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Genetic diversity and species-specific PCR-based markers from AFLP analyses of Thai bananas

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ABSTRACT

A large amount of banana genetic resource has been found in Thailand which is believed to be one of the centers of its origins. To assess genetic diversity and determine genetic relationships of edible bananas in Thailand, 110 accessions of banana species and cultivars collected from villages and natural locations were investigated. UPGMA clustering of numerical data from Amplified Fragment Length Polymorphism (AFLP) patterns showed two large groups which corresponded to genome designations of *Musa acuminata* (AA) and *Musa balbisiana* (BB), the known ancestors of most edible cultivars. The AFLP data suggested that among Thai bananas, AA and AAA cultivars were closely related to *M. acuminata* subsp. *malaccensis*, while some of 'B' genome contained ones closely related to wild *M. balbisiana* in Thailand and some may have been imported. Eight species-specific PCR-based primer pairs, generated from the AFLP results clearly identify 'A' and 'B' genomes within cultivars and hybrids. The analyses were useful to readily and easily infer progenitors of these cultivars and pronounce wide genetic diversity of the bananas in Thailand.

1. Introduction

Banana (*Musa* L.) is one of the most important tropical and subtropical food crops for millions of people worldwide (Loh et al., 2000; Noyer et al., 2005). Banana export value ranks fourth among the most important crops in the world after rice, wheat, and maize, while that of Thailand is the third in Southeast Asia (FAO, 2007). Major export cultivars are *Musa* (AAA) 'Kluai Hom Thong', *Musa* (AA) 'Kluai Khai' and *Musa* (ABB) 'Kluai Namwa' respectively (DFT, 2008; OAE, 2008).

Most cultivated bananas are seedless and belong to the *Musa* section. It is believed that they were derived or originated from parthenocarpy, seed sterility, polyploidization, and intra and interspecific hybridizations of two diploid species, *Musa acuminata* Colla (AA) and *Musa balbisiana* Colla (BB) (Simmonds and Shepherd, 1955; Simmonds, 1962; Valmayor et al., 2000; Ude et al., 2002b, 2003b). Hybrids that naturally evolved from the two species included diploids, triploids and a few

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tetraploids in various genome combinations such as AA, AB, AAB, ABB, BBB and AABB (Simmonds, 1962; Valmayor et al., 2000; Doležel et al., 1998). The dispersal of edible bananas outside Asia was accomplished solely by transport of vegetative planting materials by human agencies (Simmonds, 1962).

Conventional classification of *Musa* cultivars is based on 15 morphological traits and chromosome numbers (Simmonds and Shepherd, 1955; Silayoi and Babpraserth, 1983; Chomchalow and Silayoi, 1984; Silayoi and Chomchalow, 1987). Recently, molecular markers are commonly used not only for classifications, but also identifications and parental assessments. The markers included Restriction Fragment Length Polymorphisms (RFLPs) (Nwakanma et al., 2003a,b; Ge et al., 2005), Random Amplified Polymorphic DNA (RAPD) (Pillay et al., 2001; Onguso et al., 2004; (Uma et al., 2004)), and microsatellite markers (Creste et al., 2003, 2004). Among DNA markers, the amplified fragment length polymorphism (AFLP) technique is being widely used for genetic diversity studies because it reveals significant polymorphisms and is a reliable and robust molecular marker assay (Vos et al., 1995; Mueller and Wolfenbarger, 1999; Crouch et al., 1999; Ude et al., 2002b). It has been recently employed in many plant systematic studies, e.g. among rice varieties (Singh et al., 1999), *Coffea arabica* cultivars (Steiger et al., 2002), soybean (Ude et al., 2003a), and *Aglaonema* species and cultivars (Chen et al., 2003).

In banana, AFLP clearly distinguished taxonomical differences among sections of the genus *Musa* (Ude et al., 2002a; Wong et al., 2001b, 2002) and subspecies of *M. acuminata* (*malaccensis*, *microcarpa*, and *truncata*) (Wong et al., 2001a). The study of Ude et al. (2002b) also showed that there is wild diversity within *M. balbisiana*, as confirmed by Wang et al. (2007). AFLP could be used to identify cultivars by their unique banding patterns and develop specific probes for identification purposes (Loh et al., 2000). AFLP technique can also be used to assess genetic diversity and identify relationships between the cultivated clones of bananas (Loh et al., 2000; Rubaihayo et al., 2002; Ude et al., 2002a,b, 2003a,b; De Langhe et al., 2005).

Thailand is believed to be one of the centers of origin and possesses great diversity of banana species and cultivars. Combined with advanced molecular technology, these valuable germplasm could play an important role in modern breeding programs. The information will be beneficial for germplasm management and conservation and ensure our sustainable food security.

The objectives of this study were to assess genetic diversity and relationships among accessions of the cultivated bananas in Thailand using AFLP markers. PCR-based primer pairs designed from unique banding patterns were proved to be ready and easy molecular markers which characterize the parental taxa.

2. Materials and methods

2.1. Plant materials

A total of 110 *Musa* species and cultivars (Table 1) were collected from wild habitats and cultivations in Thailand. The *Musa* species, identified based on Simmonds (1959, 1962), Argent (1976), De Langhe et al. (2002) as described in Athawongsa (2008), included 24 accessions of *M. acuminata* from four subspecies *i.e. siamea*, *malaccensis*, *microcarpa*, and *truncata*, and 18 accessions of *M. balbisiana*. Sixty-six accessions of the banana cultivars were from six genome designations, *i.e.* AA, BB, AAA, AAB, ABB/BBA, and BBB. Two accessions of *Musa laterita* Cheesman were used as outgroup. Dry and in-spirit specimens were collected and deposited at Suan Luang Rama IX Herbarium, Prawet, Bangkok 10260, Thailand. Living specimens were planted ex situ in Nakhon Pathom province Thailand. Cigar (youngest unfurled) leaves were collected for DNA extractions.

2.2. Genomic DNA extraction and AFLP procedure

Approximately 5 g of cigar leaf was ground in liquid nitrogen with mortar and pestle. Isolation of total DNA was done following the protocol described by Doyle and Doyle (1990), which is a CTAB-based extraction. DNA concentration was estimated using spectrofluorometric measurement with H 33 258 fluorescent dye (Hoefer DQ 200 fluorometer).

The AFLP procedure was carried out as reported by Vos et al. (1995) with a few modifications. Approximately 100 ng/μl of DNA was digested by two restriction enzymes, *i.e. Eco*RI and *Mse*I or *Tru*9I in 10× buffer A (Promega) and incubated for 2 h at 37 °C or 1 h at 37 °C and 1 h at 65 °C, respectively, if digest with *Tru*9I. The restricted DNA fragment was ligated to *Eco*RI-adapter and *Mse*I-adapter overnight at 37 °C to generate template DNA for amplification. Five microliters of the 1:10 diluted DNA template generated was first pre-amplified (Px2 Thermal Cycler; Thermo Electron Corporation, USA) using *Eco*RI + A and *Mse*I + C primers (Loh et al., 2000; Wong et al., 2001a,b, 2002; Ude et al., 2002a,b, 2003b). Then the pre-amplified DNA was diluted to 1:9 with sdH_2O and 3 μl of the product were used for selective amplification in a reaction tube containing 20 μl of selective amplification mixtures. AFLP adapters and eight primer pairs (E + AAC/M + CAA, E + AAG/M + CAC, E + ACA/M + CAG, E + ACC/M + CTA, E + ACG/M + CTC, E + ACT/M + CAT, E + AGC/M + CTG and E + AGG/M + CTT) were used for the selective amplification as of Vos et al. (1995), Loh et al. (2000), Wong et al. (2001a,b, 2002), and Ude et al. (2002a,b, 2003b). The final PCR products were run on a 4.5% denaturing polyacrylamide gel electrophoresis in 1× TBE buffer in a Sequi-Gen GT Sequencing Cell (Bio-Rad, USA). DNA fragments on gels were visualized using silver nitrate staining protocol (Bassam et al., 1991). The gel was rinsed with distilled water and air-dried on mirror plates.