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Genetic relationship among subspecies of *Musa acuminata* Colla and A-genome consisting edible cultivated bananas assayed with ISSR markers

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Abstract

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Genetic relationship among subspecies of *Musa acuminata* and A-genome consisting edible cultivated bananas was investigated by ISSR (inter-simple sequence repeat) markers. Twelve samples of wild type bananas that were classified into 5 subspecies of *Musa acuminata*, thirty-three samples of edible cultivated bananas and *M. balbisiana* were used as plant materials for this study. Of a total of 36 ISSR primers screened, 6 primers revealed a total of 128 alleles, allele size varied from 200 to 3,000 bp with an average of 21.33 alleles per primer, average of allele frequency was 0.18, polymorphic percentage was 1.0 and heterozygosity was 0.29. From the dendrogram, banana samples can be divided into two main clusters with similarity coefficient value at 0.18. The first cluster belonged to the out group which included *Musa itinerans* and *Ensete glaucum*, the second cluster belonged to *Musa coccinea, M. laterita*, all subspecies of *M. acuminata, M. balbisiana* and all the cultivar groups of the edible cultivated bananas and plantains. In addition, the results indicated two *Musa* species, consisting of *M. coccinea* and *M. laterita*, were sister group of the second cluster as well. All specimens of subspecies of *M. acuminata* were related to cultivated groups of A-genome consisting of cultivated bananas in Thailand.

Key words : *Musa acuminata*, A-genome consisting edible cultivated banana, genetic relationship, ISSR marker, banana genetic resources

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บทคัดย่อ

พฤทธิ์ ราชรักษ์ และ วิชาญ เอียดทอง

ความสัมพันธ์ทางพันธุกรรมระหว่างชนิดย่อยของกล้วยป่า (*Musa acuminata* Colla) และกล้วยปลูกกลุ่มจีโนม A โดย ISSR markers

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ความสัมพันธ์ทางพันธุกรรมระหว่างชนิดย่อยของกล้วยป่า *Musa acuminata* และกล้วยปลูกที่มี A-genome เป็นองค์ประกอบโดยใช้ ISSR markers พบว่า ตัวอย่างกล้วยป่า 12 ตัวอย่าง สามารถจำแนกชนิดย่อยด้วยลักษณะสัณฐานวิทยาได้ 5 ชนิดย่อย ประกอบกับกลุ่มพันธุ์กล้วยปลูก 33 ตัวอย่างรวมทั้งกล้วยตานีถูกนำมาวิเคราะห์หาความสัมพันธ์ทางพันธุกรรม โดยใช้ไพรเมอร์ 6 ไพรเมอร์ จากทั้งหมด 36 ไพรเมอร์ ให้แถบดีเอ็นเอที่สามารถตรวจสอบได้ 128 แถบ มีขนาดระหว่าง 200-3,000 bp มีจำนวนอัลลีลต่อไพรเมอร์ 21.33 ค่า allele frequency เท่ากับ 0.18 ค่า polymorphic percentage เท่ากับ 1.0 และค่า heterozygosity เท่ากับ 0.29 จากผลการวิเคราะห์ด้วยไพรเมอร์ดังกล่าวแสดงผลในรูปแบบของแผนภาพความสัมพันธ์ทางพันธุกรรม พบว่าสามารถจัดกลุ่มตัวอย่างได้ 2 กลุ่ม จากค่า coefficient value เท่ากับ 0.18 คือ กลุ่มที่ 1 ประกอบด้วย กล้วยหกและกล้วยนวล และกลุ่มที่ 2 ประกอบด้วย กล้วยรัตกัทรี กล้วยไหล ชนิดย่อยของ *Musa acuminata* ทุกชนิดย่อย กล้วยตานี รวมทั้งกลุ่มพันธุ์กล้วยปลูก ในการศึกษาครั้งนี้ชี้ให้เห็นว่า *Musa acuminata* ทุกชนิดย่อยมีความสัมพันธ์ทางพันธุกรรมกับกลุ่มพันธุ์กล้วยปลูกจีโนม A ที่เป็นองค์ประกอบ

ภาควิชาชีววิทยาป่าไม้ คณะวนศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ จตุจักร กรุงเทพฯ 10900

The Musaceae is a large family of order Zingerberales which has been classified into three genera; *Musa, Ensete* and *Musella*. The genus *Musa* is divided five sections by chromosome number. Two sections, *Callimusa* and *Australimusa*, have a basic chromosome number of 2n = 20, the two sections, *Eumusa* and *Rhodochlamys*, have a basic chromosome number of 2n = 22, and the last section *Ingentimusa* has a basic chromosome number of 2n = 14 (Horry *et al.*, 1997). The genus *Musa* is a majority of the section *Eumusa* which included of all edible cultivated bananas, *Musa acuminata* and *M. balbisiana* (Simmond, 1962).

Five species of the genus *Ensete* were found in the world and its natural distribution areas occur in tropical countries. In Thailand, they have been reported only two species; *Ensete glaucum* and *E. superbum*, while only 1 species of *Musella* was found in Southwest Yunnan, China and it was introduced to Thailand as an ornamental banana. (Silayoi and Babpraserth, 1983).

Musa acuminata is originated in Southeast Asia and its classification is divided into 8 subspecies (Jones, 2000), namely *Musa acuminata* ssp. *banksii, M. acuminata* ssp. *errans, M. acuminata* ssp. *burmannica, M. acuminata* ssp. *malaccensis, M. acuminata* ssp. *siamea, M. acuminata* ssp. *microcarpa, M. acuminata* ssp. *truncata* and *M. acuminata* ssp. *zebrina*. In Thailand, 3 subspecies of *Musa acuminata* are naturally distributed; *Musa acuminata* ssp. *burmannica, M. acuminata* ssp. *siamea* and *M. acuminata* ssp. *malaccensis* (Silayoi, 2002); other species such as *M. acuminata* ssp. *banksii, M. acuminata* ssp. *microcarpa* and *M. acuminata* ssp. *zebrina* were introduced as genetic resources for banana improvement. *Musa balbisiana* originated in the southern part of India and it is cultivated all regions in Thailand (Swangpol, 2003). Mostly, the edible cultivated bananas and plantains are polyploidy and /or hybrid from two species, which have been classified into 2 genome types, A-genome, a representative of *Musa acuminata*, and B-genome, a representative of *M. balbisiana*. At present, the classification of edible cultivated bananas and plantains is based on ploidy

levels (diploid, triploid, tetraploid and genome differences) (Simmonds and Shepherd, 1955).

Molecular technology has been used to investigate the phenotype and genotype of gene expression, genetic variation, genetic diversity, genetic relationship and classification of species of flora and fauna because it provides more accurate results compared to other methods. Molecular techniques have been used to analyze genetic information in fruit crops such as isozyme (Bhat *et al.*, 1992), RFLP (Gawel and Jarret, 1991), RAPD (Howell *et al.*, 1994), SSR-anchored (Eiadthong *et al.*, 1999; Grapin *et al.*, 1998) and AFLP (Wong *et al.*, 2002). ISSR (inter-simple sequence repeat) is another molecular technique developed by Zietkiewicz *et al.* (1994) and is utility DNA fragments of about 100 to 3,000 bp located between adjacent, oppositely oriented microsatellite regions. ISSR is amplified by PCR using microsatellite core sequences as primers with a few selective nucleotides as anchors into the non-repeat adjacent regions (16-18 bp). About 10-60 fragments from multiple loci are generated simultaneously, separated by gel electrophoresis and scored as the presence or absence of fragments of particular size. The main advantage of ISSR is that no sequence data in required for primer construction, which is easier than SSR markers, which require knowledge of the genomic sequence data to design specific primers, because the analytical procedures of ISSR include PCR, only low quantities of template DNA are required (5-50 ng per reaction). Furthermore, ISSR is randomly distributed throughout the genome and operational costs, development costs and labor-intensity are less than AFLP markers, and can be applied in many studies involving genetic identity, parentage, clone and strain identification, and taxonomic studies of closely related species. Finally, ISSR is considered useful in gene mapping studies (Godwin *et al.*, 1997). For these reasons, ISSR marker was selected to investigate the genetic relationship among subspecies of *Musa acuminata* and A-genome consisting of cultivated bananas and to examine the genetic variation of subspecies of *M. acuminata* are naturally distributed in Thailand.

Materials and Methodology

1. Plant materials

Totally 45 samples of bananas and plantains were collected from various places in Thailand. They consisted of 12 wild type samples of *Musa acuminata*, and 33 samples of edible cultivated bananas and plantains. All wild collection samples were identified to species based on morphological characteristics according to Simmond (1957) and Daniells (2001) (Table 1).

2. DNA extraction

Young leaves of all banana samples were collected and soaked in liquid nitrogen for DNA extraction using the 2% CTAB method modified by Doyle and Doyle (1990) and Agrawal *et al.* (1992). Briefly, 100 mg of fresh leaves were ground to fine powder in liquid nitrogen, followed by the addition of 1 ml preheated (65°C) extraction buffer with further grinding. The extraction buffer consisted of 2% (w/v) CTAB, 1.4 M NaCl, 0.1% (v/v) β-mercaptoethanol, 20 mM EDTA, 100 mM Tris-HCl (pH 8.0), and 1% (w/v) PVP-40. The homogenates were incubated at 65°C for 30 min and extracted two times with a phenol:chloroform: isoamyl alcohol (25:24:1) solution and phenol was eliminated by chloroform:isoamyl alcohol (24:1) solution. Then, DNA was precipitated in cold isopropanol and treated with RNase A (37°C) for 60 min. After electrophoresis with a standard DNA on 1% agarose gels DNA concentrations were determined by comparison of the intensity of staining with ethidium bromide.

3. DNA amplification

The ISSR primers were obtained from Biotechnology Laboratory, University of British Columbia, Canada. Thirty six primers were initially screened using two banana clones, as a representative sample for *Musa acuminata* and *M. balbisiana*, and DNA amplification was carried out by a modified protocol of Anyamanee (2001). The total reaction mixture was 15 µl contained 10x PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTP mixed, 10 pmol primer, 1.25 U *Taq* polymerase and

Table 1. Check List of banana samples and their collected location used in this study.

Genus	Section[1)]	Botanical name 'Cultivar'	Genome type[2)]	Location[3)] and (Code)
Musa	Eumusa	*Musa balbisiana* Colla	BB	Srinakhon, Sukhothai (MW1)
Musa	Eumusa	*Musa balbisiana* Colla	BB	Bangkhen, Bangkok (MW2)
Musa	Eumusa	*Musa balbisiana* Colla	BB	Pak Chong, Nakhon Ratchasima (MW3)
Musa	Eumusa	*Musa acuminata* ssp. *burmannica* Simmonds	AA	Muang, Mae Hong Son (MW4)
Musa	Eumusa	*Musa acuminata* ssp. *burmannica* Simmonds	AA	Muang, Mae Hong Son (MW5)
Musa	Eumusa	*Musa acuminata* ssp. *burmannica* Simmonds	AA	Muang, Mae Hong Son (MW6)
Musa	Eumusa	*Musa acuminata* ssp. *siamea* Simmonds	AA	Nakhonthai, Phisanulok (MW7)
Musa	Eumusa	*Musa acuminata* ssp. *siamea* Simmonds	AA	Nakhonthai, Phisanulok (MW8)
Musa	Eumusa	*Musa acuminata* ssp. *siamea* Simmonds	AA	Srisatchanalai, Sukhothai (MW9)
Musa	Eumusa	*Musa acuminata* ssp. *malaccensis* (Ridl.) Simmonds	AA	Nayong, Trang (MW10)
Musa	Eumusa	*Musa acuminata* ssp. *malaccensis* (Ridl.) Simmonds	AA	Muang, Krabi (MW11)
Musa	Eumusa	*Musa acuminata* ssp. *malaccensis* (Ridl.) Simmonds	AA	Thakuapa, Phang Nga (MW12)
Musa	Eumusa	*Musa acuminata* ssp. *banksii* (F. Muell.) Simmonds	AA	Pak Chong, Nakhon Ratchasima (MW13)
Musa	Eumusa	*Musa acuminata* ssp. *banksii* (F. Muell.) Simmonds	AA	Pak Chong, Nakhon Ratchasima (MW14)
Musa	Eumusa	*Musa acuminata* ssp. *banksii* (F. Muell.) Simmonds	AA	Bang Bua Thong, Nonthaburi (MW15)
Musa	Eumusa	*Musa itinerans* Cheesm.	-	Pak Chong, Nakhon Ratchasima (MI1)
Musa	Rhodochlamys	*Musa laterita* Cheesm.	-	Thong Phapum, Khanchanaburi (ML)
Musa	Callimusa	*Musa coccinea* Andrews	-	Chatuchak, Bangkok (MCo)
Ensete	-	*Ensete glaucum* (Roxb.) Cheesm.	-	Naklang, Nong Bua Lamphu (EG)
Musa	Eumusa	*Musa* 'Kluai Hom Champa'	AA	Pak Chong, Nakhon Ratchasima (MC1)
Musa	Eumusa	*Musa* 'Kluai Nam Thai'	AA	Pak Chong, Nakhon Ratchasima (MC2)
Musa	Eumusa	*Musa* 'Kluai Khai Thong Neoy'	AA	Pak Chong, Nakhon Ratchasima (MC3)
Musa	Eumusa	*Musa* 'Kluai Khai Thong Roung'	AA	Pak Chong, Nakhon Ratchasima (MC4)
Musa	Eumusa	*Musa* 'Kluai Leb Mue Naang'	AA	Pak Chong, Nakhon Ratchasima (MC5)
Musa	Eumusa	*Musa* 'Kluai Nam Thai'	AA	Bang Bua Thong, Nonthaburi (MC26)
Musa	Eumusa	*Musa* 'Kluai Hom Khiew Kom'	AAA	Pak Chong, Nakhon Ratchasima (MC6)
Musa	Eumusa	*Musa* 'Kluai Nak Daeng'	AAA	Pak Chong, Nakhon Ratchasima (MC7)
Musa	Eumusa	*Musa* 'Kluai Khai BW. 2'	AAA	Pak Chong, Nakhon Ratchasima (MC8)
Musa	Eumusa	*Musa* 'Kluai Khai Pratabong'	AAA	Pak Chong, Nakhon Ratchasima (MC9)
Musa	Eumusa	*Musa* 'Kluai Hom Thong'	AAA	Pak Chong, Nakhon Ratchasima (MC10)
Musa	Eumusa	*Musa* 'Kluai Klaai'	AAB	Pak Chong, Nakhon Ratchasima (MC11)
Musa	Eumusa	*Musa* 'Kluai Roiwi'	AAB	Pak Chong, Nakhon Ratchasima (MC12)
Musa	Eumusa	*Musa* 'Kluai Nom Sao'	AAB	Pak Chong, Nakhon Ratchasima (MC13)
Musa	Eumusa	*Musa* 'Kluai Nam Fad'	AAB	Pak Chong, Nakhon Ratchasima (MC14)
Musa	Eumusa	*Musa* 'Kluai Nam Kaab Dam'	AAB	Pak Chong, Nakhon Ratchasima (MC15)
Musa	Eumusa	*Musa* 'Kluai Namwa Sai Leong'	ABB	Pak Chong, Nakhon Ratchasima (MC16)
Musa	Eumusa	*Musa* 'Kluai Namwa Dam'	ABB	Pak Chong, Nakhon Ratchasima (MC17)
Musa	Eumusa	*Musa* 'Kluai Namwa Khom'	ABB	Pak Chong, Nakhon Ratchasima (MC18)
Musa	Eumusa	*Musa* 'Kluai Hak Muk Kheio'	ABB	Pak Chong, Nakhon Ratchasima (MC19)
Musa	Eumusa	*Musa* 'Kluai Hak Muk Khaao'	ABB	Pak Chong, Nakhon Ratchasima (MC20)
Musa	Eumusa	*Musa* 'Kluai Theparod'	ABBB	Pak Chong, Nakhon Ratchasima (MC21)
Musa	Eumusa	*Musa* 'Kluai Thepanom'	BBB	Pak Chong, Nakhon Ratchasima (MC22)
Musa	Eumusa	*Musa* 'Kluai Phama Haek Kuk'	BBB	Pak Chong, Nakhon Ratchasima (MC23)
Musa	Eumusa	*Musa* 'Kluai Lep Chang Kut'	BBB	Pak Chong, Nakhon Ratchasima (MC24)
Musa	Eumusa	*Musa* 'Kluai Hin'	BBB	Pak Chong, Nakhon Ratchasima (MC25)

Remarks: 1) The banana classifications followed as Simmond (1957)
2) Genome type classifications followed as Silayoi (2002)
3) All collected location (District Name, Province Name)

approximately 150 ng genomic DNA. DNA amplification was obtained through 40 cycles in a DNA thermal cycler (icycler BIO-RAD). The temperature profiles were as follows: denature temperature 94°C for 1 minute, annealing temperature 45°C and 47°C (depending on primers used, Table 2) for 1 minute and extension temperature 72°C for 2 minute. After completion of the am-

Table 2. Base sequence, melting temperature (Tm) and annealing temperature (Ta) of reliable ISSR primers used in this study.

Primer	Base sequence (590'3')	Tm/Ta (°C)
UBC-814	-CTC TCT CTC TCT CTC TA-	50/45
UBC-815	-CTC TCT CTC TCT CTC TG-	52/47
UBC-835	-AGA GAG AGA GAG AGA GYC-	52/47
UBC-840	-GAG AGA GAG AGA GAG AYT-	50/45
UBC-843	-CTC TCT CTC TCT CTC TRA-	52/47
UBC-844	-CTC TCT CTC TCT CTC TRC-	52/47

plification, the PCR product was loaded into a 1.4% agarose gel in 1x TBE buffer and the electrophoresis run at 100 volts for 45 minute. Amplification products were visualized by staining a gel with ethidium bromide (0.5 µg/ml). The sizes of each fragment were estimated with reference to a size marker of 100 bp DNA ladder Plus (Fermentas).

4. Data analysis

The reproducible bands of 45 banana samples from 6 selected primers were scored as 1 (presence) or 0 (absence), and were calculated for allele size, allele frequency, polymorphic percentage and heterozygosity (Powell *et al.*, 1996). Then, the pairwise difference matrix between banana samples was determined by the index of Nei and Li (1979) and the unweighed pair-group method using arithmetic averages (UPGMA) cluster analysis was performed using the personal computer program NTSYS-pc (Numerical Taxonomy System version 2.01) (Rohlf, 1993).

Results

1. Plant identification

From 45 collected banana samples; twelve samples were wild banana relative and the rests were edible cultivated bananas. All wild bananas were collected from various places in Thailand.

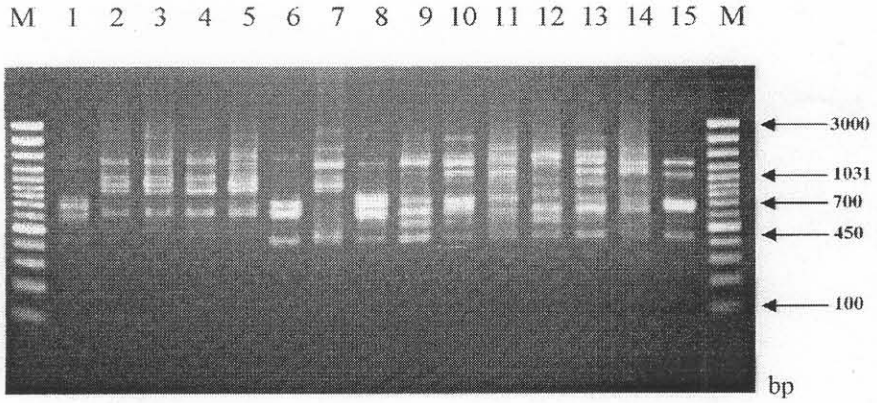

Figure 1. ISSR banding pattern from UBC-815 primer. Lane M is 100 bp DNA Ladder. Lane 1-2; *M. acuminata* ssp. *burmannica* (A- genome) and *M. balbisiana* (B- genome), lane 3-5; *M. balbisiana* (MW1, MW2 and MW3), lane 6-8; *M. acuminata* ssp. *burmannica* (MW4, MW5 and MW6), lane 9-11; *M. acuminata* ssp. *siamea* (MW7, MW8 and MW9), lane 12-14; *M. acuminata* ssp. *malaccensis* (MW10, MW11 and MW12) and lane 15; *M. acuminata* ssp. *banksii* (MW13).

Their identifications based on morphological characteristics according to Simmond (1957) and Daniells (2001) were classified into 5 subspecies of *M. acuminate*. Three samples (MW4, MW5 and MW6) were classified to be *M. acuminata* ssp. *burmannica*, collected from Mae Hong Son province (Table 1). Three samples (MW7, MW8 and MW9) were classified to be *M. acuminata* ssp. *siamea*, collected from Phitsanulok and Sukhothai province. Three samples (MW10, MW11 and MW12) were classified to be *M. acuminata* ssp. *malaccensis*, collected from Trang, Krabi and Phang Nga province. The introduced subspecies of *M. acuminata* samples for Thailand were *M. acuminata* ssp. *banksii* and *M. acuminata* ssp. *microcarpa*. The collected location of *M. acuminata* ssp. *banksii* (MW 13 and MW 14) was a banana orchard in Nakhon Ratchasima province and that of *M. acuminata* ssp. *microcarpa* (MW 15) in Nonthaburi province.

The morphological characteristics are important, and can be used to identify wild banana samples such as; the quickly deciduous and non-imbricate of bract can be used to identify *M. acuminata* ssp. *malaccensis* from *M. acuminata* ssp. *siamea*; pseudostem intensely brown-pigmented and non-imbricate can be used to identify *M. acuminata* ssp. *microcarpa* from *M. acuminata* ssp. *burmannica*. *Musa acuminata* ssp. *banksii* is easily examined as it has yellow bract, light sheath and the female flowers have shown at least the upper one; male-fertile. Classification of genome groups in edible cultivated banana is based on 15 different characters between *M. acuminata* (A-genome) and *M. balbisiana* (B-genome) (Simmond and Shepherds, 1955), and the other banana samples such as *Musa itinerans, M. laterita, M. coccinea* and *Ensete glaucum* were used as out group samples.

In addition, *Musa acuminata* was identified by using some apparent morphological characteristic which were used to simply and clearly identify to subspecies level such as; rachis pattern, shape of male bud and fruit (Ortiz and Vuylsteke, 1998). All morphological characteristics used in this research were according to Simmonds (1957) and some characteristics were confused to identify such as pigment of pseudostem and color of bract were included.

2. ISSR profiles

Thirty-six ISSR primers were first screened by using *Musa acuminata* ssp. *burmannica*, a representative of A-genome, and *M. balbisiana*, a representative of B-genome. Only six primers (16.67 percentages) were selected for further analysis (Table 2). Mostly, the yield of DNA amplifications showed a polymorphic pattern and without any monomorphic pattern (Figure 1). A total of 128 alleles polymorphic bands were selected and allele size varied from 200 to 3,000 bp with an average of 21.33 alleles per primer, average of allele frequency 0.18, polymorphic percentage 1.00 and heterozygosity 0.29 (Table 3).

The genetic relationships between subspecies of *Musa acuminata* and A-genome consisting of edible cultivated bananas were clarified by comparing 128 allele polymorphic bands, and the similarity index was calculated between all reliable allelic polymorphism. The dendrogram obtained by UPGMA clustering method revealed the genetic relationship of all banana samples in this study (Nei and Li, 1979). The results showed that the dendrogram was divided into two main clusters when considered similarity coefficient value at 0.18 (Figure 2). The first cluster was belonging to the out groups which are including *Musa itinerans* (MI) and *Ensete glaucum* (EG). All samples of this cluster had shown the similarity index range between 0.05-0.59 which was lower than the other clusters and average of similarity index at 0.25. The second cluster was belonging to *Musa acuminata* complex, *M. balbisiana* and cultivar groups of the edible cultivated bananas and plantains.

The phylogenetic relationships of second cluster can be divided into 3 sub-clusters. The first sub-cluster was *Musa acuminata* ssp. *banksii* (MW13 and MW14), *M. laterita* (ML) and *M. coccinea* (MCo); the similarity index ranged between 0.08-0.59 and average similarity index was 0.29. The second sub-cluster was *Musa*

Table 3. Number of allele, allele frequency, allele size, polymorphic percentage and heterozygosity of reproducible ISSR primers.

Primer	No. of allele	Allele frequency	Allele size (bp)	Size (bp)	Polymorphic percentage	Heterozygosity (Hn)
UBC-814	20	0.17	350-2500	2500, 2000, 1750, 1500, 1350, 1200, 1116, 1031, 950, 900, 800, 750, 700, 650, 600, 550, 500, 450, 400, 350	1.00	0.23
UBC-815	22	0.17	350-2000	2000, 1875, 1750, 1500, 1425, 1350, 1275, 1200, 1116, 1031, 950, 900, 850, 800, 750, 700, 650, 600, 550, 500, 450, 350	1.00	0.34
UBC-835	25	0.19	250-3000	3000, 2500, 2250, 2000, 1750, 1500, 1350, 1200, 1116, 1031, 950, 900, 850, 800, 750, 700, 650, 600, 550, 500, 450, 400, 350, 300, 250	1.00	0.30
UBC-840	21	0.21	200-2500	2500, 2000, 1750, 1500, 1350, 1200, 1031, 950, 900, 850, 800, 700, 650, 550, 500, 450, 400, 350, 300, 250, 200	1.00	0.32
UBC-843	23	0.17	250-3000	3000, 2500, 2000, 1750, 1500, 1350, 1200, 1116, 1031, 950, 900, 800, 750, 700, 650, 600, 550, 500, 450, 400, 350, 300, 250	1.00	0.29
UBC-844	17	0.19	350-2500	2500, 2000, 1750, 1625, 1500, 1350, 1200, 1031, 900, 850, 800, 700, 600, 550, 450, 400, 350	1.00	0.28
Total	128	-	-	-	-	-
Average	21.33	0.18	-	-	1.00	0.29

acuminata ssp. *burmannica* (MW4, MW5 and MW6), *M. acuminata* ssp. *siamea* (MW7, MW8 and MW9), *M. acuminata* ssp. *malaccensis* (MW10, MW11 and MW12), *M. acuminata* ssp. *microcarpa* (MW15) and the majority of edible cultivated banana cultivar groups of A-genome consisting of such genome as AA (MC1, MC2, MC3, MC4, MC5 and MC26) and genome AAA (MC6, MC7, MC8, MC9 and MC10); the similarity index range was between 0.47-0.93 and the average similarity index was 0.67. The third subcluster was *Musa balbisiana* (MW1, MW2 and MW3) and the majority of cultivar group of edible cultivated plantains that have B-genome consisting genome such as ABB (MC16, MC17, MC18, MC19 and MC20), genome ABBB (MC21) and genome BBB (MC22, MC23, MC24 and MC25); the similarity index ranged between 0.46-1.00 and average similarity index was 0.72. The dendrogram of genome AAB banana cultivar group revealed indistinct classification when examined with ISSR primers in this study; they showed scattered positions (Figure 2).

Discussion

Six ISSR primers were chosen to examine genetic diversity of both wild and cultivated banana, including UBC-814, UBC-815, UBC-835, UBC-840, UBC-843 and UBC-844 (16.67 %), all high potential polymorphic primers, because they have a polymorphic percentage 1.00, heterozygosity was 0.29 and no monomorphic pattern in this study. These results accorded with previous results from other studies in bananas, such as Creste *et al.* (2003) was found 67 polymorphic alleles from 11 primers using SSR marker and an average allele per primer of 6.1 and an average heterozygosity frequency of 0.90.

In this study, we found the specific alleles for A-genome, consisting of edible cultivated bananas (genome AA and AAA and all wild rela-

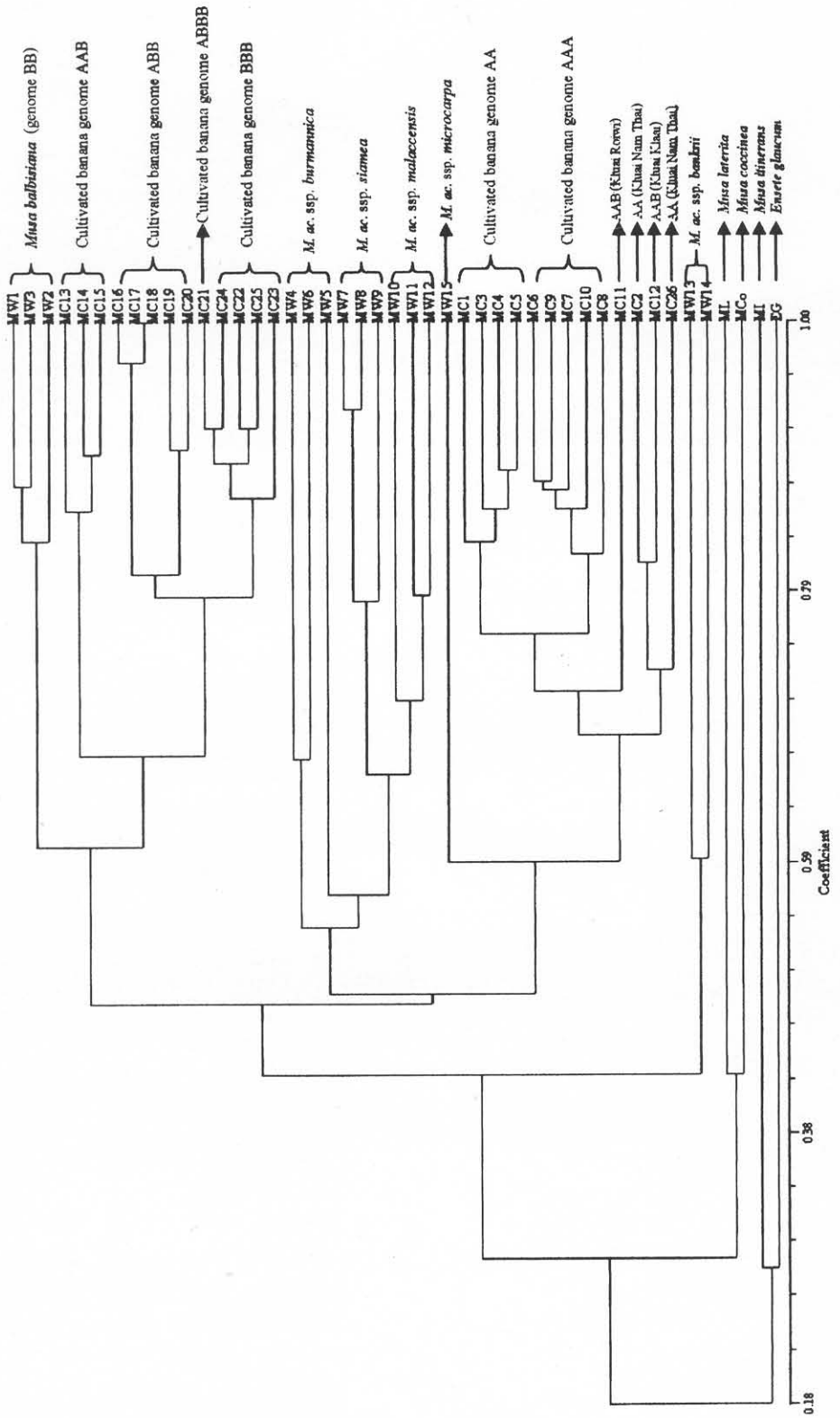

Figure 2. Dendrogram of genetic relationships among subspecies of *Musa acuminata, M. balbisiana* and cultivated bananas using ISSR primers.

tive of *Musa acuminata*), were 450 bp size and 700 bp size in primer UBC-815, 1350 bp size in primer UBC-835 and 300 bp size in primer UBC-843. The specific alleles for B-genome consisting of edible cultivated bananas (genome AAB, ABB, ABBB and BBB) and *Musa balbisiana* (genome BB), were 900 bp size in primer UBC-835, 400 bp size and 600 bp size in primer UBC-844. Another specific allele size only *Musa balbisiana* was 850 bp sizes in primer UBC-844. According to our result, *Musa acuminata* ssp. *burmannica, M. acuminata* ssp. *siamea* and *M. acuminata* ssp. *malaccensis* had a more close genetic relationship with each subspecies of *M. acuminata* that were collected in Thailand. Wong *et al.* (2002) reported the phylogenetic relationship of *Musa acuminata* ssp. *malaccensis, M. acuminata* ssp. *burmannica* and *M. acuminata* ssp. *siamea* that accorded with the result of this study.

The *Musa acuminata* ssp. *banksii* had shown the distant genetic relationship to the other subspecies of *M. acuminata*, while the similarity index range was 0.30-0.53. *Musa acuminata* ssp. microcarpa showed a close phylogenetic relationship to A-genome consisting of edible cultivated bananas more than other subspecies, and its similarity index range was 0.51-0.89. Partially, the results of ISSR marker inside B-genome consisting of edible cultivated bananas and *Musa balbisiana* were divided clearly from *M. acuminata* which was compatible with the results when considered with morphological characteristics. We found that the similarity index range of cultivar group of B-genome consisting of edible cultivated bananas (sub-cluster 3) was lower than the group of the sub-cluster 1 and the sub-cluster of *Musa balbisiana* had a similarity index of 0.85. Nevertheless, we found that the B-genome consisting of edible cultivated banana had a closer genetic relationship with *Musa balbisiana* more than *M. acuminata*. Therefore, the B-genome consisting of edible cultivated bananas may have obtained higher genetic inheritance from *Musa balbisiana* than *M. acuminata* as reported by Creste (2003). The B-genome consisting of edible cultivated bananas in our analysis such as 'Kluai Theparod' (MC21) and 'Kluai Phama Haek Kuk' (MC23) have shown close genetic relationship with *Musa balbisiana* than the other cultivars. The edible cultivated bananas genome AA and genome AAA have closer genetic relationship with the *Musa acuminata* ssp. *microcarpa* than other subspecies which indicated that 'Kluai Nam Thai' (MC2 and MC26) and 'Kluai Khai Thong Roung' (MC4) may be old ancestors of Thai cultivated bananas which have similarity index 0.71, 0.66 and 0.63, respectively. The total similarity index average in this sub-cluster was 0.72 as reported by Ude (2001). Therefore, all subspecies of *Musa acuminata* were related to cultivar groups of A-genome consisting edible cultivated bananas and plantains in Thailand.

Conclusions

Twelve collected samples of wild types of *Musa acuminata* were classified into 5 subspecies; *M. acuminata* ssp. *burmannica , M. acuminata* ssp. *siamea, M. acuminata* ssp. *malaccensis, M. acuminata* ssp. *banksii* and *M. acuminata* ssp. *microcarpa* based on morphological characteristics followed by Simmond and Shepherds (1955) and then the thirty-three collected cultivated banana samples, included genome AA, AAA, AAB, ABB, ABBB, BBB and *Musa balbisiana* (BB), were classified to genome types by Silayoi (2002) (Table 1). The results showed that the dendrogram was divided into two main clusters. The first cluster belonged to the out group which were *Ensete glaucum, M. itinerans* and the second cluster belonged to *Musa coccinea, M. laterita, M. balbisiana*, all subspecies of *M. acuminata*, and cultivar groups of the edible cultivated bananas and plantains. All specimens of subspecies of *M. acuminata* were related to cultivar group of A-genome consisting edible cultivated bananas in Thailand when investigated by using 6 ISSR primers.

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