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Genetic relationships and genome verification of Thai banana cultivars using Random Amplification of Polymorphic DNA (RAPD) markers

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Abstract. *Boonsrangsom T, Fuenghoi C, Premjet D, Suvittawat K, Ratanasut K, Sujipuli K. 2023. Genetic relationships and genome verification of Thai banana cultivars using Random Amplification of Polymorphic DNA (RAPD) markers. Biodiversitas 24: 3758-3765.* Edible bananas and plantains, belonging to the family Musaceae, genus *Musa*, represent one of the most important fruit crops, with an annual production of more than 65 million tons worldwide. Bananas have several hybrid variations since they are descended from the two species *Musa acuminata* Colla (AA genome) and *Musa balbisiana* Colla (BB genome). Different morphological traits divide almost hybrid bananas into various genomic groupings. Banana genome categorization and identification, however, have always been challenging issues. This study aimed to assess the genetic relationships and verify the genomes of Thai banana cultivars using random amplification of polymorphic DNA (RAPD) markers. Using the 15 selected RAPD markers, 149 RAPD bands were found, with sizes ranging from 0.2 to 3.2 kb, and 88.6% were polymorphic. Polymorphic information content (PIC) values ranged from 0.18 to 0.42, averaging 0.30. Based on the Jaccard coefficient, the unweighted pair-group method arithmetic average (UPGMA) analysis showed that the banana samples had a similarity range of 0.27 to 1.00 with a mean of 0.56, demonstrating an abundance of viability across six banana genomes. The dendrogram generated from RAPD data revealed that all 18 *Musa* samples could be divided into two main groups (Group I and II). Three additional subgroups were created for each primary group (A, B, and C). The accurate identification and genetic data on the available genetic resources for bananas will be beneficial for breeding and conservation programs.

Keywords: Banana cultivars, genetic relationship, genome verification, *Musa*, RAPD markers

INTRODUCTION

Edible bananas and plantains are the fourth-largest agricultural crop in developing countries, following primarily maize, wheat, and rice. These plants, members of the family Musaceae, order Scitamineae, and genus *Musa*, are grown in tropical and subtropical temperate regions in more than 130 countries across five continents. The products made from bananas not only serve as a vital source of food but also play an important economic and ecological function (Bakry et al. 2009). With an annual production of 68 million tonnes, or 54.7% of the world's total, the Asian continent leads the globe in banana production (Statista 2021). The edible banana and plantain types have been developed by crossing the two wild cultivars of *Musa acuminata* Colla (AA genome) with *Musa balbisiana* Colla (BB genome), which resulted in the establishment of different genome groups. Most of these hybrid cultivars are seedless, and they are divided into different genetic categories based on 15 morphological features, including AA/AAA, AAB, AB, ABB, ABBB, and BB/BBB, with scores 15-23, 26-46, 49, 59-63, 67, and 75, respectively (Simmonds and Shepherd 1955) (Figure 1). The expression of the 15 characters is evaluated, and a score of 1 or a maximum of 5 is assigned for each character that closely matches wild *M. acuminata* or has an extreme *M. balbisiana* expression. With this scoring system, the wild *M. acuminata* species are given a range of 15 (15 × 1) and the wild *M. balbisiana* species a 75 (15 × 5) range. The overall scores for the hybrid cultivars should be in the range of 15 and 75. However, the classification and nomenclature of bananas have always been challenging issues. Some samples had the same genome score but varied morphological and floral characteristics, while others had the same genome type but various genome scores (Boonsrangsom et al. 2020). Additionally, several researchers discovered that the results of the morphological character analysis employed to determine the genome were variable and inconsistent (Atom et al. 2015; Sunaryo et al. 2019; Premjet et al. 2022). Therefore, these need to be identified using reliable genetic markers.

Moreover, unlike morphological markers, molecular markers are more prevalent and unaffected by the environment. In recent years, plant genomic research has increasingly incorporated DNA-based markers. Many DNA-based marker techniques have been developed and are routinely used to identify a wide variety of organisms, such as inter-retrotransposon amplified polymorphism (IRAP) (Shelke and Das 2015), simple sequence repeat (SSR) (Kabir et al. 2015), inter-simple sequence repeat

(ISSR) (Babu et al. 2018; Sunaryo et al. 2020; Wahyudi et al. 2020), random amplified polymorphic DNA (RAPD) (Zozimo et al. 2018; Kanjanaphachoat et al. 2020), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (Singh et al. 2021), amplified fragment length polymorphism (AFLP) (Wongniam et al. 2010; Safhi et al. 2023), start codon targeted (SCoT) (Igwe et al. 2022), and sequence-related amplified polymorphism (SRAP) (Youssef and Escobedo-GraciaMedrano 2016; Boonsrangsom et al. 2020; Premjet et al. 2022). One of these methods is the RAPD approach, a PCR-based marker that is simple, quick, affordable, applies a single primer (typically 10 bp) of arbitrary nucleotide sequence, and only needs a tiny amount of DNA for analysis (Agarwal et al. 2008; Amiteye 2021). Recently, RAPD technique has been extensively employed in various plant species, including olive (*Olea europaea* L.) (Brake et al. 2014), sugarcane (*Saccharum officinarum* L.) (Hapsoro et al. 2015), plantains and bananas (*Musa x paradisiaca* L.) (Poerba and Ahmad 2010; Zozimo et al. 2018), *Curcuma comosa* Roxb. (Boonsrangsom 2020), hazelnut (*Corylus avellana* L.) (Felbinger et al. 2020), purple yam (*Dioscorea alata* L.) (Rao et al. 2020), shallot (*Allium ascalonicum* L.) (Hasanah et al. 2022), and the *Rhynchostylis* and *Cymbidium* orchids (Oliya et al. 2021; Pradhan et al. 2023). Nevertheless, RAPD has some limitations, such as poor repeatability and dominant features that make it difficult materials to distinguish between homozygotes and heterozygotes (Kumari and Thakur 2014; Zulfahmi et al. 2023). These problems can be addressed by selecting appropriate RAPD primers, optimizing the PCR for the target species, and repeating the experiment. Therefore, this study aimed to assess the genetic relationships among Thai banana cultivars and utilize RAPD markers to confirm their genomes.

MATERIALS AND METHODS

Plant materials and genomic DNA isolation

This work employed 18 Thai banana cultivars, which contained six distinct genomes (two of AA, two of AAA, four of AAB, seven of ABB, two of BB, and one of BBB banana genomes) (Table 1). Young and fresh leaves from banana trees were collected from two banana plantations: (i) the Plant Propagation Center No. 6, Mueang District, Phitsanulok Province, and (ii) the Pakchong Research Station of Kasetsart University, Pakchong District, Nakhon Ratchasima Province, Thailand.

Therefore, to isolate genomic DNA (gDNA), a young banana leaf sample (about 100 mg) was ground to a fine powder in liquid nitrogen and placed in the Plant Genomic DNA Isolation Kit (BIO-HELIX Co., Ltd., Taiwan) following the manufacturer's instructions. Aliquots of the extracted DNA were electrophoresed on a 0.8% (w/v) agarose gel in Tris-Acetate-EDTA (1.0x TAE) buffer and subsequently dyed with RedSafe staining (iNtRON Biotechnology, Inc., Korea). Then, gDNA was compared to 100 ng lambda DNA standards and diluted to a 25 ng/µL concentration in sterile water for RAPD analysis.

RAPD-PCR amplification and electrophoresis

Thirty RAPD primers (S1-S30) (BioBasic Inc., Ontario, Canada) were used to amplify genomic DNA samples. The total volume of the RAPD-PCR reaction was 15 µL, which contained Quick Taq™ HS DyeMix (TOYOBO Co., Ltd., Japan) (7.5 µL), primer (2 µL), DNA template (2 µL), and nuclease-free water (3.5 µL). In a thermal cycler (BIO-RAD T100™, USA), the RAPD-PCR procedure was carried out with the initial denaturation at 94°C for 3 minutes, followed by 40 cycles of 94°C for 1 minute, 37°C for 1 minute, and 68°C for 2 minutes, and the final extension at 68°C for 10 minutes. The PCR products were electrophoresed on a 1.5% (w/v) agarose gel in Tris-Acetate-EDTA (1.0× TAE) buffer and subsequently dyed with RedSafe staining (iNtRON Biotechnology, Inc., Korea). The banding patterns of the gel were visible under UV lighting and gel documentation (Thermo Fisher Scientific, Waltham, MA, USA). According to a 100 bp plus DNA ladder (OneMARK, BIO-HELIX Co., Ltd., Taiwan), the size of the PCR product was estimated.

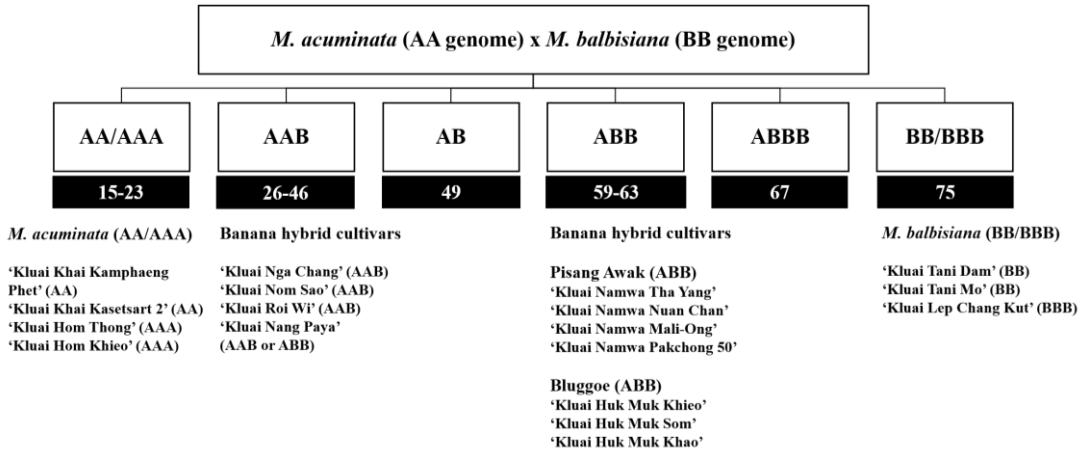

Figure 1. The genomic classification of bananas and the score ranges based on 15 morphological features for various hybrid banana cultivars were established by Simmonds and Shepherd (1955)

Table 1. Eighteen Thai banana cultivars were used in this study

Local name of Thai banana cultivars	International name of banana cultivars [a]	Genomic group [b]	Collection site [c]
'Kluai Khai Kamphaeng Phet'	Pisang Mas/ Sucrier	AA	PPC
'Kluai Khai Kasetsart 2'	Pisang Mas/ Sucrier	AA	PPC
'Kluai Hom Thong'	Gros Michel	AAA	PPC
'Kluai Hom Khieo'	Tall Cavendish	AAA	PPC
'Kluai Nga Chang'	Pisang Lang	AAB	PPC
'Kluai Nom Sao'	-	AAB	PPC
'Kluai Roi Wi'	Pisang Seribu	AAB	PPC
'Kluai Nang Paya'	-	AAB/ABB	PRSKU
'Kluai Namwa Tha Yang'	Pisang Awak	ABB	PPC
'Kluai Namwa Nuan Chan'	Pisang Awak	ABB	PPC
'Kluai Namwa Mali-Ong'	Pisang Awak	ABB	PPC
'Kluai Namwa Pakchong 50'	Pisang Awak	ABB	PRSKU
'Kluai Huk Muk Khieo'	Bluggoe	ABB	PRSKU
'Kluai Huk Muk Som'	Bluggoe	ABB	PRSKU
'Kluai Huk Muk Khao'	Silver Bluggoe	ABB	PRSKU
'Kluai Tani Dam'	*M. balbisiana*	BB	PPC
'Kluai Tani Mo'	*M. balbisiana*	BB	PRSKU
'Kluai Lep Chang Kut'	Lep Chang Kut	BBB	PRSKU

Note: [a] International name was provided by Valmayor et al. (2000); [b] Genome classification based on Silayoi (2015); [c] Collection site: PPC - the Plant Propagation Center No. 6, Mueang District, Phitsanulok Province; PRSKU - the Pakchong Research Station of Kasetsart University, Pakchong District, Nakhon Ratchasima Province

Data analysis

The amplicons produced by the RAPD markers were regarded as separate putative alleles. Manual scoring was performed on distinct and clear bands for DNA fragments' presence (1) or absence (0). The scoring results are gathered as binary data for cluster analysis using the unweighted pair group method with the arithmetic mean (UPGMA) method on the FreeTree and TreeView programs (Hampl et al. 2001). Moreover, the total number of DNA bands (TB), polymorphic bands (NPB), and the ratio of polymorphism (RP) for each marker were calculated. Polymorphic information content (PIC) was calculated as PIC = 1 - Σpi^2, where *pi* is the frequency of the i^{th} allele (Smith et al. 1997). A bootstrap analysis using 1,000 bootstrap repetitions was carried out.

RESULTS AND DISCUSSION

RAPD-PCR Analysis

This study determined the genetic relationships and genomes of 18 Thai banana cultivars using RAPD markers. Thirty RAPD primers (S1-S30) were initially screened, and 15 primers (S1, S4, S6, S8, S10, S11, S12, S17, S18, S20, S21, S23, S24, S27, and S28) that generated clear bands and polymorphic results were chosen, as shown in Table 2; the sizes of the DNA bands varied from 0.20 to 3.2 kb. Primers S17 and S23 had the highest (15) and S28 had the lowest (5) RAPD bands, respectively, with a mean of 9.93 bands per primer. Five primers (S6, S8, S20, S27, and S28) yielded a polymorphism rate of 100%. Furthermore, 149 DNA bands were identified in the RAPD data generated using 15 markers, 132 of which were polymorphic (88.6%). With a mean of 8.8 polymorphic bands per primer, there was variation between 5 and 14 polymorphic bands produced from primer S28 and S6, respectively. The RAPD profiles generated via S1, S4, S6, and S12 are shown in Figure 2. In this investigation, it was found that the RAPD primers could identify several kinds of bananas. For example, the S12 primer could distinguish between 'Kluai Namwa' and 'Kluai Hak Muk' bananas with ABB genomes apart. Several *Musa* AAB genotypes produced distinct bands, including 'Kluai Nga Chang' (5), 'Kluai Roi Wi' (7), and 'Kluai Nang Paya' (8). Moreover, the banding pattern varied among the bananas with the AAB genome. These findings suggested that the RAPD technique will be helpful for banana genome characterization because it detected significant polymorphism (88.6%) among six Thai banana genomes, in comparison to findings from earlier studies of banana and plantain genotypes using RAPD, ISSR, SRAP, and SCoT markers (Poerba and Ahmad 2010; Zozimo et al. 2018; Boonsrangsom et al. 2020; Igwe et al. 2022; Premjet et al. 2022).

The effectiveness of polymorphic loci in identifying genetic variation among genotypes was evaluated using polymorphic information content (PIC) value. The PIC value is important when selecting markers for study in genetics since it evaluates a marker's ability to detect polymorphisms. The PIC values for the 15 RAPD markers in this investigation ranged from 0.18 to 0.42. Four of these primers had a PIC of less than 0.25, and eleven had a PIC range of 0.28 to 0.42 with an average of 0.30. That suggests the potential of RAPD markers to generate medium locus polymorphism, which benefits the genetic variety of *Musa* genotypes. The highest PIC value for dominant markers is 0.5 (Guo et al. 2014); therefore, the average PIC in this study supports the notion that RAPD is an effective marker system for differentiating across cultivars.

Table 2. The findings of PCR amplification using 15 selected RAPD primers

Primer name	Sequence (5' - 3')	Allele size (bp)	TB	NPB	RP (%)	PIC
S1	CTTTCGCTCC	450 - 3200	10	9	90.0	0.28
S4	GGACTGGAGT	550 - 2900	8	6	75.0	0.23
S6	TGCTCTGCCC	500 - 3200	14	14	100.0	0.33
S8	GTCCACACGG	350 - 2500	6	6	100.0	0.42
S10	CTGCTGGGAC	450 - 1700	8	7	87.5	0.18
S11	GTAGACCCGT	500 - 3000	11	10	90.9	0.35
S12	CCTTGACGCA	500 - 1500	7	6	85.7	0.31
S17	AGGGAACGAG	300 - 2800	15	12	80.0	0.30
S18	CCACAGCAGT	400 - 3000	12	11	91.7	0.28
S20	GGACCCTTAC	250 - 2000	8	8	100.0	0.37
S21	CAGGCCCTTC	600 - 2500	7	5	71.4	0.22
S23	AGTCAGCCAC	200 - 2600	15	13	86.7	0.31
S24	AATCGGGCTG	250 - 2500	12	9	75.0	0.24
S27	GAAACGGGTG	300 - 2800	11	11	100.0	0.39
S28	GTGACGTAGG	300 - 1800	5	5	100.0	0.36
Sum			**149**	**132**	-	-
Average			**9.93**	**8.80**	**88.6**	**0.30**

Note: TB: total DNA bands for each marker; NPB: no. of polymorphic bands for each marker; RP: ratio of polymorphism; PIC: polymorphic information content

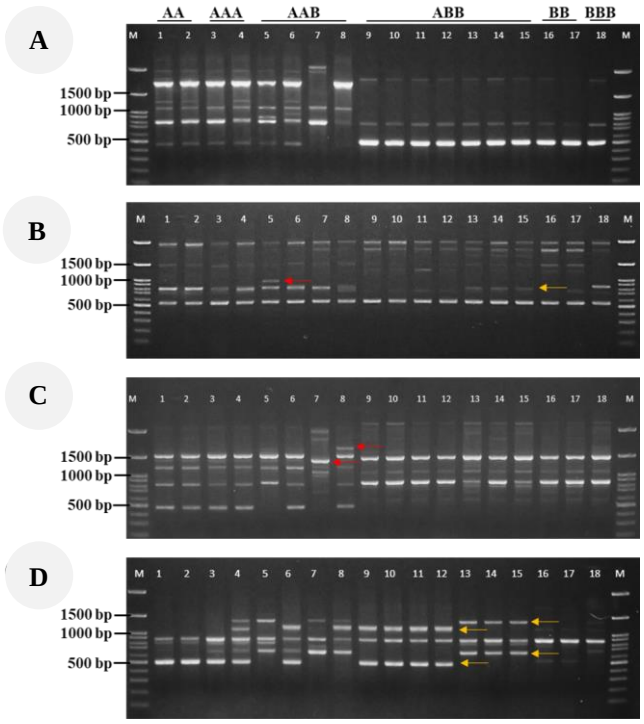

Figure 2. RAPD amplification of 18 Thai banana cultivars using four primers: A. S1; B. S4; C. S6; and D. S12. Red arrows indicate bands unique to *Musa* AAB samples, whereas yellow arrows refer to bands distinct between 'Namwa' (lanes 9-12) and 'Hak Muk' (lanes 13-15) bananas. Lane M is 100 bp Plus DNA ladder (OneMARK, BIO-HELIX, Taiwan), and lanes 1-18 correspond to the *Musa* accessions lists in Table 1

Normally, 15 key morphological traits are utilized to distinguish between *Musa* species. However, due to a slight variance in physical characteristics, varietal identification among hybrid bananas is complicated. Therefore, it is crucial to use PCR-based approaches like RAPD markers to resolve species identification and genetic categorization. Much information about an organism's genes can be quickly obtained using RAPD markers. According to other investigations, RAPD is more informative than other techniques, such as ISSR and SRAP. For instance, 132 polymorphic fragments in total, or 8.8 polymorphic fragments on average per marker, were obtained for this study. This number is higher than the 7.75 and 8.14 polymorphic bands per marker found in the 36 Thai *Musa* samples using ISSR and SRAP markers, respectively (Premjet et al. 2022). Therefore, this study proves that the RAPD marker is substantially easy, rapid, inexpensive, and instructive. The usefulness of RAPD markers for identifying genetic variation and molecular characterization was also documented in saffron (*Crocus sativus* L.) (Mir et al. 2021), pepper (*Capsicum annuum* L.) (Niklas and Olszewska 2021), rice (*Oryza sativa* L) (Epe et al. 2021), and the *Gymnema* species (Tung et al. 2022).

Genetic relationships and cluster analysis

A genetic relationship assessment was performed on 18 samples of various banana cultivars utilizing DNA fingerprint data from 15 RAPD markers. The Jaccard coefficient method (Jaccard 1908) was used to calculate the genetic similarity coefficient employing the FreeTree program jointly with the TreeView tool. The genetic similarity coefficient determines how closely the 18 Thai *Musa* species are genetically related. This measure for the 18 *Musa* accessions analyzed with RAPD markers was discovered to have a range of 0.27 to 1.00, with a mean value of 0.56 (Table 3), showing a wide range of viability among six banana genomes. These results are consistent with those of Kundu et al. (2018) and Boonsrangsom et al. (2020), who observed that banana plants in India and Thailand have significant genetic variation. The lowest genetic similarity was found (0.27) between the 'Kluai Khai Kamphaeng Phet' (AA group) (1) and the 'Kluai Tani Mo' (BB group) (17), as well as between the 'Kluai Khai Kasetsart 2' (AA group) (2) and the 'Kluai Tani Mo' (BB group) (17), implying their lack of close relationships because they belong to two distinct chromosomal

groupings (AA and BB genomes). The highest genetic similarity (1.00), however, was found between 'Kluai Khai Kamphaeng Phet' (AA group) (1) and 'Kluai Khai Kasetsart 2' (AA group) (2), as well as 'Kluai Namwa Tha Yang' (ABB group) (9) and 'Kluai Namwa Nuan Chan' (ABB group) (10). These findings support the proof of close cultivar relations between genome groupings.

Using the dendrogram generated from the RAPD data, the 18 *Musa* species in this study were divided into two major groups (Figure 3). For each primary group, three further subgroups were formed. The first large group comprised three subgroups, I-A to I-C, and the second major group comprised subgroups II-A to II-C. Therefore, bootstrap values were applied to verify the consistency and dependability of the nodes' data. The bootstrap values in this analysis were comparatively high within and between groups, ranging from 39% to 100%. Group I could be further separated into the following three subgroups: banana genomes ABB from Group I-A, such as 'Kluai Huk Muk Khieo,' 'Kluai Huk Muk Som,' and 'Kluai Huk Muk Khao' were included. Banana BB and BBB, including 'Kluai Tani Dam,' 'Kluai Tani Mo,' and 'Kluai Lep Chang Kut,' were part of Group I-B. Bananas in the I-C group included 'Kluai Namwa Mali-Ong,' 'Kluai Namwa Pakchong 50', 'Kluai Namwa Nuan Chan,' and 'Kluai Namwa Tha Yang.' They all had the ABB genome. Group II could be broken down into three subgroups: II-A included only one variety of bananas (AAB), known as 'Kluai Roi Wi.' 'Kluai Nom Sao' (AAB), 'Kluai Hom Khieo' (AAA), 'Kluai Hom Thong' (AAA), 'Kluai Khai Kasetsart 2' (AA) and 'Kluai Khai Kamphaeng Phet' (AA) comprised Group II-B, while 'Kluai Nga Chang' (AAB) and 'Kluai Nang Paya' (AAB) formed up Group II-C.

Due to the close genetic relationship among bananas, several were grouped. For instance, the 'Kluai Khai Kasetsart 2' and 'Kluai Khai Kamphaeng Phet' are grouped. This could be because the 'Kluai Khai Kasetsart 2' originated via radiation mutations of the original variety ('Kluai Khai Kamphaeng Phet'), resulting in a high degree of genetic relatedness. However, it was discovered that 'Kluai Khai' carrying the AA genome had various exterior traits. In comparison to the other AAB cultivars, 'Kluai Nom Sao' (AAB) was more closely related to the *M. acuminata* 'Kluai Hom Khieo' (AAA) and 'Kluai Hom Thong' (AAA) cultivars. Similarly, three of the 'Kluai Huk Muk' cultivars were more genetically related to the BB and BBB cultivars of *M. balbisiana* than the 'Kluai Namwa' with ABB genome.

According to the results of this study's genetic correlation clustering and DNA fingerprint analysis, the 'Kluai Nang Paya' should have an AAB rather than an ABB genome, with a genetic similarity coefficient of 0.63. Bananas with the AAB genome displayed different banding patterns, demonstrating the high genetic diversity of the cultivars having an AAB genomic group. These results agree with those of Kundu et al. (2018), who discovered that the 16 AAB banana genotypes were divided into eight groups with a broad genetic background. Additionally, the *Musa* AAB cultivars generated particular bands. Therefore, more banana cultivars with the AAB group need to be examined to confirm the distinctive bands generated from the *Musa* AAB genome in this work. Moreover, in this study, 'Kluai Namwa,' known as 'Pisang Awak,' and 'Kluai Huk Muk,' also known as 'Bloggoe,' were distinguished using RAPD primers among the *Musa* ABB group. Nevertheless, utilizing DNA markers to differentiate between numerous 'Kluai Namwa' sub-cultivars is difficult. These results are consistent with Premjet et al. (2022), who observed that it is difficult to identify between the 'Kluai Namwa' sub-cultivars since there are few physical distinctions and little genetic variation. Generally, bananas and plantains often reproduce through budding, which has led to little genetic variety. Furthermore, natural mutations have the potential to cause genetic differences. However, with only 18 Thai banana cultivar results, it might not be sufficient to differentiate the species of bananas clearly. According to Sahu et al. (2020), it is essential to identify the genetic mutations and genes that cause phenotypic alterations to comprehend various biological processes.

Table 3. Coefficients of similarity among 18 Thai banana cultivars using 15 RAPD primers

No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1.00																	
2	1.00	1.00																
3	0.69	0.69	1.00															
4	0.65	0.65	0.80	1.00														
5	0.60	0.60	0.64	0.62	1.00													
6	0.62	0.62	0.68	0.74	0.61	1.00												
7	0.44	0.44	0.46	0.42	0.49	0.48	1.00											
8	0.55	0.55	0.60	0.64	0.63	0.61	0.58	1.00										
9	0.32	0.32	0.38	0.37	0.36	0.36	0.39	0.47	1.00									
10	0.32	0.32	0.38	0.37	0.36	0.36	0.39	0.47	1.00	1.00								
11	0.32	0.32	0.35	0.36	0.37	0.35	0.37	0.46	0.88	0.88	1.00							
12	0.31	0.31	0.35	0.38	0.36	0.35	0.37	0.45	0.90	0.90	0.88	1.00						
13	0.33	0.33	0.41	0.38	0.45	0.35	0.45	0.44	0.64	0.64	0.60	0.64	1.00					
14	0.33	0.33	0.42	0.37	0.42	0.36	0.45	0.45	0.69	0.69	0.61	0.65	0.90	1.00				
15	0.34	0.34	0.40	0.37	0.45	0.33	0.46	0.45	0.67	0.67	0.63	0.65	0.92	0.92	1.00			
16	0.28	0.28	0.31	0.28	0.29	0.29	0.37	0.40	0.64	0.64	0.62	0.64	0.64	0.70	0.66	1.00		
17	0.27	0.27	0.30	0.28	0.29	0.29	0.36	0.39	0.62	0.62	0.60	0.62	0.62	0.69	0.64	0.97	1.00	
18	0.32	0.32	0.34	0.33	0.38	0.32	0.41	0.44	0.58	0.58	0.57	0.57	0.61	0.67	0.65	0.75	0.73	1.00

Note: The lists of *Musa* accessions in Table 1 are referenced by the sample name (1-18)

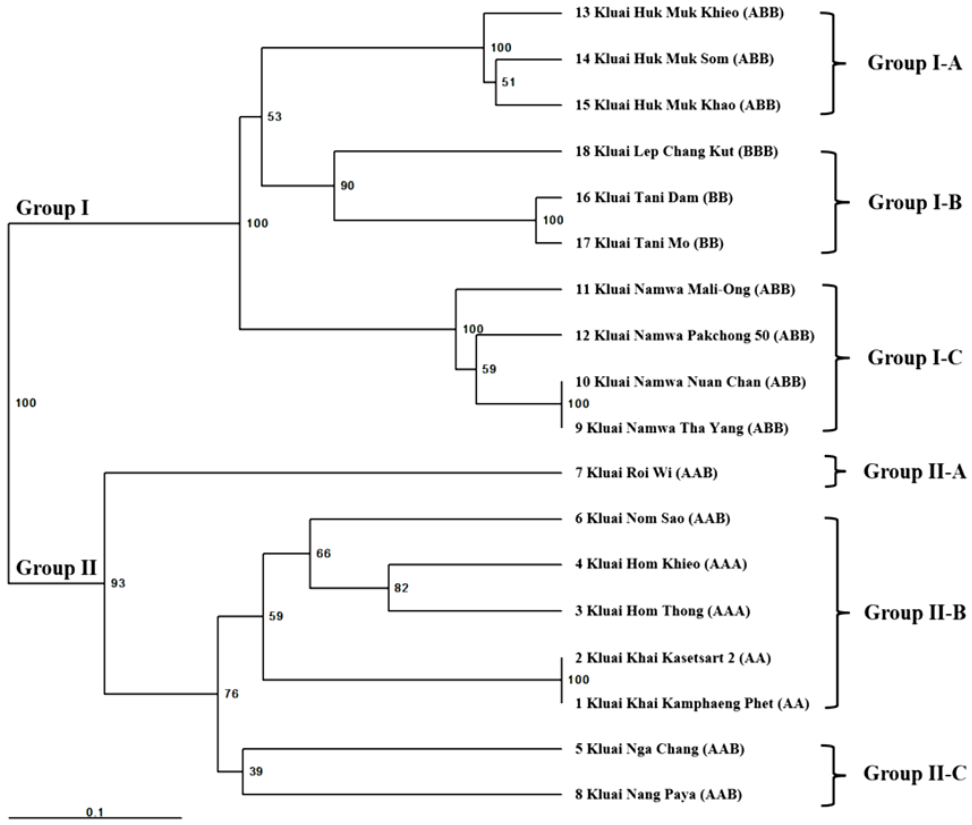

Figure 3. Eighteen accessions of Thai banana cultivars were used to generate a dendrogram of genetic relationships using 15 RAPD markers, where bootstrap values (30% and higher are shown) from 1,000 replications are represented at nodes

Overall, the outcomes of the RAPD analysis and the morphological classification were in agreement in the present study. It is crucial for gathering fundamental morphology information, molecular techniques, and specific traits to investigate genetic diversity, species identification, and future breeding. Wahyudi et al. (2020) proposed combining molecular markers and morphological features to classify banana species. According to Hinge et al. (2022), scientists used several molecular markers to assess the genetic diversity among commercial banana cultivars and the HS-SPME and GC-MS to create the volatile profiles of the genotypes. Therefore, this information helps plant breeders to develop new banana cultivars with good flavor, pleasant aroma, soft food texture, and attractive color. Additionally, scientists and farmers could guarantee the genetic stability of commercially available cultivars.

In conclusion, this study used RAPD markers to evaluate the genomes and genetic relations among 18 Thai banana cultivars. Based on the results of 15 RAPD markers, there were 149 DNA bandings with band sizes ranging from 0.2 to 3.2 kb, 132 of which were polymorphic (88.6%). Values of polymorphic information content (PIC), with an average value of 0.30, ranged from 0.18 to 0.42. According to the UPGMA analysis using the Jaccard coefficient method, the 18 *Musa* genotypes had a similarity range between 0.27 and 1.00 with a mean of 0.56, indicating a high degree of genetic diversity across the six *Musa* genomic groupings. The dendrogram generated from RAPD markers in the current study revealed that all samples could be divided into the ABB-BB-BBB and AA-AAA-AAB genome groups, the two main groupings. This study provides convincing evidence that the RAPD marker is simple, quick, affordable, and productive. The RAPD analyses will be useful for future data collection, environmental preservation, and reproductive purposes.

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REFERENCES

Agarwal M, Shrivastava N, Padh H. 2008. Advances in molecular marker techniques and their applications in plant sciences. Plant Cell Rep 27 (4): 617-631. DOI: 10.1007/s00299-008-0507-z.

Amiteye S. 2021. Basic concepts and methodologies of DNA marker systems in plant molecular breeding. Heliyon 7 (10): e08093. DOI: 10.1016/j.heliyon.2021.e08093.

Atom AD, Lalrinfela P, Thangjam R. 2015. Genome classification of banana genetic resources of Manipur using morphological characters. Sci Vis 15 (4): 189-195.

Babu AG, Prabhuling G, Karani RS, SATISH D, Patil RK, Mulla SR, Raghavendra G, Jagadeesha RC. 2018. Genetic diversity analysis among banana cultivars through ISSR markers. J Pharmacogn Phytochem 7 (6): 1576-1580.

Bakry F, Carreel F, Jenny C, Horry JP. 2009. Genetic improvement of banana. In: Jain SM, Priyadarshan PM (eds) Breeding plantation tree crops: tropical species. Springer, New York. DOI: 10.1007/978-0-387-71201-7_1.

Boonsrangsom T, Phetnin B, Ratanasut K, Sujipuli K. 2020. Assessment of genetic diversity among Musa cultivars based on sequence-related amplified polymorphism technique. Naresuan Univ J Sci Technol 28 (2): 52-61. DOI:10.14456/nujst.2020.15.

Boonsrangsom T. 2020. Genetic diversity of 'Wan Chak Motluk' (*Curcuma comosa* Roxb.) in Thailand using morphological characteristics and random amplification of polymorphic DNA (RAPD) markers. S Afr J Bot 130: 224-230. DOI: 10.1016/j.sajb.2020.01.005.

Brake M, Migdadi HM, Al-Gharaibeh MA, Ayoub S, Haddad NJ, Oqlah AA. 2014. Characterization of Jordanian olive cultivars (*Olea europaea* L.) using RAPD and ISSR molecular markers. Sci Hortic 176: 282-289. DOI: 10.1016/J.SCIENTA.2014.07.012.

Epe IA, Bir MSH, Choudhury AK, Khatun A, Aktar MM, Arefin MS, Islam MA, Park KW. 2021. Genetic diversity analysis of high yielding rice (*Oryza sativa*) varieties cultivated in Bangladesh. Korean J Agric Sci 48: 283-297. DOI: 10.7744/kjoas.20210022.

Felbinger C, Kutzsche F, Mönkediek S, Fischer M. 2020. Genetic profiling: differentiation and identification of hazelnut cultivars (*Corylus avellana* L.) using RAPD-PCR. Food Control 107: 106791. DOI: 10.1016/J.FOODCONT.2019.106791.

Guo ZH, Fu KX, Zhang XQ, Bai SQ, Fan Y, Peng Y, Huang LK, Yan YH, Liu W, Ma X. 2014. Molecular insights into the genetic diversity of *Hemarthria compressa* germplasm collections native to southwest China. Molecules (Basel, Switzerland) 19 (12): 21541-21559. DOI: 10.3390/molecules191221541.

Hampl V, Pavlícek A, Flegr J. 2001. Construction and bootstrap analysis of DNA fingerprinting-based phylogenetic trees with the freeware program Free Tree: Application to trichomonad parasites. Intl J Syst Evol Microbiol 51 (3): 731-735. DOI: 10.1099/00207713-51-3-731.

Hapsoro D, Warganegara H, Utomo S, Sriyani N, Yusnita Y. 2015. Genetic diversity among sugarcane (*Saccharum officinarum* L.) genotypes as shown by randomly amplified polymorphic DNA (RAPD). Agrivitas J Agric Sci 37 (3): 247-257. DOI: 10.17503/agrivita.v37i3.499.

Hasanah Y, Mawarni L, Hanu H, Lestami A. 2022. Genetic diversity of shallots (*Allium ascalonicum* L.) from several locations in North Sumatra, Indonesia based on RAPD markers. Biodiversitas 23: 2405-2410. DOI: 10.13057/biodiv/d230518.

Hinge VR, Shaikh IM, Chavhan RL, Deshmukh AS, Shelake RM, Ghuge SA, Dethe AM, Suprasanna P, Kadam US. 2022. Assessment of genetic diversity and volatile content of commercially grown banana (*Musa* spp.) cultivars. Sci Rep 12 (1): 7979. DOI: 10.1038/s41598-022-11992-1.

Igwe DO, Ihearahu OC, Osano AA, Acquaah G, Ude GN. 2022. Assessment of genetic diversity of *Musa* species accessions with variable genomes using ISSR and SCoT markers. Genet Resour Crop Evol 69: 49-70. DOI: 10.1007/s10722-021-01202-8.

Jaccard P. 1908. Nouvelles recherches sur la distribution florale. Bull Soc Vaud Sci Nat 44: 223-270.

Kabir MH, Mamun ANK, Fatema H, Amin R. 2015. Assessment of genetic diversity in 13 local banana (*Musa* spp.) cultivars using simple sequence repeat (SSR) markers. Intl J Rec Res Life Sci 2 (1): 65-69.

Kanjanaphachoat P, Topoonyanont N, Jantawichai P. 2020. RAPD marker analysis of genetic variation in Kluai Nam Wa (*Musa* ABB group) banana plants regenerated after 8 and 9 subcultures. Maejo Intl J Sci Technol 14 (2): 130-140.

Kumari N, Thakur SK. 2014. Randomly amplified polymorphic DNA - a brief review. Am J Anim Vet Sci 9 (1): 6-13. DOI: 10.3844/ajavsp.2014.6.13.

Kundu P, Bauri FK, Maji S. 2018. Genetic diversity study of some banana genotypes collected from various parts of India through RAPD analysis. Afr J Agric Res 13: 248-257. DOI: 10.5897/AJAR2015.10350.

Mir MA, Mansoor S, Sugapriya M, Alyemeni MN, Wijaya L, Ahmad P. 2021. Deciphering genetic diversity analysis of saffron (*Crocus sativus* L.) using RAPD and ISSR markers. Saudi J Biol Sci 28 (2): 1308-1317. DOI: 10.1016/j.sjbs.2020.11.063.

Niklas A, Olszewska D. 2021. Application of the RAPD technique to identify genetic diversity in cultivated forms of *Capsicum annuum* L. Biotechnol 102 (2): 209-223. DOI: 10.5114/bta.2021.106523.

Oliya BK, Chand K, Thakuri LS, Baniya MK, Sah AK, Pant B. 2021. Assessment of genetic stability of micropropagated plants of *Rhynchostylis retusa* (L.) using RAPD markers. Sci Hortic 281: 110008. DOI: 10.1016/J.SCIENTA.2021.110008.

Poerba YS, Ahmad F. 2010. Genetic variability among 18 cultivars of cooking bananas and plantains by RAPD and ISSR markers. Biodiversitas 11 (3): 118-123. DOI: 10.13057/biodiv/d110303.

Pradhan S, Paudel YP, Qin W, Pant B. 2023. Genetic fidelity assessment of wild and tissue cultured regenerants of a threatened orchid, *Cymbidium aloifolium* using molecular markers. Plant Gene 34: 100418. DOI: 10.1016/j.plgene.2023.100418.

Premjet D, Boonsrangsom T, Sujipuli K, Rattanasut K, Kongbangkerd A, Premjet S. 2022. Morphological and molecular characterizations of *Musa* (ABB) 'Mali-Ong' in Thailand. Biology 11 (10): 1429. DOI: 10.3390/biology11101429.

Rao GK, Kapadia C, Patel NB, Desai KD, Narasimha Murthy PN. 2020. Genetic diversity analysis of greater yam (*Dioscorea alata* L.) genotypes through RAPD and ISSR markers. Biocatal Agric Biotechnol 23: 101495. DOI: 10.1016/j.bcab.2020.101495

Safhi FA, Alshamrani SM, Alshaya DS, Hussein MAA, Abd El-Moneim D. 2023. Genetic diversity analysis of banana cultivars (*Musa* sp.) in Saudi Arabia based on AFLP marker. Curr Issues Mol Biol 45 (3): 1810-1819. DOI: 10.3390/cimb45030116.

Sahu PK, Sao R, Mondal S, Vishwakarma G, Gupta SK, Kumar V, Singh S, Sharma D, Das BK. 2020. Next generation sequencing based forward genetic approaches for identifcation and mapping of causal mutations in crop plants: a comprehensive review. Plants 9: 1355. DOI: 10.3390/plants9101355.

Shelke RG, Das AB. 2015. Analysis of genetic diversity in 21 genotypes of Indian banana using RAPDs and IRAPs markers. Proc Natl Acad Sci, India Sect B Biol Sci 85 (4): 1027-1038. DOI: 10.1007/s40011-015-0505-1

Silayoi B. 2015. Banana. Kasetsart University Publishing, Bangkok, 512 p. [Thai]

Simmonds NW, Shepherd K. 1955. The taxonomy and origins of the cultivated bananas. Bot J Linn Soc 55 (359): 302-312. DOI: 10.1111/j.10958339.1955.tb00015.x.

Smith JS, Chin EC, Shu H, Smith OS, Wall SJ, Senior ML, Mitchell SE, Kresovich S, Ziegle J. 1997. An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPS and pedigree. Theor Appl Genet 95: 163-173. DOI: 10.1007/s001220050544.

Singh WA, Singh NS, Devi EJ, Handique PJ, Devi HS. 2021. Collection and characterization of banana gene pools (*Musa* spp.) in Manipur (N.E. India) using PCR-RFLP and RAPD and ISSR markers. Braz J Bot 44: 671-684. DOI: 10.1007/s40415-021-00722-y.

Statista. 2021. Banana production worldwide by region. https://www.statista.com/statistics/264003/production-of-bananas-worldwide-by-region/

Sunaryo W, Mulyadi A, Nurhasanah. 2019. Genome group classification and diversity analysis of talas and rutai banana, two local cultivars from East Kalimantan, based on morphological characters. Biodiversitas 20: 2355-2367. DOI: 10.13057/biodiv/d200834.

Sunaryo W, Wahida, Idris SD, Pratama AN, Ratanasut K, Nurhasanah. 2020. Genetic relationships among cultivated and wild bananas from East Kalimantan, Indonesia based on ISSR markers. Biodiversitas 21: 824-832. DOI: 10.13057/biodiv/d210250.

Tung PHT, Van T, Huong PT. 2022. Assessment of genetic variation of the genus *Gymnema* in Vietnam, using RAPD and ITS-rDNA markers. J Herbs Spices Med 28 (3): 281-292. DOI: 10.1080/10496475.2022.2058672.

Valmayor RV, Jamaluddin SH, Silayoi B, Kusumo S, Danh LD, Pascua OC, Espino RRC. 2000. Banana Cultivar Names And Synonyms In Southeast Asia. International Network for Improvement of Banana and Plantain-Asia and the Pacific Office, Laguna (Philippines).

Wahyudi D, Rifliyah K, Uslan. 2020. Genome evaluation of banana cultivars based on morphological character and inter-simple sequence repeat)ISSR(molecular marker. Biodiversitas 21: 2982-2990. DOI: 10.13057/biodiv/d210715.

Wongniam S, Somana J, Swangpol SC, Seelanan T, Chareonsap PP, Chadchawan S, Jenjittikul T. 2010. Genetic diversity and species-specific PCR-based markers from AFLP analyses of Thai bananas. Biochem Syst Ecol 38: 416-427. DOI: 10.1016/J.BSE.2010.03.015.

Youssef M, Escobedo-GraciaMedrano RM. 2016. Genetic relationship among *Musa balbisiana* accessions and identification of SRAP markers linked to *Musa* B genome. J Agric Chem Biotechnol 7 (2): 39-47.

Zozimo ROB, Ratanasut K, Boonsrangsom T, Sujipuli K. 2018. Assessment of genetic diversity among Thai banana cultivars (*Musa* spp.) based on RAPD and SRAP markers. Intl J Biosci 12 (4): 172-180. DOI: 10.12692/ijb/12.4.172-180.

Zulfahmi, Pertiwi SA, Rosmaina, Elfianis R, Gulnar Z, Zhaxybay T, Bekzat M, Zhaparkulova G. 2023. Molecular identification of mother trees of four matoa cultivars (*Pometia pinnata* Forst & Forst) from Pekanbaru City, Indonesia using RAPD markers. Biodiversitas 24: 1524-1529. DOI: 10.13057/biodiv/d24032.