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Cultivar identification and genetic relationship of mango (*Mangifera indica*) in Taiwan using 37 SSR markers

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ABSTRACT

The genetic relationship of twenty-two mango (*Mangifera indica* L.) cultivars/lines was examined based on 37 SSR markers. Numbers of alleles per locus of the 37 SSR markers ranged from 2 to 11, and a total of 182 alleles with an average of 4.86 alleles per locus. Banding patterns obtained from 37 SSR primers allowed for each cultivar/line to be distinguished from the others with the exception of vegetative clones. The result indicates that SSR analysis is an efficient method for cultivar identification. Similarity coefficients were calculated based on 182 amplified bands. The dendrogram and genetic structure was showed according to those SSR markers. As expected, the genetic relationship between derived cultivars/lines and their parental cultivars are closely related based on the dendrogram. However, the SSR markers tested cannot separate monoembryonic from polyembryonic seeds of the mango cultivars/lines based on both dendrogram and genetic structure in this study.

1. Introduction

Mango belongs to the genus *Mangifera* of the family Anacardiaceae. The genus contains 41 species (Mukherjee, 1949), including several kinds of fruit trees. Nearly all mango varieties belong to one single species *Mangifera indica* L., which is the most important economic species in the family Anacardiaceae (Singh, 1968). Mango is a diploid fruit tree with $2n = 2x = 40$ chromosomes (Mukherjee, 1957). Its genome size is approximately 4.39×10^8 bp (Arumuganathan and Earle, 1991). This plant originated in tropical Asia, including the north-eastern region of India, the western region of Myanmar, and Bangladesh (Mukherjee, 1953). Mango cultivars are usually obtained through open pollination and seedling selection. There are more than a thousand varieties of mango under cultivation, but only a few of them are grown on a commercial scale. Commercialized mango cultivars have been propagated and cultivated all over the world (Ravishankar et al., 2000). It has been cultivated for over 4000 years (Mukherjee, 1972).

In general, mango cultivars belong to two distinct groups, i.e. polyembryonic types and monoembryonic types (Mukherjee, 1949). The seeds of the monoembryonic type contain only a zygote embryo, while the seeds of polyembryonic type contain several nucellar embryos (Iyer and Degani, 1997). Both polyembryonic and monoembryonic mango cultivars are commercially grown (Vasanthaiah et al., 2007). Traditionally, morphological and agronomical characters have been employed as criteria for mango cultivar identification (Singh, 1968). Nowadays, DNA markers have been widely used for genetic relationship and genotyping. Several types of DNA markers have been applied for cultivar identification, such as amplified fragment length polymorphisms (AFLPs) (Kashkush et al., 2001), random amplified polymorphic DNAs (RAPDs) (Adato et al., 1995; Schnell et al., 1995; Ravishankar et al., 2000), inter-simple sequence repeats (ISSRs) (Eiadthong et al., 1999), and microsatellites (i.e. simple sequence repeats, SSRs). SSRs are a class of molecular markers based on tandem repeats of short (2–6 bp) DNA sequences (Litt and Luty, 1989). The copy number of repeats is highly polymorphic, even among closely related genotypes (Brown et al., 1996). The codominant and high polymorphic characteristics of microsatellite loci make them useful for cultivar identification (Chiou et al., 2012) and hybrid evaluation (Liao et al., 2012; Chiang et al., 2013). Recently, microsatellite markers of mangoes have been developed by several research groups (Viruel et al., 2005; Schnell et al., 2005; Ravishankar et al., 2011; Chiang et al., 2012).

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Table 1
Mango cultivars/lines used in this study.

Code number	Cultivars/lines	Geographic origin	Embryo type
M1	Tsar-Swain	Southeast Asia	Polyembryonic
M2	Haden	Florida	Monoembryonic
M3	Jin-Mi	Taiwan	Monoembryonic
M4	Tainung No. 1	Taiwan	Monoembryonic
M5	Jin-Huang	Taiwan	Monoembryonic
M6	Irwin	Florida	Monoembryonic
M7	Keitt	Florida	Monoembryonic
M8	Red Keitt	Taiwan	Monoembryonic
M9	Sensation	Hawaii	Monoembryonic
M10	Yu-Win No. 6	Taiwan	Monoembryonic
M11	Jin-Xing	Taiwan	Monoembryonic
M12	Shan-Lin No. 1	Taiwan	Monoembryonic
M13	Kaohsiung No. 3	Taiwan	Monoembryonic
M14	Kaohsiung No. 3-clone 1	Taiwan	Monoembryonic
M15	Local line-8302	Taiwan	Polyembryonic
M16	Local line-8304	Taiwan	Monoembryonic
M17	Local line-8310	Taiwan	Polyembryonic
M18	Chok-Anan	Southeast Asia	Polyembryonic
M19	Kaohsiung No. 3-clone 2	Taiwan	Monoembryonic
M20	Kaohsiung No. 3-clone 3	Taiwan	Monoembryonic
M21	Local line-8302-1	Taiwan	Monoembryonic
M22	Tsar-Swain-clone 1	Southeast Asia	Polyembryonic

Mango is one of the important economic fruit crops in Taiwan. The cultivation area of mango in Taiwan was around 12,000 ha in 2011 (Yearly Report of Taiwan's Agriculture, 2012). The breeding program of mango in Taiwan has been conducted since 1970 (Lee et al., 2009). There are over 40 different mango cultivars/lines in Taiwan. Most of them were selected by growers derived from open pollination with unknown pollen sources (Lee et al., 2009), or lines with unknown genetic background. Therefore, there is much difficulty in identifying the existing cultivars/lines in Taiwan. In the present study, 37 SSR primers were used to investigate the genetic relationship among 22 mango cultivars/lines in Taiwan.

2. Materials and methods

2.1. Plant materials

Twenty-two mango samples were collected and cultivated at the Kaohsiung District Agricultural Research and Extension Station (KDARES), Taiwan and growers' orchards. Among them, five are polyembryonic and seventeen are monoembryonic. The cultivars used in this study are listed in Table 1. Voucher specimens were deposited at the herbarium of the National Museum of Natural Science, Taiwan (TNM).

2.2. DNA extraction and PCR amplification

Genomic DNA was extracted from mature leaf powder using the protocol from the Plant Genomic DNA Miniprep System Kit (Viogene, Taipei, Taiwan). Five (MiSHRS series), seventeen (MiI-IHR series), and fifteen (Min series) polymorphic SSR markers were respectively derived from Schnell et al. (2005), Ravishankar et al. (2011), and Chiang et al. (2012) for evaluating the genetic relationship of the 22 cultivars/lines in the study.

In the study, the designed forward primers for the 37 SSR markers were elongated from the M13 (-21) 18 bp sequence (5′-TGTAAAACGACGGCCAGT-3′) by fluorescent labeling (Schuelke, 2000). The designed primer pairs were first tested for PCR amplification and then used to amplify the 22 mango cultivars/lines after optimization. PCR conditions were as follows: total volume 25 μl with 20 ng of template DNA, 1× PCR buffer, 0.2 mM of each dNTP, 0.2 mM of each SSR specific primer and, 0.25 U *Taq* DNA polymerase (Promega, Madison, Wisconsin, USA). Two-step PCR amplification was conducted. The first thermocycling profiles were initial denaturation at 94 °C for 3 min, followed by 20 cycles of 30 s denaturation at 94 °C, 30 s annealing at 58 °C, 40 s extension at 72 °C, and a final extension for 7 min at 72 °C. After that, 0.075 mM M13 primer 5′-labeled with IRDye was added into the above PCR reaction mixture. The second thermocycling profiles were initial denaturation at 94 °C for 3 min, followed by 10 cycles of 30 s denaturation at 94 °C, 30 s annealing at 58 °C, 40 s extension at 72 °C, and a final extension for 7 min at 72 °C. Samples were denatured in loading dye (10 mg/ml blue dextran in formamide) and separated using 6.5% polyacrylamide gel (19:1, 7 M urea) electrophoresis in a LI-COR 4300 DNA analyzer (LI-COR, Lincoln, Nebraska USA). Fragment lengths were determined with the aid of an external standard (50–500 bp, GE Healthcare, USA) and with an in-house amplified internal standards using Allele Locator 1.03 software (Amersham Biosciences).

2.3. Data analysis

One hundred eighty-two reproducible bands from 37 SSR primers were scored by length variation as codominant markers for the 22 cultivars/lines tested. The genetic dissimilarity between mango individuals was calculated using the methods developed by Bowcock et al. (1994) and Ciampolini et al. (1995) based on pair wise inter-individual comparisons resulting in a multilocus genetic similarity value complementary to the multilocus genetic distance (Dm) and modified to the genetic dissimilarity by (1-Dm). Cluster analysis was generated from the pair-wise dissimilarity matrix by the unweighted pair-group method (UPGMA) using a molecular evolutionary genetics analysis program (MEGA, version 5.05, Tamura et al., 2011).

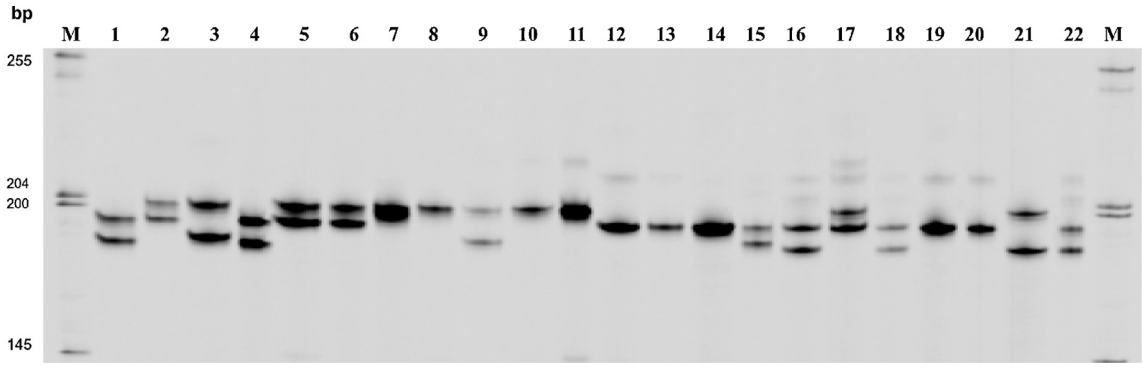

Fig. 1. Min-2 SSR locus analysis of polymorphism in 22 mango cultivars/lines. M: DNA marker. Lanes 1–22 represent different cultivars/lines (M1–M22, see Table 1).