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Full Paper

DNA Fingerprinting and Genetic Diversity Analysis of Philippine Saba and Other Cultivars of *Musa balbisiana* Colla Using Simple Sequence Repeat Markers

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Recognizing the importance of the indigenous Philippine cultivars of *Musa balbisiana* Colla, which include the popular Saba varieties, it is essential to correctly identify these cultivars for various applications. Simple sequence repeat markers were used to generate DNA fingerprints and characterize the genetic diversity among 13 Philippine *Musa balbisiana* Colla cultivars and 5 other banana cultivars. Fifty-one primer pairs (45 from Musa and 6 from other species) were screened for PCR amplification and polymorphism across 18 cultivars. Fourteen primers were found to be polymorphic markers. A total of 49 alleles were generated, with a mean of 3.77 alleles per locus, ranging from 1-8 alleles. The resolving power of molecular markers measured as the Polymorphism Information Content (PIC) ranged from 0.23 to 0.80. Mean gene diversity ranged 0.08 to 0.49 indicating high diversity. The dendrogram using UPGMA-SAHN cluster analysis based on microsatellite polymorphism showed that the Saba cultivars clustered into two groups at 56% similarity level. Cluster analysis separated the cultivars of *Musa balbisiana* Colla from the other genotypes of *Musa acuminata* Colla and *M. textilis*. The 13 polymorphic SSR primers were shown to be able to identify and differentiate the 13 cultivars suitable for specific needs of the industry.

Keywords: Saba, *Musa balbisiana*, DNA fingerprints, diversity, SSR

INTRODUCTION

The Philippines has 14 cultivars of *Musa balbisiana* Colla although only five of these are economically important, foremost of which is Saba. In 2016, the Philippines had a production of 8.903 million tons of which 2.47 million tons consisted of Saba banana (PSA 2017), making it one of the top 5 banana producing countries in the world. The Saba variety is used primarily for manufacturing banana chips and banana ketchup, and in various food products such as snacks (boiled or raw), viands such as *Arroz ala Cubana* and beef stew, *desserts* such as *turron, maruya, halo-halo,* banana cue, and *ginataan.* The flowers of Saba called *puso* (heart) are popularly used as vegetable in the Philippines. The total export of banana chips in 2015 was 50,262 metric tons which amounted to USD55.2 M and although the amount decreased to 36,633 metric tons in 2016, the value was higher at USD68.5 M. From January to October 2017 the export of banana chips reached 218,438 metric tons worth USD70.5 M (DTI 2018).

The seeded banana locally known as *butuhan* (*Musa balbisiana* Colla) is one of the progenitors of cultivated bananas and plantains. Fourteen edible *Musa balbisiana* cultivars have been recognized in the Philippines (Valmayor et al. 2002). These include one diploid, Abuhon (BB), and 13 triploids (BBB) namely: Saba or Dippig, Cardaba, Gubao, Bigihan, Pa-a Dalaga, Saba sa Hapon, Pondol, Turangkog/ Sab-a, Sabang puti, Mundo, Dali-an, Kalimpos, and Inabaniko/Binendito. However, the existence of pure *balbisiana* cultivars has been questioned by some banana taxonomists. Thus, several studies have been conducted to elucidate and prove the existence of *balbisiana* cultivars using several techniques. Espino and Pimentel (1998) and Espino (2000) confirmed the differences in banding patterns of isozymes exhibited by *acuminata* clones from *balbisiana* and their hybrids. Pure *balbisiana* cultivars were shown to be different and distinct from pure *acuminata* clones using random amplified polymorphic DNA (RAPD) (Sales and Espino 2001). Sales et al. (2011) confirmed the triploid B nature of the Philippine Saba group using flow cytometry analysis and DArT (diversity array technology) analysis. These findings provided evidence of pure *balbisiana* cultivars of banana as distinct from the more pure *acuminata* cultivars and their hybrid clones. However, inspite of the aforementioned studies, the question and researches on the purity of the BBB clones continue. Christelova et al. (2017) reported Saba (S14 ITC1745) to be ABB using flow cytometry.

The correct identification of cultivars is essential for the evaluation of genetic variability and differentiation between species and subspecies (Lu et al. 2010). Recognizing the economic and genetic importance of Saba, it is necessary to identify cultivars with good fruit quality and high potential for processing and industrial application. This will also help plant breeders properly select suitable parental lines in the development of mapping populations for crop

improvement. Reliable methods of identification are also required for the establishment of plant variety rights (Kjedlgaard et al. 1994) and for the protection and conservation of the Philippine Saba cultivars.

The taxonomy of cultivated bananas has long been a contentious issue as available literature are contradicting (Brown et al. 2009; Al-Saddy et al. 2010). Banana classification and identification have been complicated by numerous common names and synonyms in different languages and dialects (Valmayor et al. 2000). Sotto and Rabara (2000) revealed sufficient variability within *Musa balbisiana* Colla accessions in terms of bunch appearance, fruit color, shape and size, male bud characteristics as well as color of compound and free tepals of the male flowers. Current identification and classification scheme of banana relies on plant morphology. However, plant morphology is often easily influenced by the environment. To overcome the limitations of morphology-based classification scheme, molecular markers have been used in studying various aspects of plant genomes such as fingerprinting, genetic variability characterization, genome mapping, gene localization and genome evolution analysis, population genetics, taxonomy, and diagnostics.

Simple sequence repeat (SSR) or microsatellite markers have become the marker system of choice for banana genotyping (Grapin et al. 1998). These markers are based on tandem repeats of (2 - 6 bp) DNA sequences (Litt and Lutty 1989) which show simple repeat sequence polymorphism (Tautz 1989). The SSR markers are commonly used because they are highly polymorphic, show a co-dominant mode of inheritance, are abundant in the genome, reproducible and easy to interpret (Grapin et al. 1998; Selvi et al. 2002). They demonstrate a high degree of transferability between species, and are thus useful markers for comparative genetic and genome analysis (McCouch et al. 1997; Dirlewanger et al. 2002; Saja et al. 2006). This marker system has been proven to be useful for genetic analysis in a number of crops including *Musa* (Kaemmer et al. 1997; Crouch et al. 1998). Several studies have shown that microsatellite markers developed for one species could be used in related plant species (Dayanandan 1997). Microsatellite markers transferability was previously reported by Dirlewanger et al. (2002) in their study in peach (*Prunus* spp) species and by Saha et al. (2006) in grass species.

Dacumos et al. (2011) investigated the variability and genetic diversity among 61 genotypes of Philippine bananas using eight SSR markers. However, the variability and DNA fingerprints of cultivated Saba-type genotypes and cultivars of *M. balbisiana* in the Philippines were not fully studied. Other studies reported the use of SSR primers in genetic diversity studies in bananas. Creste et al. (2003) reported 67 polymorphic alleles using 11 SSR primers and an average of 6.10 alleles per primer in their study of genetic diversity among 58 Brazilian banana accessions. Oriero et al. (2006) used nine SSR primer pairs and obtained 23 alleles while Ning et al. (2007) reported 92 alleles from 10 SSRs in their study of 216 banana accessions.

Fourteen cultivars of *Musa balbisiana* have been collected from various parts of the Philippines and these include the different Saba genotypes (Valmayor et al. 2002). Based on the classifications and descriptions of Valmayor et al. (2002), Abuhon is the only edible diploid with pure balbisiana genome (BB) whose fruit is covered with wax. The variety got its name from "abu" which locally means ash. Saba or locally known as Dippig in the Ilocos region which means angular in the Ilocano dialect is probably the most important cultivar that is grown throughout the country. Cardaba, on the other hand, is more vigorous than Saba/Dippig and is popular in the Visayas and Mindanao areas. Turangkog/Sab-a is also popular cooking banana in the South with small fruits and has an erect leaf orientation while Mundo is almost similar except that it has yellow-orange fruit flesh with excellent eating quality. Other cultivars like Gubao, Bigihan, Pa-a Dalaga, Saba Hapon, Pondol, Sabang puti, Dali-an, and Kalimpos are not so common. Binendito is also known as Inabaniko because of the appearance of the hands which resemble a fan and the fingers appear to be fused together. Morphological and horticultural characteristics of these *Musa balbisiana* cultivars are listed in the book by Valmayor et al. (2002).

This paper reports on the DNA fingerprinting and analysis of genetic diversity among 13 Saba and *Musa balbisiana* cultivars determined using SSR markers. Selected cultivars of *Musa acuminata* and *Musa textilis* have been included as control or reference materials.

MATERIALS AND METHODS

Materials

Young leaves from 13 banana (*Musa* spp.) cultivars classified as BBB and other genotypes representing AA, ABB, ABBB genomic groups (Table 1) were obtained from the banana germplasm collection of the National Plant Genetic Resources Laboratory (NPGRL), Institute of Plant Breeding (IPB), College of Agriculture and Food Science, University of the Philippines Los Baños, Laguna. Only Bigihan cultivar was not included for lack of sample. The genomic classification of the sample bananas according to Valmayor et al. (2002) was used in this study.

All chemicals used were of the highest purity or molecular biology grade and obtained from Sigma-Aldrich (St. Louis, MO, USA), Vivantis (Selangor Darul Ehasan, Malaysia), and Invitrogen (Carlsbad, CA, USA), unless stated otherwise.

Genomic DNA Isolation

Genomic DNA was extracted from homogenized fresh young leaf tissue (500 mg) using modified CTAB-based protocol described by Doyle and Doyle (1990). The DNA pellet was washed with 500 µL of 70% (v/v)

ethanol; the supernatant was discarded and the remaining pellets were air dried, resuspended in 30 µL of sterile nano pure water and treated with 0.1 volume RNase A (10 mg mL^{-1}) at 37°C for 1 hr. DNA concentration was estimated from the absorbance at 260 nm using the conversion factor of 50 ng uL^{-1} and the quality was assessed by obtaining the A260/A280 ratio (SmartSpec 3000, Bio-Rad, Hercules, CA, USA). The DNA samples were subjected to electrophoresis on 1% agarose gel, stained with ethidium bromide and viewed under UV light using a Gel Doc 1000 gel documentation system (Bio-Rad).

PCR-based Screening

A total of 45 *Musa*-based SSR primer pair sequences (Supplement Table 1) synthesized by Invitrogen (Singapore) were tested for amplification and polymorphism on the 18 banana samples. Six SSR primers previously used for papaya (Cimagala 2011) and *Garcinia* species (Wittayawannakul et al. 2010) were also tested for cross-species amplification.

Polymerase Chain Reaction (PCR) was conducted based on the PCR conditions described by Kaemmer et al. (1997) with some modifications. The reaction solution contained 50 ng of template DNA, in 12.5 µL reaction containing 1X PCR buffer (10 mM Tris-HCl (pH 8.3), 50 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, and 0.4 µM each of forward and reverse primers and 0.05 unit of *Taq* polymerase. The PCR amplification using *Musa* based primers was performed at 94°C for 4 min for the initial denaturation and then 35 cycles of 94°C for 30 s denaturation, 30 s at annealing temperature, T*a* (depending on primer pair, Supplement Table 1), and 30 s at 72°C extension with a final extension at 72°C for 10 min. Annealing temperature for each primer was optimized for optimum band resolution.

PCR amplification using polymorphic markers from papaya and Garcinia species was carried out in a C1000 Touch Thermal Cycler (Bio-Rad) using the optimized protocol for amplification. The reaction mixtures contained the following at their final concentration: DNA (100 ng μL^{-1}), 1 X PCR buffer, 0.1 mM dNTPs, 1.5 mM $MgCl_2$, 0.4 µM each of forward and reverse primers and 1.0 U *Taq* polymerase (Vivantis). PCR cycling consisted of 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing temperature of 50°C, 53°C, 54°C and 55°C according to each SSR primer for 1 min, and extension at 72°C for 2 min, with a final extension at 72°C for 5 min. PCR products were mixed with 2.5 µL gel loading dye (5 X dye: 0.25% bromophenol blue, 0.25% xylene cyanol FF, 30% glycerol, (w/v) and centrifuged briefly in a microfuge before loading. The bands were resolved using 2% agarose (Invitrogen) in 0.5 X TAE buffer at 70 V for 1 h and 30 min. The gels were stained with 0.005% (w/v) ethidium bromide for 1-2 min, de-stained for 15-20 min and viewed under UV light using Gel Doc 1000 (Bio-Rad) gel documentation system.

Molecular Marker Scoring

PCR was done at least 3 times to check the reproducibility of each marker and a representative electrophoretogram was used for molecular marker scoring. Each polymorphic marker was identified and only clear and unambiguous bands were scored. Bands were scored "1" for the presence and "0" for absence of a DNA fragment. The presence or absence of the amplified band (similar size) in all genotypes indicates similarity, whereas presence in one and absence in the other indicates dissimilarity.

Polymorphism Information Content (PIC)

Polymorphism information content (PIC), a measure of the allelic diversity at a locus as described by Saal and Wricke (1999), was calculated according to the following formula:

$$PIC = 1 - \sum_{i=1}^{n} pi^2$$

Where p is the frequency of the ith allele out of the total number of alleles at an SSR locus and n is the total number of different alleles for that locus. PIC refers to the value of a marker for detecting polymorphism within population. It is the probability that an individual is informative with respect to the segregation of its inherited alleles and measures the usefulness of a marker.

Similarity Index (SI)

The similarity indices were obtained using the formula of Dice (1945).

$$S_d = \frac{2N_{xy}}{(N_x + N_y)}$$

where N_x and N_y are the number of bands observed in clones X and Y, respectively and N_{xy} is the number of bands common to both clones. The relationships of different Saba banana accessions were determined based on the SI values. The data obtained were analyzed using NTSYS PC (NYTSYS 2002) to obtain the similarity indices and to create a consensus dendrogram of the saba cultivars in the country.

Genetic Diversity

The genetic diversity or expected heterozygosity between the populations was calculated using the unbiased diversity index equation by Nei (1973):

$$He = 1 - \sum_{n=1}^{n}(\mathrm{qi})2$$

where: qi = frequency of the ith allele of n alleles at a locus.

To get the average expected heterozygosity this equation was used:

$$He(ave) = \sum_{n=1}^{n} \mathrm{He/L}$$

where: He = heterozygosity equation per locus and;
L = total number of loci

The maximum genetic diversity is given as "1", while the genetic diversity of "0" indicates uniform population

composed of a single lineage (Ma et al. 2001). Manual computations using the formulas were done to estimate the level of genetic diversity.

RESULTS AND DISCUSSION

SSR Characterization and Banding Pattern Analysis

A total of 18 banana cultivars representing different genomes were analyzed in this study: one BB genome, 12 BBB genome, two AAB, one AA genome, and one ABBB genome (Table 1) based on the classification of Valmayor et al. (2002). *Tinawagang Pula*, a variety of abaca, was included in the sample population. High quality genomic DNA was obtained and used for PCR amplification of microsatellites.

Fourteen out of 51 SSR primer pairs screened showed polymorphism. Only clear and reproducible bands ranging from 100 to 500 bp were scored. An SSR marker was considered polymorphic if it exhibited different amplification patterns across the 13 cultivars tested. Among the 14 polymorphic loci, a total of 47 alleles were detected with a mean value of 3.36 alleles per locus (Table 2). The number of DNA amplified products per primer pair ranged from 1-8 alleles. The largest allele size (500 bp) was observed with primer mMaCIR 25 and primer mMaCIR 45, whereas, the smallest allele (100 bp) was observed using primer mMaCIR 03. The maximum number of bands obtained was eight using primer mMaCIR 25, whereas several primers provided at least two bands.

Six SSR primer pairs from papaya and Garcinia were tested on the 18 banana cultivars DNA samples and only the BGal primer from papaya amplified robust, polymorphic bands. The two SSR primers from Garcinia (Wittayawannakul et al. 2010) were not able to produce scorable bands. Cross-species amplification may fail due to distant genetic relationship between the source species and the target species (Lopez-Vinyallonga et al. 2011). The authors further explained that the similarities in the flanking regions of the microsatellite, where the PCR primers bind to the DNA, are higher in phylogenetically close species than in phylogenetically distant species. In the present study, Garcinia and Saba belong to different families, thus, may share few conserved sequences flanking SSRs, and this could explain why the two SSR primers from Garcinia did not work.

The polymorphism information content (PIC) of the 14 microsatellite markers used in this study ranged 0.23 to 0.80 (Table 2). Of the 14 markers 10 were rated highly informative (PIC > 0.5), three markers were reasonably informative (0.5 > PIC > 0.25) and one marker was slightly informative (PIC < 0.25). The highest PIC value of 0.80 was obtained with primer mMaCIR 25 and the lowest PIC value of 0.23 with primer Ma 1-32. The average PIC value of 0.55 is considered highly informative. The observed high level of information content by each microsatellite marker indicates the microsatellites' capability to quantify genetic diversity and potential to discriminate between Saba cultivars. According to Botstein et al. (1980), a PIC value >0.5 indicates a highly polymorphic locus while a value of <0.25 denotes lower polymorphic locus, thus, indicating the highly informative nature of the SSR markers used in this study.

Table 1. Different banana cultivars used in the study.

Cultivar Name	Scientific Name	Genomic Group[a]	Type
1. Saba	*M. balbisiana*	BBB	Cooking
2.Cardaba	*M. balbisiana*	BBB	Cooking
3.Abuhon	*M. balbisiana*	BB	Cooking
4. Pa-A Dalaga	*M. balbisiana*	BBB	Cooking
5. Sabang Puti	*M. balbisiana*	BBB	Cooking
6. Saba sa Hapon	*M. balbisiana*	BBB	Cooking
7. Mundo	*M. balbisiana*	BBB	Cooking
8. Gubao	*M. balbisiana*	BBB	Cooking
9. Dali-An	*M. balbisiana*	BBB	Cooking
10. Pondol	*M. balbisiana*	BBB	Cooking
11. Turangkog	*M. balbisiana*	BBB	Cooking
12. Kalimpos	*M. balbisiana*	BBB	Cooking
13. Binendito/ Inabaniko	*M. balbisiana*	BBB	Cooking
14. Pisang Seribu	*M. acuminata* x *M. balbisiana*	AAB	Dessert
15. Tiparot	*M. acuminata* x *M. balbisiana*	ABBB	Cooking
16. Lakatan	*M. acuminata*	AA	Dessert
17. Latundan	*M. acuminata* x *M. balbisiana*	AAB	Dessert
18. Abaca	*M. textilis*	T genome	For fiber

[a]Classification used based on Valmayor et al. (2002)

In a previous study on the diversity of Philippine bananas, the microsatellite markers used revealed high PIC ranging 0.66 to 0.94 (Dacumos et al. 2011) indicating high discriminatory potential of the primers. Yllano (2010) revealed a high polymorphism information content (PIC) value of 0.48 to 0.79 among abaca accessions in the Philippines.

DNA Fingerprints of Saba and *M. balbisiana* Cultivars

The number of band patterns produced by the 14 SSR markers across the 18 cultivars ranged 2-8 (Table 2, Figure 1). The most number of patterns was observed using primer mMaCIR 25 with 8 banding patterns from 8 alleles. This is followed by mMaCIR 01 and mMaCIR 13 which generated 7 and 5 banding patterns, respectively. Ten markers exhibited 2 banding patterns from combinations of 2-3 alleles. A total of 42 banding patterns were generated using the 14 polymorphic markers in 18 banana cultivars. The use of SSR with many alleles per locus enables uniquely fingerprinting large number of accessions by relatively few loci (McCouch et al. 1997).

Table 2. Molecular and genetic characteristics of the 14 microsatellite markers.

Microsatellite markers	Number of alleles	Number of banding patterns	Relative molecular weight (bp)	Polymorphic Information Index (PIC)	Gene diversity (He)
mMaCIR 01	5	7	240-390	0.78	0.31
mMaCIR 03	2	2	100-120	0.50	0.44
mMaCIR 25	8	8	220-500	0.80	0.27
mMaCIR 152	2	2	150-230	0.47	0.27
mMaCIR 260	5	5	130-380	0.65	0.21
pMaCIR 348b	3	2	180-270	0.62	0.40
pMaCIR 108	3	2	250-300	0.63	0.42
mMaCIR 22	3	2	200-450	0.52	0.18
mMaCIR 45	2	2	200- 380	0.36	0.14
mMaCIR 13	6	5	200- 380	0.70	0.35
mMaCIR 39	2	2	180-200	0.50	0.41
MA 3-90	2	2	150-200	0.51	0.49
Ma 1-32	2	2	250-300	0.23	0.08
Bgal	2	2	250-280	0.48	0.25
Mean	3.36	3.21		0.55	0.31

Table 3. DNA fingerprints of the 13 *M. balbisiana* and other banana cultivars using 14 microsatellite markers.

Cultivar	Primers													
	1	3	25	152	260	348b	108	22	45	13	39	3-90	1-32	Bgal
Abuhon	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Saba	0	2	2	1	1	1	2	2	2	2	1	1	1	2
Cardaba	2	0	2	0	2	2	2	2	2	2	0	0	1	1
Paa-dalaga	3	2	3	0	2	0	1	1	2	1	1	2	1	1
Saba sa Hapon	0	2	3	0	3	1	1	1	1	0	1	0	1	1
Dali-an	4	2	2	0	3	1	2	2	1	1	0	1	1	2
Kalimpos	4	2	2	0	3	2	2	2	2	1	1	1	1	2
Gubao	5	2	3	2	3	0	1	1	1	1	1	1	1	1
Turangkog	3	2	0	0	1	1	0	2	2	1	1	1	1	1
Saba sa Puti	5	2	4	0	1	2	0	2	2	0	1	1	1	1
Mundo	0	2	4	2	3	2	1	2	2	0	0	1	1	1
Pondol	3	2	0	2	3	2	0	1	2	2	2	0	2	1
Inabaniko	5	2	4	1	4	2	0	2	2	2	0	1	2	2
Pisung Seribu	6	0	5	0	3	0	0	0	0	3	1	0	1	1
Tiparot	5	2	6	1	3	2	0	0	0	2	1	1	1	1
Musa acuminata 'Lakatan'	6	0	0	0	4	1	0	0	0	0	0	1	1	1
Latundan	7	2	7	0	5	0	0	0	0	4	0	0	1	1
Musa textilis	0	0	8	0	3	1	0	0	0	5	0	0	1	1

Note: The numbers represent unique banding patterns using specific primers. The "0" denotes no amplification.

To differentiate the Saba and other cultivars, the banding patterns generated by the 14 primers across the sample cultivars were analysed. Each banding pattern was designated with a number and the DNA fingerprint of each cultivar was assigned as the banding pattern observed for the 14 microsatellite markers used (Table 3). Unique DNA fingerprints were obtained for all banana cultivars studied as well for *Musa textilis* showing that the microsatellite markers used in this study can effectively differentiate the different banana cultivars and can be used to identify the different Philippine Saba and *M. balbisiana* cultivars.

The results also showed that a number of the SSR markers can identify specific cultivars with only one unique banding pattern using a particular marker. Markers mMaCIR 03, mMaCIR 260, mMaCIR 39 and Ma 3-90 can identify Abuhon, Inabaniko, Pondol and Paa-dalaga, respectively. However, a combination of all the markers gives a more definitive identification of the cultivar. Fingerprinting with molecular markers allows precise, objective and rapid variety identification.

Cluster Analysis of the Saba and other *Musa* samples using Microsatellite Markers

The genetic similarities of the banana cultivars were computed using the Dice coefficient because it excludes 'negative matches' or both isolates with no distinguishable bands. The Dice coefficient of similarity is advantageous to other commonly used similarity

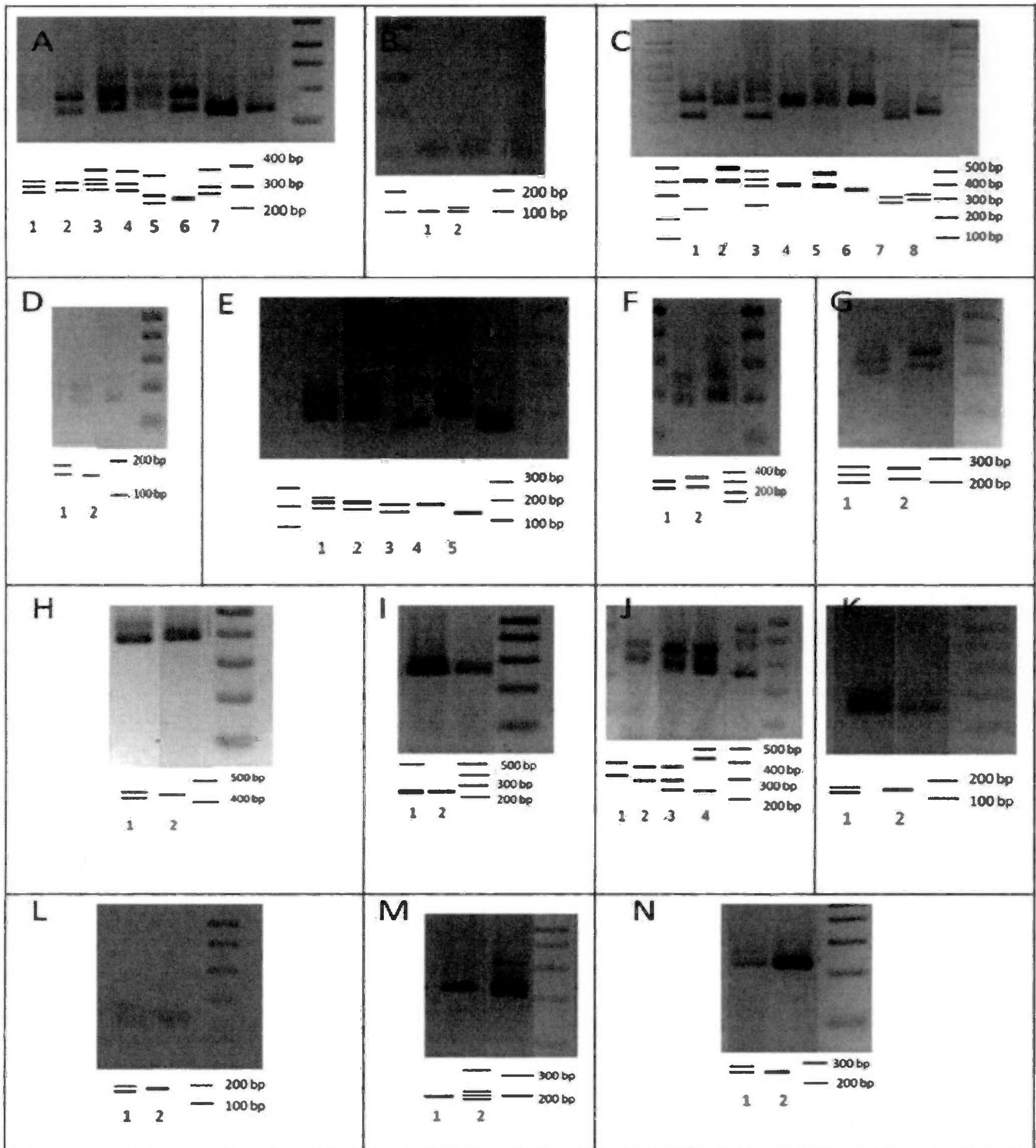

Figure 1. Representative gels showing the banding patterns generated using the 14 polymorphic markers. (A) mMaCIR 01; (B) mMaCIR 03; (C) mMaCIR 25; (D) mMaCIR 152; (E) mMaCIR 260; (F) pMaCIR 348b; (G) pMaCIR 108; (H) mMaCIR 22; (I) mMaCIR 45; (J) mMaCIR 13; (K) mMaCIR 39; (L) MA 3-90; (M) Ma 1-32; and (N) Bgal.

coefficients such as the simple matching coefficient especially when using banding pattern data where potentially infinite number of negative matches ends may exist.

The dendrogram constructed from the cluster analysis of the 18 banana cultivars using UPGMA-SAHN is shown in Figure 2. The similarity indices obtained ranged 0.43 to 0.92 coefficient value (Supplement Table 2). UPGMA cluster analysis generated three major clusters at the 60% similarity level. Cluster I is composed of all *M. balbisiana*-genome (BB and BBB) containing cultivars studied together with Tiparot (ABBB). On the other hand, the *M. acuminata* and *Musa* x *paradisiaca* cultivars formed two clusters. Pisang Seribu (AAB) was the lone cultivar in Cluster II

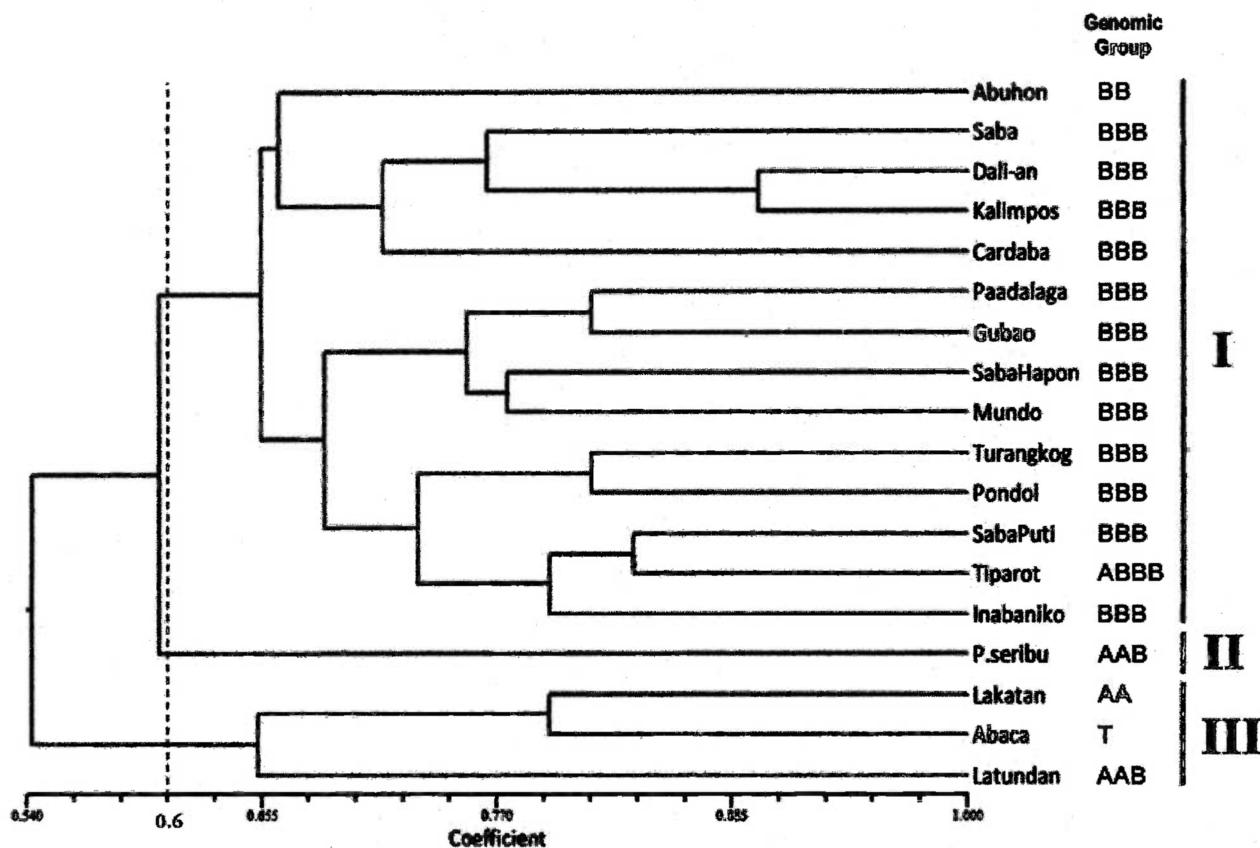

Figure 2. Dendrogram of 18 banana cultivars showing the genetic relationship generated by UPGMA SAHN cluster analysis.

while Lakatan (AA) and Latundan (AAB) was grouped with *M. textilis* (T genome) in Cluster III. The grouping of *M. textilis* with Lakatan and Latundan may be due to the inadequacy of the primers to separate the former from the *M. acuminata* varieties. Yllano et al. (2010) using a different set of primers were able to show the separation of the *M. textilis* accessions from Lakatan and Latundan.

Highest genetic similarity was observed between Kalimpos and Dali-an cultivars at 92% (Figure 2) indicating a very close evolutionary relationship. Moreover, the Dice genetic distance and the DNA fingerprints (Table 3) of Saba and Cardaba indicate that they are different cultivars with only 67% similarity contrary to previous report of Espino (2000) that Saba and Cardaba clustered together using isozymes. These results confirm, at the molecular level, the report by Valmayor et al. (2002) that Saba/Dippig and Cardaba were different based on morphological characteristics. The lowest similarity index value of 0.43 was observed in four combinations, namely, Latundan (AAB) and Saba (BBB), Latundan and Cardaba (BBB), Latundan and Dali-an (BBB), and Pisang Seribu (AAB) and Inabaniko (BBB) (Supplement Table 2). This demonstrated the ability of the SSR markers used to differentiate between the triploid cultivars AAB and BBB.

Furthermore, the cluster analysis showed that the 14 polymorphic markers used in this study were able to separate cultivars containing the *Musa acuminata* genome (AA) and the *Musa balbisiana* genome (BB) which affirmed the results of the studies conducted by Sales et al. (2001) using isozymes and Du (2003) using RAPD markers. The separation of the two genomes based on microsatellite polymorphism is in agreement with characterization based on morphological descriptors (Sotto and Rabara 2000).

Genetic Diversity of Philippine Saba (*Musa balbisiana* Colla) Cultivars

The 14 polymorphic SSR markers were used for genetic diversity analysis among the 13 Saba cultivars, 3 *Musa acuminata* Colla cultivars and 1 *Musa textilis* Nee cultivar. The gene diversity computed ranged 0.08 to 0.49, with a mean value of 0.31 (Table **2**) indicating the strong genetic diversity of Saba (*Musa balbisiana* Colla) in the Philippines. SSR marker Ma 3-90 showed the highest gene diversity (0.49) while Ma 1-32 (0.08) gave the lowest gene diversity value. The 14 SSR markers demonstrated the genetic diversity of the *M. balbisiana* samples and the other cultivars studied as shown by the clustering into 3 groups according to their genome composition (Figure 2). Furthermore, the cultivars carrying the BBB genomes showed genetic variability at 0.60 similarity

index. Christelova et al. (2017) observed high genetic diversity in Musa germplasm using 19 SSR markers. Similarly, Ravishankar et al. (2013) reported a high gene diversity of 0.138 to 0.934 for 63 SSR markers used in characterizing 30 *M. balbisiana* accessions belonging to the AA, BB or AB genome types. Many aspects, such as the breeding system, plant longevity, and agricultural practices, influence genetic diversity, including the proportion of variation distributed within and between populations (Hamrick and Godt 1996). Ge et al. (2005) observed a high level of intra-population genetic diversity using cpDNA PCR-RFLP with Gst value of 0.77 in *M. balbisiana* in China using SSR markers in *M. balbisiana*.

Overall, the genetic diversity observed in *Musa balbisiana* indicates the presence of a large gene pool which may include important genes that can be utilized in the improvement of banana genotypes.

CONCLUSIONS AND RECOMMENDATIONS

This study generated definitive DNA fingerprints using microsatellite markers which could identify each of the 13 Saba and *Musa balbisiana* cultivars and differentiate them from other banana cultivars of different genome composition. Further, the 14 SSR markers used in the study revealed that the 13 Saba and *Musa balbisiana* cultivars and five other banana cultivars were genetically diverse. Thus, these cultivars may be useful sources of desirable traits for Musa breeding and improvement.

These DNA fingerprints can be utilized in the proper identification of the *M. balbisiana* cultivars especially the economically important Saba cultivars used for processed food and industrial applications. Plant breeders can also use the DNA fingerprints and diversity data for proper selection of suitable parental lines in the development of mapping populations for crop improvement. The Philippine Department of Agriculture and its attached agencies can utilize these molecular markers to identify mislabelled varieties or identify varieties in a mixture. Moreover, these DNA fingerprints will be useful in the protection and conservation of the Philippine Saba and other cultivars.

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Supplement Table 1. List of SSR primers tested and the degree of polymorphism obtained among the different Philippine *M. balbisiana* and other cultivars

Marker Name	Reference	Ta (°C)	Characteristics
mMaCIR 01	Lagoda et al. (1998)	55	Polymorphic
mMaCIR 03	Lagoda et al. (1998)	55	Polymorphic
mMaCIR 07	Lagoda et al. (1998)	53	No Amplification
mMaCIR 08	Lagoda et al. (1998)	55	Monomorphic
mMaCIR 12	-		No Amplification
mMaCIR 13	Lagoda et al. (1998)	53	Polymorphic
mMaCIR 22	-		No Amplification
mMaCIR 24	Lagoda et al. (1998)	48	Polymorphic
mMaCIR 25	-		Polymorphic
mMaCIR 27	Lagoda et al. (1998)	58	To be verified
mMaCIR 39	Lagoda et al. (1998)	52	No Amplification
mMaCIR 40	Lagoda et al. (1998)	54	Polymorphic
mMaCIR 45	Lagoda et al. (1998)	57	To be verified
mMaCIR 150	Hippolyte et al. (2010)	54	Polymorphic
mMaCIR 152	Hippolyte et al. (2010)	54	Polymorphic
mMaCIR 164	Hippolyte et al. (2010)	55	Polymorphic
mMaCIR 195	Hippolyte et al. (2010)	54	Polymorphic
mMaCIR 196	Hippolyte et al. (2010)	55	Polymorphic
mMaCIR 214	Hippolyte et al. (2010)	53	Polymorphic
mMaCIR 231	Hippolyte et al. (2010)	55	Monomorphic
mMaCIR 260	Hippolyte et al. (2010)	55	Polymorphic
mMaCIR 264	Hippolyte et al. (2010)	53	Polymorphic
pMaCIR 332a	-	53	Polymorphic
pMaCIR 348b	-	53	Polymorphic
pMaCIR 327a	-	53	Polymorphic
pMaCIR 631a	-	53	Polymorphic
MA 1-32	Crouch et al. (1998)	60	Polymorphic
Ma 3-90	Crouch et al. (1998)	53	Polymorph
mMaCIR 307		53	Monomorphic

Supplement Table 2. Similarity index of Philippine *Musa balbisiana* (Colla) and Other Cultivars using the 14 polymorphic markers.

	Abuhon	Saba	Cardaba	Paadalaga	SabaHapon	Dali-an	Kalimpos	Gubao	Turangkog	SabaPuti	Mundo	Pondol	Inabaniko	P.seribu	Tiparot	Lakatan	Latundan	Abaca
Abuhon	1																	
Saba	0.653061	1																
Cardaba	0.653061	0.673469	1															
Paa dalaga	0.734694	0.591837	0.632653	1														
SabaHapon	0.632653	0.693878	0.571429	0.77551	1													
Dali-an	0.77551	0.795918	0.795918	0.673469	0.612245	1												
Kalimpos	0.693878	0.795918	0.795918	0.632653	0.612245	0.918367	1											
Gubao	0.673469	0.571429	0.571429	0.857143	0.755102	0.653061	0.693878	1										
Turangkog	0.755102	0.653061	0.530612	0.693878	0.673469	0.653061	0.653061	0.714286	1									
SabaPuti	0.653061	0.632653	0.632653	0.632653	0.653061	0.673469	0.755102	0.734694	0.816327	1								
Mundo	0.673469	0.693878	0.693878	0.77551	0.795918	0.693878	0.693878	0.673469	0.673469	0.734694	1							
Pondol	0.612245	0.632653	0.632653	0.673469	0.77551	0.55102	0.591837	0.612245	0.734694	0.673469	0.77551	1						
Inabaniko	0.55102	0.571429	0.571429	0.612245	0.55102	0.612245	0.612245	0.673469	0.591837	0.734694	0.673469	0.693878	1					
P.seribu	0.591837	0.612245	0.612245	0.612245	0.632653	0.530612	0.571429	0.632653	0.632653	0.530612	0.55102	0.653061	0.428571	1				
Tiparot	0.632653	0.612245	0.612245	0.653061	0.673469	0.653061	0.734694	0.755102	0.755102	0.897959	0.714286	0.734694	0.755102	0.510204	1			
Lakatan	0.632653	0.612245	0.612245	0.571429	0.673469	0.571429	0.489796	0.510204	0.714286	0.612245	0.714286	0.693878	0.510204	0.673469	0.591837	1		
Latundan	0.489796	0.428571	0.428571	0.591837	0.571429	0.428571	0.387755	0.571429	0.530612	0.510204	0.612245	0.591837	0.530612	0.489796	0.571429	0.653061	1	
Abaca	0.55102	0.489796	0.530612	0.530612	0.632653	0.44898	0.367347	0.510204	0.591837	0.489796	0.632653	0.693878	0.510204	0.632653	0.55102	0.755102	0.612245	1