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Genetic Analyses of Abaca (*Musa textilis* Née) Germplasm from its Primary Center of Origin, the Philippines, Using Simple Sequence Repeat (SSR) Markers

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This study presents the first extensive genetic diversity assessment of abaca (*Musa textilis* Née) germplasm in the Philippines using simple sequence repeat (SSR) markers. Southeast Asia is the recognized center of origin of the genus *Musa*, and the Philippines has the most number of abaca cultivars and wild genotypes in the world. In this study, a total of 150 accessions of abaca from Luzon, Visayas, and Mindanao, Philippines and two banana cultivars were analyzed using SSRs to examine their genetic diversity and similarity relationships. Six of 44 banana-based SSR primers were highly polymorphic, detecting 28 alleles with a mean of 4.55 alleles per locus and polymorphism information content (PIC) mean of 0.56. Genetic diversity of abaca in three main islands was high (I = 0.68). Abaca genotypes from Luzon had higher genetic diversity compared to Visayas and Mindanao. Ninety-five (95) percent was attributed to molecular variance within the population and only 5% of the molecular variance to variation among populations. Genetic analysis by Unweighted Pair Group Method with Arithmetic Mean (UPGMA) revealed nine clusters consisting of two big groups and seven small groups, irrespective of geographical origin. Using a combination of SSR markers, the abaca accessions and closely related species could be effectively distinguished and identified putative abaca duplicates. Our results provide genetic evidence of the high diversity of abaca germplasm in the Philippines. The characterization of abaca germplasm using SSR markers will aid in the identification of superior genotypes and for improving the *in situ* and *ex situ* abaca germplasm conservation and optimal utilization of abaca genetic resources.

Key Words: abaca, Manila hemp, germplasm, genetic diversity, molecular markers

Abbreviations: Simple Sequence Repeat (SSR), polymorphism information content (PIC), Unweighted Pair Group Method with Arithmetic Mean (UPGMA), cetyl trimethylammonium bromide (CTAB), tris-ethylenediaminetetraacetic acid (TE), ribonuclease (RNase), ethidium bromide (EtBr), polymorphism information content (PIC)

INTRODUCTION

Abaca, the source of a strong fiber called the Manila hemp, is the Philippines' most economically important fiber crop used as cordage, nets, cloth, handicrafts, furnishings, geotextile, a part of paper currency, structural composites, non-structural car parts, and a promising spacesuit component. Due to high demands, production of abaca increased from 68,100 to 71,500 metric tons from 2014 to 2018, worth PhP 2.948 billion to PhP 4.982 billion (PSA 2019). Abaca exports also increased from USD 14.71 million in 2014 to USD 36.77 million in 2018 (PSA 2019). With this, the Philippines remains the top producer and exporter of this fiber crop worldwide.

The family Musaceae, which includes banana and abaca, has the richest diversity in the Indo-Malay region

(Chakravorti 1951). Abaca is native to the Philippines, and the Bicol region in Southern Luzon boasts of the greatest number of abaca (*M. textilis* Née) cultivars and wild types and is thus considered the center of origin of abaca. The abaca spread from the Philippines to neighboring countries like Borneo, and then to the East Indies, India, Ceylon, Fiji Islands, Jamaica, Trinidad, the Dominican Republic, and the Western World (Copeland 1911; Spencer 1951).

Presently, different abaca cultivars are grown in various areas of the Philippines. They are improved through classical selection of existing varieties and accessions, clonal selections, and hybridization. Since abaca has always been propagated vegetatively, this has resulted in numerous cultivars and accessions that are phenotypically similar to one another with synonymous and homonymous local names. Some wild abaca accessions possess morphological attributes similar to those of the cultivated varieties. This limits the understanding of the genetic resource and raises problems in germplasm improvement and management.

Breeders are now employing various strategies in broadening the genetic base of abaca and exploiting its wild relatives to enhance its resistance to diseases and improve its fiber quality. Abaca varieties have a haploid chromosome number of 10 (2n=20) (Valmayor et al. 1956; Pancho and Capinpin 1959). Natural hybrids of abaca with other native *Musa* species, *M. balbisiana* Colla and *M. acuminata* Colla, exist in the Philippines. Abaca varieties Canton and Mimay are natural hybrids of Pacol, a cultivar of *M. balbisiana* Colla, and abaca (*M. textilis* Née) (Valmayor et al. 1956). Pacol and Canton exhibit a high degree of resistance to abaca mosaic and bunchy-top virus diseases, the major diseases of abaca. First-generation hybrids of Pacol and abaca exhibited traits and fiber quality intermediate between Pacol and abaca and similar to those of Canton (Bernardo 1957) and with varying resistance to said diseases (Bernardo and Umali 1956; Bernardo et al. 1965). Backcrossing Pacol x abaca hybrid to abaca has generated BC_2 plants with strong resistance against bunchy-top virus and good fiber quality (Lalusin 2010).

Morphological and biochemical markers are used to assess the genetic diversity of various crops (Wittayawannakul et al. 2010; Major et al. 2018). However, because these markers are affected by environmental changes, molecular markers such as simple sequence repeats (SSRs) which occur abundantly throughout eukaryotic genomes and demonstrate a high degree of transferability between species (Powell et al. 1996; Jewell et al. 2006; Abdi et al. 2019; Lebedev et al. 2020) are now utilized. SSRs are inherited in a Mendelian manner and are codominant and hypervariable (Saghai-Maroof et al. 1994). SSR markers have been identified and used in a number of valuable plant species (Kaemmer et al. 1997; Cipriani et al. 2002; Oriero et al. 2006; Zhu et al. 2018). Dacumos et al. (2011) reported the variability and genetic diversity among 61 genotypes of Philippine bananas using eight SSR markers. Doloiras-Laraño et al. (2018) showed that 13 polymorphic SSR primers were able to identify and differentiate 13 Philippine *M. balbisiana* Colla and Saba cultivars. SSR markers were showed to be effective in marker-assisted breeding, linkage mapping, genetic diversity analysis, and phylogenetic studies (Kalia et al. 2011; Vieira et al. 2016).

Lasalita-Zapico et al. (2010) assessed the level of morphological diversity among variant strains of three abaca varieties (Maguindanaon, Tangongon, and Bongolanon) from several areas in Southern Mindanao, Philippines. Using six quantitative and 25 qualitative morpho-agronomic traits, their study showed morphological homogeneity for the traits evaluated. On the other hand, Boguero et al. (2016) reported that 57 accessions of Philippine abaca associated with bunchy top virus resistance were highly diverse with a Shannon-Weaver index of 0.92 using six SSR markers. Their results further showed 56% and 45% similarity of two abaca hybrids with Pacol (*M. balbisiana* Colla) which has been used as a source of bunchy top virus resistance in abaca breeding.

This study aimed to determine the genetic diversity and relationships of the abaca germplasm from the three major islands of Luzon, Visayas, and Mindanao of the Philippines using SSRs. Information derived from this study may be applied in setting effective breeding strategies and germplasm conservation of abaca.

MATERIALS AND METHODS

Plant Materials

A total of 150 accessions of *M. textilis* Née, 78 from Luzon, 48 from the Visayas and 24 from Mindanao, and two relatives — *M. balbisiana* Colla and *Musa acuminata* Colla (Table 1), were obtained from various sources, namely, the Institute of Plant Breeding, College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños (UPLB), Abaca Experimental Station of the Philippine Fiber Industry Development Authority (PhilFIDA) in Bicol, National Abaca Research Center (NARC) at the Visayas State University in Baybay Leyte, Murcia Negros Occidental and PhilFIDA in Davao City, Mindanao. Some of these accessions were planted at

Table 1. List of Philippine abaca accessions, origin, and corresponding code.

Accession	Origin	Code	Accession	Origin	Code
AB San Miguel	Luzon	L1	Negro	Luzon	L42
Abuab Rapu	Luzon	L2	Nilagonoy	Luzon	L43
Baguisan Puti	Luzon	L4	Parang	Luzon	L44
Bongliuan	Luzon	L5	Pisgan	Luzon	L45
Bongolanon	Luzon	L6	Puti	Luzon	L46
Bontimag	Luzon	L7	Putian	Luzon	L47
Bulac-luno	Luzon	L8	Salibaonon	Luzon	L48
Bulao	Luzon	L9	Samura	Luzon	L49
Bulaoluno	Luzon	L10	Sil-on	Luzon	L50
Butikon	Luzon	L11	Sognod	Luzon	L51
Canarahon	Luzon	L12	T. pula	Luzon	L52
Canton	Luzon	L13	T. outi	Luzon	L53
Casilihon	Luzon	L14	Tangongon	Luzon	L54
Gaes	Luzon	L15	Tosoy	Luzon	L56
Guakon	Luzon	L16	Turutagakon	Luzon	L57
Igit	Luzon	L17	Yoga	Luzon	L58
Inotang	Luzon	L19	Ogarum	Luzon	L59
Kaulayan	Luzon	L20	Popoonan	Luzon	L60
Kurukutuhan	Luzon	L21	Javaque	Luzon	L61
Kutay-Kutay	Luzon	L22	Binongongoran	Luzon	L62
Lagunuyon itom	Luzon	L23	Del monte puti	Luzon	L63
Lagunuyon puti	Luzon	L24	Laguis	Luzon	L64
Lausigon Red	Luzon	L26	Bongotsanon	Luzon	L65
Layas	Luzon	L27	Agutayon	Luzon	L66
Laylay	Luzon	L28	Binagakay	Luzon	L67
Linlay	Luzon	L29	Samina	Luzon	L68
Linoluban	Luzon	L30	Layahon	Luzon	L60
M-50	Luzon	L31	Lunhan	Luzon	L70
M-82	Luzon	L32	Sugmad pula	Luzon	L71
Maginoo	Luzon	L33	Lawaan x Tangongon	Luzon	L72
MINC	Luzon	L34	Casilihon	Luzon	L73
MTP	Luzon	L35	Amokid	Luzon	L74
NAC 12	Luzon	L36	Libutanay	Luzon	L75
Nam 10	Luzon	L37	Baunan	Luzon	L76
NARC 51	Luzon	L38	Itolaus	Luzon	L77
NARC 60	Luzon	L39	Pakil	Luzon	L78
NARC 61	Luzon	L40	Kirayan	Luzon	L79
Natural Tobacco	Luzon	L41	Goa-x	Luzon	L80

Table 1 continued on p. 314.

the Institute of Plant Breeding, CAFS, UPLB, Los Baños, Laguna, Philippines (Fig. 1).

Primers

This study screened a total of 140 SSR primers: 39 pairs for the repetitive sequences of the A and B genome of *Musa*, five pairs targeting the B genome (*Musa* Genome Consortium; Crouch et al. 1999), 89 pairs of rice (*Oryza sativa* L.) SSR primers, four pairs of papaya (*Carica papaya* L.) SSR primers (Cimagala et al. 2019) and three pairs of gene-specific malunggay (*Moringa oleifera* Lam.) primers. Primer pairs that worked for abaca are listed in Table 2. The *Musa* Genome Consortium has been integrated into the global Musa genetic resources network MusaNet (https://www.promusa.org/MusaNet).

DNA Isolation

Leaf genomic DNA from 150 abaca accessions from Luzon, Visayas, and Mindanao and two banana relatives (*M. balbisiana* Colla and *M. acuminata* Colla) were isolated using the modified cetyl trimethylammonium bromide (CTAB) method (Doyle and Doyle 1987). The pellet obtained was resuspended in 50–100 µl of tris-ethylenediaminetetraacetic acid (TE) buffer and treated with 0.1 volume ribonuclease (RNase) A (10 mg ml^{-1}) at 37°C for 1 h. The quantity and quality of all DNA samples were determined spectrophotometrically and on agarose gels. Bands were resolved using 1.5% agarose gels and stained with ethidium bromide (EtBr) and viewed under ultraviolet (UV) light.

Polymerase Chain Reaction (PCR) Amplification

PCR amplification using a Biorad iCycler Thermal Cycler was carried out following the conditions described by Kaemmer et al. (1997) with some modifications. Each 20 µl reaction contained ~30 ng of template DNA, 1 × PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl), 1.5 mM $MgCl_2$, 0.15 mM dNTPs, and 0.2 µM each of forward and reverse primers and 0.4 U of *Taq* DNA polymerase. Amplification conditions had the following profile: initial denaturation, 94°C for 4 min, 35 cycles of 94°C for 30 s, 50–60°C for 30 s and 72°C for 30 s, and a final extension at 72°C for 10 min. The soaking temperature was set at 4°C. The annealing temperature for each primer was modified for optimum band resolution. Bands were resolved

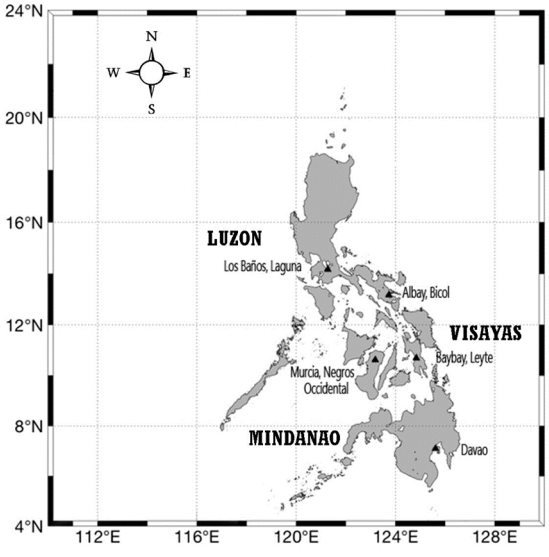

Fig. 1. Sampling sites of *Musa textilis* Née accessions in the Philippines.

Table 1. Continuation

Accession	Origin	Code	Accession	Origin	Code
Karaykayon	Luzon	L81	Dehayo	Visayas	V37
Samoro	Luzon	L82	Bool	Visayas	V38
Lagurhuan	Visayas	V1	Linuy-a	Visayas	V39
Minenonga	Visayas	V2	Sangalang	Visayas	V40
Putian	Visayas	V3	Pulang bato	Visayas	V41
Inosa	Visayas	V4	Wild 1	Visayas	VW1
Itesog	Visayas	V5	Wild 2	Visayas	VW2
Alman	Visayas	V6	Wild 3	Visayas	VW3
Linino	Visayas	V7	Wild 4	Visayas	VW4
Sinamoro	Visayas	V8	Wild 5	Visayas	VW5
Itihenbalod	Visayas	V9	Wild 6	Visayas	VW6
Minorado	Visayas	V10	Wild 7	Visayas	VW7
Baguisan	Visayas	V11	Bongolanon	Mindanao	M1
Wild	Visayas	V12	I39 x Mg3	Mindanao	M2
Minsog	Visayas	V13	Agutay (ITC)	Mindanao	M3
Lagursog	Visayas	V14	Maguino	Mindanao	M4
Suglin	Visayas	V15	Kaunayan	Mindanao	M5
Malaniceron	Visayas	V16	Halayhay	Mindanao	M6
Hanginan	Visayas	V17	Putian	Mindanao	M7
Kabasnon	Visayas	V18	Musatex 118	Mindanao	M8
Bisaya	Visayas	V19	Mamakaw/Mosolong	Mindanao	M9
Luno luno1	Visayas	V20	Samponganon	Mindanao	M10
Luno luno2	Visayas	V21	Laguis Agusan	Mindanao	M11
Lagwis kilayan	Visayas	V22	Danulon	Mindanao	M12
Baunan leyte	Visayas	V23	Maguindanao	Mindanao	M13
ACC #2	Visayas	V24	Mag-ino	Mindanao	M14
Laguis Natural	Visayas	V25	Lintang	Mindanao	M15
Aurelio	Visayas	V26	Tansug	Mindanao	M16
Tagbinonga	Visayas	V27	Maguindanao black	Mindanao	M17
Bila	Visayas	V28	UP-USF-Caf2	Mindanao	M18
Sagunaa	Visayas	V29	UP-USF-Caf4	Mindanao	M19
Hinagikhik	Visayas	V30	Danganon	Mindanao	M20
Balunan-white	Visayas	V31	Davao	Mindanao	M21
Tanglaw x Nagahakno	Visayas	V32	Bunguiao	Mindanao	M22
No ID253	Visayas	V33	Tanglawan x Inosa	Mindanao	M23
Lawisig pula	Visayas	V34	Maguindanao x Inosa	Mindanao	M24
Anondi	Visayas	V35			
Calbayognon	Visayas	V36			

using 2% agarose gels, stained with ethidium bromide (EtBr) and viewed under UV.

Data Scoring and Analysis

Each polymorphic marker was identified and scored. The Shannon's Diversity Index (*I*) was computed using the equation below as described by Peakall and Smouse (2006):

$$I = -1\sum pi\ Ln\ pi$$

where p_i is the allele frequency of the i^{th} allele at a particular locus and Ln is equal to the log of *pi* to the base e (~2.718). Shannon's could distinguish the level of variation between populations with the same number of alleles, or in cases where populations are dominated by few common alleles or contributed more evenly by all alleles (Konopiński 2020). Observed Heterozygosity (H_o) for a genetic marker was computed using the formula:

$$H_o = \frac{No.\ of\ Hets}{N}$$

where the number of heterozygotes (Hets) is determined by direct count. N is the number of sample size (Peakall and Smouse 2006). Expected Heterozygosity (H_e) was calculated using the equation:

$$He = 1 - \sum pi^2$$

where *pi* is the frequency of the i^{th} allele at any particular locus (Peakall and Smouse 2006). The polymorphism information content (PIC) equation derived from Botstein et al. (1980) was utilized to measure the allelic diversity at a locus:

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

Where P_i is the frequency of the i^{th} allele at any particular locus. PIC takes into account both the number of alleles detected by the marker at a given locus and the respective frequency of each allele in the subset of germplasm tested (Panaud et al. 1996; Peakall and Smouse 2006). For the analysis of the molecular variance (AMOVA), equations described by Excoffier et al. (1992) were utilized using the Genetic Analysis in Excel (GenAlex v. 6.3) (Peakall and Smouse 2006).

The Numerical Taxonomy and Multivariate Analysis System (NTSYS) software package version 2.02f (Rolf 1998) was utilized to obtain the similarity index. Dice coefficient (D) is a statistic used to compare the number of bands shared between individuals, and thus, define the similarity between samples (Dice 1945). The equation is described as: D = 2a/2a + b + c, where a, b, and c are the number of bands shared between individuals. A dendrogram was then constructed based on the matrix of similarity coefficients using the Unweighted Pair Group Method with Arithmetic Average (UPGMA) to show the genetic relationships among abaca genotypes and closely related species.

RESULTS AND DISCUSSION

Transferability of Banana SSR Markers to Abaca

Of the 44 pairs of *Musa* SSR primers evaluated, seven generated scorable SSR bands. This represents 16% effectivity of banana SSR primers on abaca. The results reveal interspecies transferability of banana SSR primers to abaca. Cross-species transferability of SSR markers has been reported in different crops, including *Cenchrus*

Table 2. *Musa*-derived SSR markers successfully used in screening the abaca genotypes.

SSR Name	Upper Size	Motif	Forward	Reverse	TM (°C)
mMaCl R07	165	(GA)13	AACAACTAGGATGGT AATGTGTGGAA	GATCTGAGGATGGT TCTGTTGGAGTG	53
mMaCl R39	400	(CA)5GATA(GA)5	AACACCGTACAGGG AGTCAC	GATACATAAGGCA TCACATTG	52
mMaCl R40	540	(GA)13	GGCAGCAACAACAT ACTACGAC	CATCTTCACCCCCAT TCTTTTA	54
mMaCl R45	275	(TA)4CA(CTCGA)4	TGCTGCCTTCATCGCT ACTA	ACCGCACCTCCACC TCCTG	57
mMaCl R150	270	(CA)10	ATG CTG TCA TTG CCT TGT	GAA TGC TGA TAC CTC TTT GG	54
mMaCl R231	286	(TC)10	GCA AAT AGT CAA GGG AAT CA	ACC CAG GTC TAT CAG GTC A	55
mMaCl R264	274	(CT)17	AGG AGT GGG AGC CTA TTT	CTC CTC GGT CAG TCC TC	53

Primers sources: Global *Musa* Genomics Consortium and Crouch et al. (1999).

ciliaris (Abdi et al. 2019), *Fragaria*, and *Rubus* species (Lebedev et al. 2020). The effectivity of banana-derived SSR markers could reach up to 20%–33% when applied to banana (Creste et al. 2003, Oriero et al. 2006, Ning et al. 2007). The *Musa*-derived SSR primers were primarily designed to target the A and B genomes of banana. This indicates some degree of variation of the *M. textilis* (T) genome compared with the A and B genomes of banana.

SSRs derived from distant taxa [rice (*Oryza sativa* L.), papaya (*Carica papaya* L.), and horseradish tree (*Moringa oleifera* Lam.)] did not produce scorable bands. The absence of amplified abaca SSR product using rice and papaya SSR primers and gene-specific *Moringa* primers may indicate different SSR flanking regions present among these crops.

Six SSR primers (mMaCIR39, mMaCIR40, mMaCIR45, mMaCIR150, mMaCIR231, mMaCIR264) successfully amplified the desired microsatellites and were polymorphic across the screened genotypes, and one (mMaCIR7) produced a monomorphic profile. In most of the orphan crops and not fully elucidated taxa, only a few genomic markers are available (Armstead et al. 2009). In the case of Tunisian olives, only five SSR markers were utilized in fingerprinting its 34 varieties (Taamalli et al. 2008). For grape (*Vitis vinifera* L) cultivars, four to five moderately polymorphic loci were recommended (Bowers et al. 1996), while for hazelnut (*Corylus avellana* L.) five loci (Boccacci et al. 2006) had been proven to be effective in cultivar's certification.

Genetic Diversity

Allelic polymorphisms, availability of endemic genotypes, and genetic diversity are valuable attributes of a population to adapt to changing environments. A total of 28 alleles were recorded, with a mean value of 4.55 alleles per locus (Table 3). The recorded number of alleles per locus was relatively higher than those reported for abaca with a mean value per locus of 3.7 alleles (Boguero et al. 2016), 3.8 for 13 Philippine *M. balbisiana* cultivars (Doloiras Laraño et al. 2018), for plantain, 2.4 (Noyer et al. 2005), for B-genome *Musa*, 2.1 (Oriero et al. 2006), and wild bananas (*M. balbisiana*), 2.3 (Ge et al. 2005) but lower than the reported 10–15 for Brazilian banana genotypes (Creste et al. 2003).

The mMaCIR150 locus generated the highest number of alleles (10) followed by mMaCIR264 with seven alleles. The rest of the polymorphic loci — mMaCIR39, mMaCIR231, mMaCIR40, and mMaCIR45, had alleles ranging from two to three per locus. The presence of polymorphic alleles may indicate allelic diversity in the abaca genome. Polymorphic microsatellites are essential for mapping studies since they are useful even in narrow intra-gene pool crosses and in analyzing recent changes in population structure and in determining the history of selection among closely related germplasm (Blair et al. 2006).

The polymorphism information content (PIC) of SSR markers for abaca ranged from 0.48 to 0.65, with an average of 0.56 (Table 3). Around eighty-six percent (6/7) of these markers were able to amplify their corresponding loci, and most of them were considered as informative (PIC>0.5) (Botstein et al. 1980). mMaCIR39 and mMaCIR231 exhibited moderately informative PIC value

Table 3. List of working SSR primers for abaca, repeat motif, allele size range and number of alleles (Na), and polymorphism information content (PIC).

SSR Code	Motif	Allele Size Range (bp)	Na	PIC
mMaCIR39	(CA)5GATA(GA)5	300-400	2	0.48
mMaCIR40	(GA)13	250-550	3	0.63
mMaCIR45	(TA)4CA(CTCGA)4	275-600	3	0.50
mMaCIR150	(CA)10	150-1650	10	0.65
mMaCIR231	(TC)10	200-250	2	0.48
mMaCIR264	(CT)17	250-400	7	0.63
mMaCIR7	(GA)13	170	1	NA
Mean			4.55	0.56

Na, Number of alleles
PIC, Polymorphism Information Content
NA, Not Applicable

(0.48). In the diversity study of Philippine bananas, the PIC ranged from 0.66 to 0.94 for 10 SSR primers (Dacumos et al. 2011), 0.23 to 0.80 with a mean of 0.55 for 14 SSR markers (Doloiras-Laraño et al. 2018), and 0.09 to 0.48 with a mean of 0.38 for 11 SSR markers (dela Cruz et al. 2020). PIC analysis is utilized to determine the informativeness of the markers to differentiate various genotypes. The high level of informativeness of most of these SSR markers indicates their capability to quantify genetic diversity and differentiate different abaca genotypes.

The abaca populations in Luzon, Visayas, and Mindanao exhibited high genetic diversity (Table 4). Among the three, Luzon had the highest genetic diversity (I) of 0.74. According to Valmayor et al. (2002), the Bicol region in Luzon is the recognized center of diversity for abaca. The abaca populations from Luzon (mostly coming from the Bicol region) exhibited the highest genetic diversity (0.74) although Visayas and Mindanao also showed relatively high genetic diversity of 0.67 and 0.63, respectively. A greater number of abaca cultivars and accessions were present in Luzon compared with Visayas and Mindanao. Likewise, the presence of wild genotypes in the area enhances heterozygosity and may contribute to more considerable allele variability and genetic diversity of the population. The observed values for heterozygosity (H_o) of alleles in Luzon, Visayas, and Mindanao of 0.51, 0.57, and 0.49, respectively, are higher than the expected heterozygosity (H_e) of 0.40 for both Luzon and Visayas and 0.38 for Mindanao (Table 3), indicating an excess of heterozygotes and generally low inbreeding.

Genetic diversity (I) estimates per locus ranged from 0.11 to 1.32. The mMaCIR264 and mMaCIR40 loci exhibited higher average variability (1.32 and 0.97, respectively). These values were higher as compared to that of cauliflower (0.51) (Zhu et al. 2018) and relatively lower than the SSR olive marker DCA15 (I = 1.51) (di Rienzo et al. 2018). Likewise, the average diversity index (I) of mMaCIR264 (0.98), mMaCIR40 (0.94), mMaCIR45 (0.72), mMaCIR150 (0.72) and mMaCIR39 (0.56) were also shown to be diverse, while mMaCIR231 had the lowest genetic diversity value of 0.17 (Table 4).

The mean observed heterozygosity (H_o) of alleles in Luzon, Visayas, and Mindanao were 0.51, 0.57 and 0.49, respectively, while the mean expected heterozygosity (H_e) ranged from 0.38 in Mindanao to 0.40 in Luzon and Visayas (Table 4). The high level of overall observed heterozygosity in abaca populations (mean H_o = 0.52> mean H_e 0.39) may indicate greater allelic polymorphism present in these populations and the possible mixing of

Table 4. Diversity index (I), observed heterozygosity (H_o) and expected heterozygosity (H_e) of Luzon, Visayas, and Mindanao abaca genotypes.

Population	Locus	I[a]	H_o[b]	H_e[c]
Luzon	mMaCIR39	0.50	0.40	0.32
	mMaCIR40	0.88	0.77	0.53
	mMaCIR45	0.86	0.74	0.52
	mMaCIR150	0.77	0.26	0.39
	mMaCIR231	0.11	0.05	0.05
	mMaCIR264	1.32	0.84	0.62
Mean		0.74	0.51	0.40
Visayas	mMaCIR39	0.63	0.64	0.44
	mMaCIR40	0.95	0.95	0.58
	mMaCIR45	0.73	0.80	0.49
	mMaCIR150	0.66	0.43	0.37
	mMaCIR231	0.20	0.02	0.10
	mMaCIR264	0.87	0.56	0.45
Mean		0.67	0.57	0.40
Mindanao	mMaCIR39	0.57	0.51	0.38
	mMaCIR40	0.97	0.79	0.55
	mMaCIR45	0.57	0.51	0.38
	mMaCIR150	0.73	0.68	0.47
	mMaCIR231	0.21	0.06	0.10
	mMaCIR264	0.74	0.40	0.36
Mean		0.63	0.49	0.38
Total Mean		0.68	0.52	0.39

[a]Shannon's Diversity Index
[b]Observed Heterozygosity
[c]Expected Heterozygosity

previously isolated populations as reflected in the breeding management of abaca in various national breeding centers in the country. Moreover, inbreeding, which would reduce the proportion of heterozygotes and reproductive fitness of species is low in the three abaca populations. This may be due to its cross-pollinated nature and self-incompatibility. This result was supported by the study of Oriero et al. (2006) on the low inbreeding coefficient (-0.28) of *Musa* compared to the other crops (0.34).

In the three abaca populations, higher mean observed heterozygosity versus the expected heterozygosity was noted (0.51>0.40; 0.57>0.40; 0.49>0.38). This may be due to the presence of excess heterozygotes in the population and confirms *Musa*'s (e.g., *M. textilis*) highly heterozygous nature (Oriero et al. 2006). In contrast, mMaCIR150 in Luzon and mMaCIR231 in Visayas and Mindanao had higher expected heterozygosity than the observed heterozygosity. Higher He values suggest the multiallelic property of the particular SSR locus under the Hardy-Weinberg equilibrium (Weising et al. 2005). Higher heterozygosity in these loci was supported by the high genetic diversity values.

The estimated molecular variance among the abaca population was 0.16 and 3.03 for the variance within populations with a total of 3.19 (Table 5). A large

Table 5. Analysis of molecular variance among and within abaca populations.

Source	df	SS	MS	Est. Var.	%
Among populations	2	20.79	10.39	0.16	5%
Within populations	147	445.61	3.03	3.03	95%
Total	149	466.40		3.19	100%

percentage (95%) of the molecular variance was due to the variation within populations, and a small fraction (5%) was attributed to variation among the abaca populations in Luzon, Visayas, and Mindanao. The greater molecular variance reflects the high allelic variability of abaca accessions within Luzon, Visayas, and Mindanao populations.

Abaca parentals flower and fruit regularly and are considered cross-pollinated in nature due to self-incompatibility. This mode of fertilization exemplified by abaca may explain the high observed heterozygosity values. The high level of genetic diversity among the three islands and high genetic polymorphism within populations are of particular importance in maintaining the genetic variability of abaca germplasm in various *ex situ* genebanks in the country. Brewbaker and Gorrez (1956) established through detailed morphological studies that Canton and Minay are natural hybrids of *M. balbisiana* x *M. textilis* and Alinsanay is a hybrid of *M. textilis* x *M. acuminata*. Pacol (*M. balbisiana*) has been used in the breeding for bunchy top virus-resistant abaca, resulting in hybrids similar to the natural hybrids (Bernardo and Umali 1956; Bernardo et al. 1965).

The genetic variation present in *ex situ* and *in situ* abaca germplasm is an excellent resource to enrich further the genetic pool of commercial abaca cultivars that are generally produced by backcrossing. This rich genetic resource will also sustain the long-term yield performance of the cultivars and at the same time, mitigate the risk of anthropogenically-derived bottleneck brought by backcrossing. A typical case is the backcrossing of Pacol x abaca hybrid to abaca that has generated BC2 plants with strong resistance against bunchy-top virus and good fiber quality (Lalusin 2010). Likewise, genetic diversity of a species represents the essential raw material for species to adapt to changing environments (Frankham et al. 2010) and the foundation in the development of new lines and varieties towards sustainable crop production.

UPGMA Cluster Analysis

Using Unweighted Pair Group Method with Arithmetic Mean (UPGMA), the 150 abaca and two banana genotypes from Luzon, Visayas, and Mindanao were clustered into nine groups at 0.63 similarity coefficient (Figure 2). Group I included 98 abaca accessions from Luzon, Visayas, and Mindanao and could be further separated into two subgroups (SG1A and SG1B). Group II comprised of 28 accessions with two subgroups (SC2A and SC2B). Most of the accessions in Group II were from Luzon and Visayas with a few accessions originating from Mindanao. Group III had five accessions from the three islands while Group IV comprised of 11 abaca accessions. Groups V, VI, VII, and VIII formed the small clusters with three, two, one, and two accessions, respectively. Groups IV and VII were composed of accessions emanating from Luzon and Visayas while groups V and VI had genotypes from Visayas and Mindanao. Kaunayan from Mindanao represented group VII. Group VIII had two accessions, one each from Luzon and Visayas. As expected, the two banana genotypes, *M. acuminata* Colla and *M. balbisiana* Colla represented by cultivars Señorita and Pacol, respectively, formed group IX separate from all abaca genotypes. The similarity index coefficients of abaca genotypes from the three islands ranged from 0.43 to 1.

The clustering of abaca genotypes may indicate the sharing of common alleles or genomic regions present across the genotypes. The results support the observation that in the domestication and hybridization of abaca genotypes in the Philippines, some of the alleles were transferred from wild genotypes to several abaca landraces and vice versa. Likewise, the exchange of genetic materials occurred among the three populations. This is possibly due to the breeding strategy being employed by various research agencies in the country, where superior genotypes are being shared and introduced among the stakeholders. Extensive trading of abaca cultivars for several decades may also explain the mixed clustering between and among the three populations. Likewise, the suitable tropical condition of abaca-planting areas in the country makes it conducive for the introduced genotypes to easily adapt and promote gene flow. In general, there is no distinct clustering of abaca genotypes based on geographical location. Similar results were obtained in *Vicia fava* and *Pisum sativum* (Martos-Fuentes et al. 2019), however, geographical grouping pattern was observed in birch (Hao et al. 2015) and olives (di Rienzo et al. 2018).

The dendrogram (Fig. 2) also reveals several putatively identical abaca accessions. In Cluster 1, Subcluster 1A, accessions L51 and V39; L23, L35, L80, V35, M7, and M12; L36 and V37; M4, M8, M9, and M13; L57, L70, and *VW6; V8 and *VW5; L82 and *VW2; L60 and L66 had genetic similarity coefficient of 1.0. This similarity was also noted in genotypes L50 and L52; V17 and V40; L15 and V21; L62, V14, and V31; L63 and V30

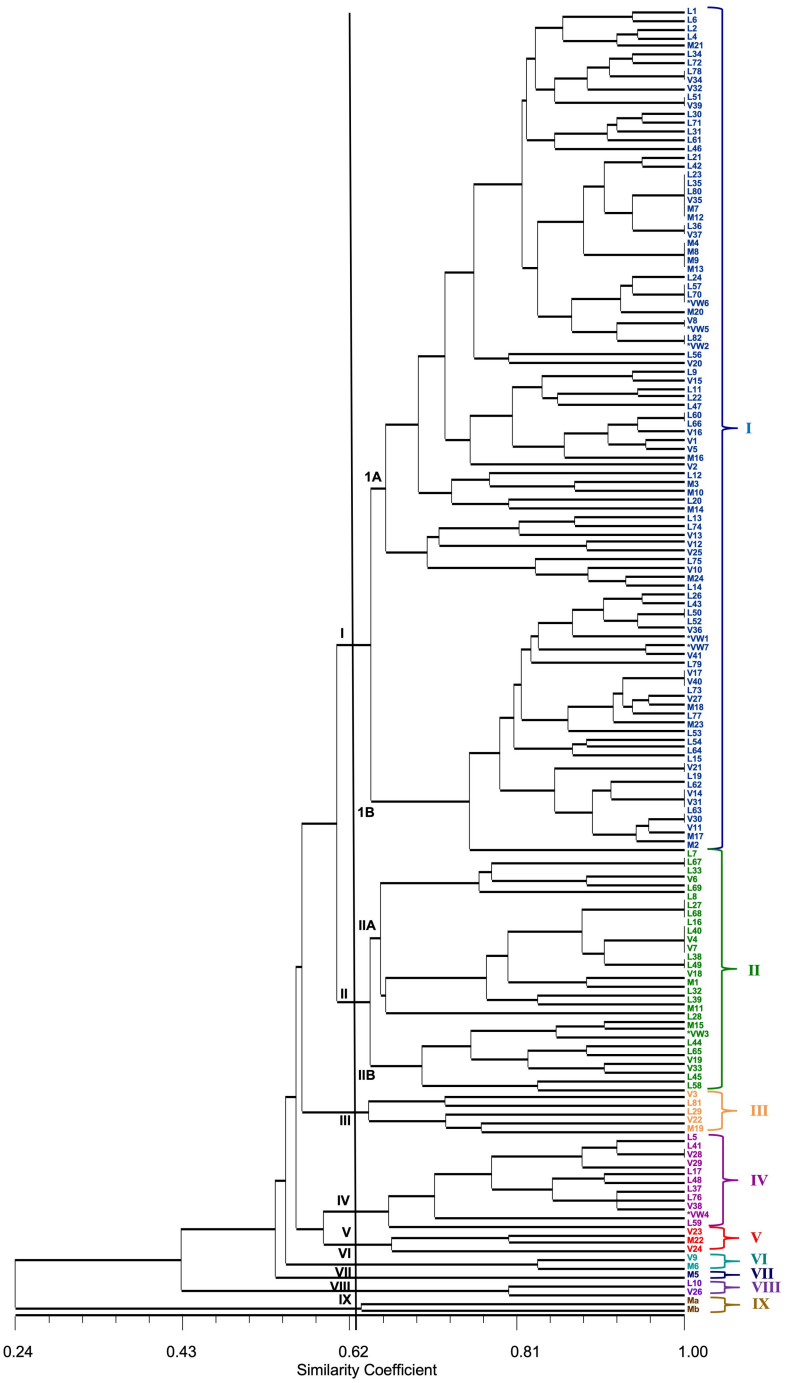

Fig. 2. The Unweighted Pair Group Method with Arithmetic Average (UPGMA) based dendrogram of abaca genotypes and two closely related species [*Musa acuminata* (Ma); *Musa balbisiana* (Mb)] in the Philippines.

belonging to Subcluster 1B. In Cluster 2, putatively identical genotypes were L7 and L67; L8, L27, and L68; L40, V4 and V7; and L38 and L49. The same is true with L41 and V28 in Cluster 4.

Presence of duplicate accessions could happen during movement and sharing of germplasm among stakeholders and across genebanks. During the sharing of acquisitions, these newly acquired genetic resources are often re-marked and annotated with local identifiers. With time, the associated original records and identifiers are lost, resulting in inaccurate classification and duplications across collections (Singh et al. 2019). Likewise, the presence of morphological homogeneity (Lasalita-Zapico et al. 2010) in the abaca population may also result in misidentification of abaca genotypes. The presence of duplicates in the genebank would entail high costs in storing and maintaining those accessions. In the long run, proper identification and maintaining of unique abaca genotypes would lead to the efficient management of *in situ* and *ex situ* genebanks, cost reduction in storing and maintaining of germplasm, and optimizing the conservation and utilization of genetic resources for breeding of superior abaca cultivars.

CONCLUSION

The present study identified and utilized SSR primers for abaca derived from its related taxa, banana, which were highly polymorphic and had a high number of alleles per locus, which confirm their suitability in genetic analysis of abaca germplasm. The abaca genotypes were found to have high diversity and heterozygosity. Alleles among abaca genotypes were observed to be shared with those from the three islands. Genetic analysis confirmed the high variability of the abaca genotypes clustering into two large groups and seven small groups. Using a combination of SSR markers, the two banana species — *M. balbisiana* and *M. acuminata* were differentiated from the rest of the abaca genotypes. These SSR markers also

identified putative abaca duplicates in several clusters. Proper identification of duplicates is a prerequisite in optimizing the abaca genetic resources in genebanks. The high genetic variability of abaca populations in the Philippines could be considered as a valuable genetic resource for selection of superior genotypes, for development of hybrids with a high level of heterosis and outstanding traits, *in situ* and *ex situ* germplasm conservation, and improvement of abaca breeding programs toward enhanced competitiveness of abaca sector in the global market.

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