Retz. – Combretaceae (TC) and *Woodfordia fruticosa* (L.) Kurz. – Lythraceae (WF) were collected from the same locality in the Udupi district of Karnataka, India during September 2011. The plant materials were authenticated by Dr. Jacob Thomas, Plant Taxonomist and Curator of the Herbarium of the King Saud University, Riyadh, Saudi Arabia. The specimens were stored separately in a deep freezer at −20 °C immediately after collection until isolation of genomic DNA.

2.2. Genomic DNA isolation

The leaves were carefully crushed to a powder using dry ice. 50 mg of this powder was taken in an eppendorf tube. 1 ml of extraction buffer was added and macerated using the tissue homogenizer. The tubes were incubated at 60 °C for 1 h in a dry bath with intermittent mixing and centrifuged at 10,000 rpm for 10 min to separate the unlysed cells. Supernatant was transferred to a fresh eppendorf tube carefully. Equal volumes of Tris saturated phenol:chloroform:isoamyl alcohol (25:24:1) was added and mixed well and centrifuged at 10,000 rpm for 10 min. The aqueous layer was pipetted out into the fresh eppendorf tube without taking the interface. Equal volumes of isopropanol and 1/10th volume of 3 M Sodium acetate were added and mixed well and left at room temperature to stand for 5–10 min again, centrifuged at 10,000 rpm for 10–15 min. Then the supernatant was discarded. The pellet was washed with 300 µl of 70% ethanol, air dried and suspended in 30 µl of 1× Tris–ethylenediaminete traacetic acid (EDTA) buffer.

2.3. Purification of DNA

To the DNA solution 5 µl of RNAase was added and incubated in a water bath at 37 °C for 1 h. After 1 h it was removed from the water bath and 1 ml volume of chloroform: isoamyl alcohol (24:1) was added and gently mixed for 10 min. The solution was then centrifuged at 10,000 rpm for 20 min and the aqueous phase was pipetted out. The upper aqueous phase was separated after centrifugation and mixed with 1/10th volume of 3 M sodium acetate (pH 4.8). The DNA was precipitated by adding 2.5 ml of chilled absolute ethanol. The DNA pellet was carefully dried in laminar airflow. The dried DNA was dissolved in minimum amount of TE buffer (pH 8.0). The quantity of total DNA isolated was checked by adding 2 µl 6× orange loading dye (Fermentas, USA) to 2 µl of isolated DNA. Four micro liters of this isolate was loaded in a well of 0.8% w/v agarose gel containing 0.05% of ethidium bromide. Undigested lambda DNA (Fermentas, USA) was used as marker. Agarose gel electrophoresis was carried out for approx. 1 h at 50 V. The gel was visualized under UV light in a gel documentation system (Syngene, UK). The intact double-stranded DNA forming a thick single band of high molecular weight confirmed good quality DNA.

2.4. RAPD analysis

RAPD analysis was performed using 4 randomly and arbitrarily selected 10-base primers (A and B series) obtained from Operon Technologies Inc., Alameda, California. The four random decamer primers used were OPA-02 (TGCCGAGCTG), OPA-13 (CAGCACCCAC), OPA-18 (AGGTGACCGT) and OPB-10 (CTGCTGGGAC). polymerase chain reaction (PCR) was performed based on the protocol of Williams et al. (1990) with some modifications. All 5 DNA samples were diluted to 50 ng/µl and set for PCR. Amplification reactions were performed with 2.5 µl of 10× PCR buffer with 15 mM $MgCl_2$, 0.2 µl of dNTPs mixtures containing 0.2 mM