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Genetic diversity of mango cultivars estimated using SCoT and ISSR markers

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ABSTRACT

Two molecular marker systems, SCoT and ISSR were used for identification and genetic comparison analysis of 23 mango germplasm accessions collected within Guangxi province of China. Using 18 selected SCoT primers 158 bands were generated, of which 104 (65.82%) were polymorphic. Eighteen selected ISSR primers amplified 156 bands with 87 (55.77%) being polymorphic. The cultivars of Xiang Ya Mango type and their progeny have high genetic similarity with each other. The 23 cultivars were clustered into two major groups based on the SCoT analysis and three major groups based on the ISSR analysis with UPGMA. These clusters are in accordance with their known origins and main phenotypic characteristics. Our results indicated that the SCoT analysis better represents the actual relationships than ISSR analysis, although both analyses give similar results. The results also demonstrate that the SCoT marker system is useful for identification and genetic diversity analysis of mango cultivars.

1. Introduction

Mango (*Mangifera indica* L.) is an important member of the family Anacardiaceae and is an important fruit tree crop grown commercially in tropical and subtropical regions of the world (Rajwana et al., 2011). Mango genotypes are divided into two distinct categories: monoembryonic mangos, which are mostly subtropical (Indian types) and polyembryonic mangos, which are mostly tropical (Southeast Asian types). The seeds of Indian types characteristically contain a zygote embryo and the fruit skin is highly colored (mixes of red, purple, and yellow), while the seeds of Indo-Chinese types contain several nucellar embryos and the skin is not highly colored (green to light green to yellow) (Crane et al., 1997; Iyer and Degani, 1997; Viruel et al., 2005).

Mango is a highly cross-pollinated plant and most cultivars have arisen through selection of desirable types among naturally produced seedlings (Karihaloo et al., 2003). In the various mango growing regions, breeding programs are in progress to develop new disease resistant and productive cultivars. Widespread hybridization and recombination of characters has contributed extensively to the existing diversity in mango (Ravishankar et al., 2000). In addition, there has been a great degree of confusion in the identity of mango cultivars due to the use of synonyms for a single cultivar (Krishna and Singh, 2007). Understanding the genetic relationships among mango accessions through the use of DNA molecular fingerprinting techniques will assist breeders use mango germplasm collections for breeding programmes. Many different molecular markers have been used in mango for cultivar identification, such as RAPD (Rajwana et al., 2008; Marcela et al., 2009); AFLP (Yamanaka et al., 2006); ISSR (Sagar et al., 2007; Pandit et al., 2007) and SSR (Schnell et al., 2005; Schnell et al., 2006).

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The ISSR marker system detects polymorphisms in inter-microsatellite DNA regions without any prior sequence knowledge (Zietkiewicz et al., 1994). Primers are based on a repeat sequence, often with a degenerate 3′ anchor, and amplify the sequence between two microsatellites. Large numbers of amplification products per primer are produced, providing high reproducibility and low cost. ISSR markers have been widely used for cultivar identification in many species including banana, sorghum, Arabidopsis, sunflower, potato and others (Bornet and Branchard, 2001; Godwin et al., 1997). ISSR markers have been used for cultivar identification in mango. Eiadthong et al. (1999) utilized ISSR markers to analyze 22 mango cultivars and were able to distinguish genotypes, but were unable to find markers unique to either monoembryonic or polyembryonic types, or for the Thai cultivars selected for green harvest, or for the cultivars selected for ripe fruit production. Pandit et al. (2007) analyzed 70 mango cultivars using 33 polymorphic ISSR markers, and they were able to distinguish Indian from non-Indian cultivars, though they were not able to find marker differences between cultivars from South and North India.

The start codon targeted (SCoT) polymorphism is a novel, simple and reliable gene targeted marker technique based on the translation start codon (Collard and Mackill, 2009; Xiong et al., 2009). Primers for SCoT marker analysis were designed based on the conserved region surrounding the translation initiation codon, ATG (Joshi et al., 1997; Sawant et al., 1999). Using a single 18-mer primer as a forward and reverse primer in the PCR, and an annealing temperature of 50 °C, amplicons are resolved using standard agarose gel electrophoresis. Thirty six primers were designed by Collard and Mackill (2009) and successfully utilized in rice for cultivar identification and genetic diversity analysis. We designed another 44 new primers and successfully using these primers to analysis mango cultivars (Luo, 2010).

In Guangxi province of China, there are many mango cultivars which have been planted for several decades. Some of these mango cultivars are of the Xiang Ya Mango type. 'Xiang Ya Mango' is not a single mango cultivar, but a general name of which the shape of the fruit is similar to Ivory. Many mango growers and researchers cannot distinguish Xiang Ya Mango type from each other; so many different cultivars of Xiang Ya Mango type were directly called and recorded 'Xiang Ya Mango'. Moreover, there are many mango cultivars that originate from Xiang Ya Mango type; but there is little information about the genetic relationships about this group. Though a few cultivars of Xiang Ya Mango type have been analysis in the past, extensive studies with large collections of Xiang Ya Mango type are rather needed. In this study, SCoT and ISSR markers were applied to elucidate genetic diversity and genetic relationship within Xiang Ya Mango type. Our objectives are: (1) to access the genetic diversity and relationship within Xiang Ya Mango type for the purpose of providing appropriate germplasm management and clonal identification at field breeding stations; (2) to evaluate the usefulness of SCoT versus ISSR markers for identification the closest mango cultivars. To the best of our knowledge, this is the first report of employing SCoT marker to estimate the genetic diversity of Xiang Ya Mango type, and the first comparative analysis between SCoT and ISSR markers.

2. Materials and methods

2.1. Plant material

The experimental material used in the present study consisted of 23 mango cultivars (Table 1). The germplasm included collections from different mango growing regions of Thailand (3), Vietnam (1) and China (19). Four groups of cultivars that have a known family structure and a set of five unrelated cultivars were included (Fig. 1). The first family contains four maternal half-sibs (MHS) and the maternal parent 'Nang Klang Wan', as well as 3 s generation MHS. The second family contains one seedling from the maternal parent 'Aroemwnis No. 26' and two MHS of this seedling, while the third family contains two MHS and one paternal half-sib. The fourth family contains two MSH and the maternal parent 'Yellow Aroemwnis'. Young and healthy leaf samples were collected from the germplasm collections of Guangxi Academy of Agricultural Sciences, Guangxi University and Guangxi Subtropical Crops Research Institute (Fig. 2).

2.2. DNA extraction

From each accession, 0.5 g of young and healthy leaf tissue was collected and was either immediately used for DNA extraction, or stored at −30 °C before DNA isolation. Genomic DNA was isolated from the leaf tissues according to the CTAB method described by Doyle and Doyle (1990) with minor modifications (He et al., 2005). DNA samples were stored at −30 °C and the quality verified by electrophoresis on ethidium bromide stained 1% agarose gel.

2.3. SCoT analysis

All Start Codon Targeted (SCoT) primers were synthesized by Shanghai Sangon Biological Engineering Technology & Services (Shanghai, China). Eighty primers were initially screened, using three cultivars, for polymorphism and reproducibility. Each 20 μL amplification reaction consisted of 10 mM Tris–HCl (pH8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.25 μM primer, 1 unit rTaq polymerase and approximately 50 ng template DNA. rTaq polymerase and buffer were purchased from BoRi Biological Engineering company (BoRi; Hangzhou, China), dNTPs and agarose were purchased from Shanghai Sangon Biological Engineering Technology & Services (Shanghai, China). The PCR reaction was carried out in a T-Professional thermocycler (Biometra; Goettingen, Germany) as follows: an initial denaturation step at 94 °C for 3 min, followed by 36 cycles of 94 °C for 50 s, 50 °C for 1 min, and 72 °C for 2 min; the final extension at 72 °C was held for 5 min. PCR amplifications for all the primers were processed using the same procedure and all amplified products were resolved on 1.5% agarose