SciMedCentral

International Journal of Plant Biology & Research

Research Article

Accurate Identification of Abaca (Musa textilis Née) Cultivars Using Single Nucleotide Polymorphisms (SNP) Markers Developed for Banana (Musa acuminata Colla)

Leny C Galvez[1], Lyndel W Meinhardt[2], Ricardo Goenaga[3] and Dapeng Zhang[2]*

[1]*Philippine Fiber Industry Development Authority, Diliman, Philippines*
[2]*USDA-ARS, Sustainable Perennial Crops Laboratory, USA*
[3]*USDA-ARS, Tropical Agriculture Research Station, Mayaguez*

***Corresponding author**
Dapeng Zhang, USDA-ARS, Sustainable Perennial Crops Laboratory, Beltsville Agricultural Research Center, Beltsville, MD 20705, USA, Tel: 301-504-7477; Fax: 301-504-1998; Email: Dapeng.Zhang@ars.usda.gov

Submitted: 14 February 2021

Accepted: 10 April 2021

Published: 12 April 2021

ISSN: 2333-6668

Keywords
- Manila hemp
- Molecular markers
- Natural fiber
- Industrial crop
- The Philippines

Abstract

Abaca (*Musa textilis* Née) is a diploid *Musa* species native to the Philippines that is used to produce abaca fibers. The Philippines supplies 85 percent of the world supply for raw fiber, fiber craft, cordage and pulp, which provide livelihood opportunities for 1.5 million Filipinos. Cutting edge molecular markers is needed to support germplasm management and crop improvement of this understudied crop. The objective of the study is to adapt a set of SNP markers from banana and validate the efficacy of the SNP markers for abaca genotype identification. Using a nano-fluidic genotyping platform, we evaluated 384 putative Single Nucleotide Polymorphism (SNP) markers developed for diploid banana (*Musa acuminata* Colla), based on 62 abaca germplasm accessions. The cross-species transfer of nuclear SNP markers showed a 15.6% success rate, resulting in the selection of 60 polymorphic SNPs. The generated SNP profiles enabled accurate identification of all tested abaca cultivars and detection of homonymous naming mistakes. Cultivars with a background of inter-specific hybrid (e.g. *M. textilis* x *M. balbisiana*) were differentiated using multi-variant analysis and Bayesian stratification. These selected SNP markers will be highly useful for downstream applications in abaca industry, including cultivar identification, nursery accreditation, and authentication of abaca product and protection of intellectual property rights.

ABBREVIATIONS

SNP: Single Nucleotide Polymorphism; DNA: Deoxyribonucleic Acid; RNase: A Ribonuclease I or Ribonucleate 3′- pyrimidinooligonucleotidohydrolase; PCR: Polymerase Chain Reaction; IFC: Integrated Fluidic Circuit; PCoA: Principle Coordinates Analysis; PIC: Polymorphism Information Content; PCO: Principle Coordinates; STA: Specific Target Amplification

INTRODUCTION

Abaca (*Musa textilis* Née) is a monocotyledonous plant that is closely related to banana [1,2]. It is a member of the Order *Zingiberales*, Family *Musaceae,* under the section *Callimusa/ Australimusa* and has a diploid chromosome number of 20 (2n=2x=20) [3-6]. Abaca plant is the source of the world's strongest natural fiber, internationally known as Manila hemp [1,7].

The abaca fiber market is expected to grow considerably due to an increasing global demand from pulp manufacturing and from abaca fiber industries [7,8]. The abaca is indigenous to the archipelagos of the Philippines and was grown in the country even before Spanish colonization. The cultivation, fiber extraction and weaving into cloth was widespread in the islands and abaca has been introduced into Sumatra, British Borneo, Malaya [9], Central America [10] and New Caledonia and Queensland [9]. After World War II, abaca was introduced into and cultivated in Ecuador and other tropical American countries [11].

Since the onset of the 20th century, abaca fiber has become the premier export commodity of the Philippines with the abaca industry makes up a substantial part of the national GDP (US$131 M in export earnings in 2016) [12]. Abaca production in the Philippines accounts 87% of the world supply for raw fiber, fiber craft, cordage and pulp [12]. Moreover, the

abaca industry is a major source of livelihood for nearly 1.5M Filipinos, which consist of 124,063 abaca farmers cultivating a total area of 141,614 hectares [12].

Due to current environmental concerns that focus on biodegradable products and forest conservation, abaca is a superior natural material that has an expanding industrial potential [12]. Abaca is now a preferred material in the production of pulp for specialty papers like tea bags, meat/sausage casings, cigarette paper, filter papers, currency notes, stencil paper and non-woven product applications and local and international companies are continuously developing new specialized products [7,8]. To cope with the growing demands for high quality fiber in the international market, abaca cultivars with favorable agronomic traits and quality attributes are needed. Systematic characterization of abaca genetic resources is a pre-requirement for effective selection and use of abaca germplasm [8].

Abaca is taxonomic complex because of the hybridization and polyploidization that had occurred naturally among abaca (i.e. cv Lausmag) and *Musa* species [13] including *Musa balbisiana* and *Musa acuminata* [1,2]. There are as many as 200 cultivars of abaca in the Philippines, mostly landraces, which are attributed to the planting of seeds in the early days of its domestication [9]. Duplications are possible because the same cultivar may be given a different name in different regions. There are more than 700 accessions of abaca maintained in field gene banks in the country [14], and there are still abaca plants in the wild [15]. To take fully advantage of the rich genetic diversity in the abaca germplasm collections, high standard of accuracy is essential for gene bank management. Each accession must be a true-to-type genetic identity, be accurately labeled and have intact database records.

As part of the PhilFIDA's project, abaca germplasm is being characterized phenotypically. However, the phenotypic characteristics appear to be influenced by environmental conditions and geographic location where they are grown. Evolutionary processes driven by environment factors that are influenced by geographical and physical differences have caused changes in some morphological characteristics but the genotype remains unchanged [16]. Moreover, the environmental effects on phenotypic traits can be confused by somaclonal mutations, which have been commonly reported in vegetative propagated crops, including other *Musa* species. Subsequently, both molecular and phenotypic characterizations are needed to accurately identify abaca genetic resources in both genebanks and farmer's fields [8].

Molecular markers have been proposed for the identification of mislabeling, parentage and sibship analysis for quality control in breeding and seeds programs, and characterization of farmer selections of new varieties for abaca production [8]. However, published research on molecular characterization of abaca germplasm is limited. Boguero et al. [17], used six SSR markers to genotype 57 abaca accessions and could identify resistant and susceptible accessions for bunchy top virus, thus established a genetic pool of germplasm for breeding resistance to bunchy top virus.

Single nucleotide polymorphisms (SNPs) are a highly abundant class of DNA sequence polymorphisms found in plant genome [18]. SNP markers have become the preferred method for accurate genotype identification in tropical crops, as recently demonstrated in tea [19], Pumelo [20], longan [21], pineapple [22] and coffee [23]. The development of the draft genomes of several Musa species, including the diploid banana [24], paved the way to the development of putative SNP markers using next generation sequencing [26,27]. The online database, Banana Genome Hub http://banana-genome-hub.southgreen.fr/, provides possible tools for other related *Musa* species, including abaca.

The objective of the study is to adapt a set of SNP markers from banana and validate the efficiency of the SNP markers for abaca genotype identification. We conducted a pilot study to evaluate a set of banana SNP markers for abaca genotyping using a nanofluidic array. The cross-species transfer of nuclear SNP markers, as well as the genotyping method, will be useful for intellectual property rights in cultivar protection, germplasm management, and genetic improvement of abaca.

MATERIALS AND METHODS

Abaca leaf samples collection and DNA extraction

A total of 62 abaca accessions, most of which were landraces and farmer selections, were used in this pilot study (Table 1). These abaca accessions were collected from the abaca germplasm repository maintained by the Philippines Fiber Industry Development Authority at Diliman, Quezon City. Young and healthy leaf samples were harvested and dried in silica gel and DNA was extracted from dried abaca leaves with the DNeasy Plant Mini kit (Qiagen Inc., Valencia, CA, USA). The dry leaf tissue was placed in a 2-mL microcentrifuge tube with oneJ-inch ceramic sphere and 0.15 g garnet matrix (Lysing Matrix A; MP Biomedicals. Solon, OH, USA). The leaf samples were disrupted by high-speed shaking in a TissueLyser II (Qiagen Inc.) at 30 Hz for 1 min. Lysis solution, along with RNase A, was added to the powdered leaf samples and the mixture was incubated at 65 C, as specified in the kit instructions. The remainder of the extraction method followed manufacturer's suggestions. DNA was eluted from the silica column with two washes of 50 mL Buffer AE, which were pooled, resulting in 100 mL DNA solution.

DNA concentrations were determined by measuring absorbance at 260 nm, using a NanoDrop spectrophotometer (Thermo Scientific™, Wilmington, DE, USA). DNA purity was estimated by the 260:280 ratio and the 260:230 ratio of absorbance maximums.

SNP markers and genotyping

All putative SNP markers were downloaded from Banana Genome Hub http://banana-genome-hub.southgreen.fr/. A total of 384 putative SNPs were selected based on genome distribution, with the number of SNPs ranged from 33 to 36 in each of the 11 chromosomes. The 384 SNP sequences were submitted to the Assay Design Group at Fluidigm Corp. (South San Francisco, CA, USA) for final design and synthesis for the development of the SNP type genotyping panel.

The validation assays were based on competitive allele-specific PCR, and they enable bi-allelic scoring of SNPs at specific loci (KBioscience Ltd, Hoddesdon, UK). Specific Target

Table 1: List of 62 accessions of Musa textilis, Musa balbisiana and hybrid (Musa textilis x Musa balbisiana) used in the present experiment

Accessions	Recorded type	Recorded identity/ pedigree
Abuab Labo	farmer's cultivar	Abaca
Abuab Rapu-rapu	farmer's cultivar	Abaca
Abuab SanMiguel	farmer's cultivar	Abaca
Abuab TC	farmer's cultivar	Abaca
Agpas	hybrid	abaca x banana
Batayan	farmer's cultivar	Abaca
Binagakay	farmer's cultivar	Abaca
Bongolanon	farmer's cultivar	Abaca
Bonliwon	farmer's cultivar	Abaca
Buributikon	farmer's cultivar	Abaca
Canarahon	farmer's cultivar	Abaca
Canton	hybrid	abaca x banana
Casilihon	farmer's cultivar	Abaca
Daratex	hybrid	abaca x banana
Gais # 2	hybrid	abaca x abaca
Hybrid 2	hybrid	abaca x banana
Hybrid I	hybrid	abaca x banana
Igit 1	farmer's cultivar	Abaca
Igit 2	farmer's cultivar	Abaca
Inlabo	farmer's cultivar	abaca
Kaunayan	farmer's cultivar	abaca
Kur x Kan	hybrid	abaca x abaca
Lacatan	edible banana	banana (*Musa acuminata*)
Lagonoyon Puti	farmer's cultivar	abaca
Lausigon	farmer's cultivar	abaca
Lausigon Red	farmer's cultivar	abaca
Layas	farmer's cultivar	abaca
Linawaan	farmer's cultivar	abaca
Linino	hybrid	abaca x abaca
Linobloban San Andres	farmer's cultivar	abaca
Litom	hybrid	abaca x abaca
Maguindanao Bicol	farmer's cultivar	abaca
Maguino	hybrid	abaca x abaca
Mamakaw	hybrid	abaca x banana
Mi-NC	hybrid	abaca x banana
MTP	hybrid	abaca x banana
Musa tex 51	hybrid	abaca x abaca
Musa Tex 82	hybrid	abaca x banana
Musa text 50	hybrid	abaca x abaca
NAM ID	hybrid	abaca x abaca
Natural Tobacco	farmer's cultivar	abaca
Negro	farmer's cultivar	abaca
Parang	farmer's cultivar	abaca
Pisgan	hybrid	abaca x abaca
Puti 1	farmer's cultivar	abaca
Puti 2	farmer's cultivar	abaca
Putian sfsb	farmer's cultivar	abaca
Putie	farmer's cultivar	abaca
Samtang	farmer's cultivar	abaca
Samtong	farmer's cultivar	abaca
Samuro	farmer's cultivar	abaca
Silibaonan	farmer's cultivar	abaca
Sogmad	farmer's cultivar	abaca
St. Vincent	farmer's cultivar	abaca
T.Pula	farmer's cultivar	abaca
Tan Mag	hybrid	abaca x abaca
Tangongon	farmer's cultivar	abaca
Tuod	farmer's cultivar	abaca
Tu-soy	farmer's cultivar	abaca
Unidentified Bacon	farmer's cultivar	abaca
Yoga	farmer's cultivar	abaca
Zamboanga Pula	farmer's cultivar	abaca

Amplification [28] was performed to enrich SNP sequences in the sample DNAs. Amplified samples were then genotyped using the nanofluidic 96.96 Dynamic Array™ IFC (Integrated Fluidic Circuit; Fluidigm Corp., South San Francisco, CA). The architecture, mechanics and analysis of the system using Fluidigm IFCs for SNP genotyping was described by Wang et al. [28]. End-point fluorescent images of the 96.96 array were acquired on a Fluidigm EP1™ imager, and the data was recorded and analysed with Fluidigm Genotyping Analysis Software [29]. The data were then exported in Excel format.

Data analysis

Raw data were first analyzed for call rate. Markers with call rate < 90% were removed. Duplicate accessions were identified using pairwise multilocus matching among all individual samples. The program GenAlEx 6.5 [30, 31] was used for computation and samples that fully matched at the tested SNP loci were designated as identical cultivars or clones. After duplicate identification, the redundant samples were removed and descriptive statistics for measuring the informativeness of the SNP markers were calculated based on the remaining distinctive cultivars. Using the same program key descriptive statistics were measured such as, minor allele frequency, observed heterozygosity, expected heterozygosity and Shannon's information index.

Distance-based multivariate analysis was used to assess the relationship among the individual abaca samples, as well as their relationships with reference samples from the USDA *Musa* germplasm collection. Pairwise genetic distances were computed using the Distance option and Principal Coordinates Analysis (PCoA), based on the pairwise distance matrix were measured using the GenAlEx 6.5 program [30,31]. Both distance and covariance were standardized. In addition, a cluster analysis using the UPGMA (unweight pair group method with arithmetic mean) method was performed to further examine the genetic relationship among the 62 abaca accessions. First, the distance between individuals was calculated with 100 bootstrapping

using the shared proportion of alleles distance measurement in the program Microsatellite Analyser [32]. The resulting distance matrix was used to generate a consensus dendrogram using the program PHYLIP [33]. Thereafter, the dendrogram was visualized using the FigTree program version 1.3.1 [34].

The population structure of the abaca samples was analysed using model-based Bayesian cluster analysis software STRUCTURE v2.3.4 [35]. The admixture model was applied and the number of clusters (K-value), indicating the number of subpopulations set from 1 to 10. The analyses were carried out without assuming any prior information about the genetic group or geographic origin of the samples. Ten independent runs were assessed for each fixed number of clusters (K value), each consisting of 100,000 iterations after a burn-in of 200,000 iterations. The Delta K value [36] was used to detect the most probable number of clusters using the online program STRUCTURE HARVESTER [37]. Permutation was performed using the computer program Clumpp v1.1.1 [38] and the resultant outputs were then visualized using computer program Distruct v1.1 [39].

RESULTS AND DISCUSSION

SNP validation, cultivar identification and descriptive statistics

Out of the 384 SNP markers selected from the Banana genome hub, 258 markers were found monomorphic across the 62 abaca accessions and 32 markers had no amplified products. These markers, together with those had low call rate (<90%), were excluded from data analysis. The final 60 polymorphic SNPs were reliably scored across the validation panel and were used in data analysis. The 60 SNPs and their flanking sequences are listed in Table 2.

An example of SNP profiles for abaca cultivars was presented in Table 3. No duplicates were identified among the 62 abaca accessions by multi-locus matching and all cultivars could be differentiated by the 60 SNP markers. The two homonymous pairs ('Igit' and 'Puti'; Figure 1) were found having distinguished SNP profiles (Table 3).

Descriptive statistics were then computed for the 60 polymorphic SNPs across the 62 abaca accessions and the result

Table 2: Flanking sequences and SNPs of the 60 banana SNP markers that are suitable for abaca genotype identification.

SNP ID	Flanking sequences and SNPs
Mt006	A G T A G C A T C A C G C A A T A A A A A C T G T C C G T C T G C A G G G M C G T C G A A T G C C C T A G T T G T C G T [A / C] GGTACTTTCAATTGGCATAATGACACCATATTCTTGGGTGATGTTAACTGCCATCCAATG
Mt010	G G T C A T G A A T C C A A M C G A G A G G A A G A C A A G A G C T G G T C G A G G G T C A G C A A C C G A T C C A C A [A / C] AGCCTCTATGCGAGGGTAGAAACCTGATTCAGAGTAACCATTGTTTTCGATATGTTGCCT
Mt012	G A C T G C A G G G M G A T C A A T T A C A G G T A C A A A C C A A A T C C A G G A C A T C A A G C T C G T C A G C T T [A / C] TGTGAAAGCCCAAGTCTGTATCAGTGAATCCTCCAAAACGAAACATTAAATATGTGTCTG
Mt020	G C C T G C A G A A T A C C A C C G T C A C T C G A G T A T C G A A C G G A A G C A A A A C T C A T T T C T A G C A T G [A / C] CTGCCCCAACCATGTTATCTAGCATGACCGTCCGACCCTTTGGTATAGTAACTTTTTTAG
Mt036	G A A T C C A G C C G A R G G A A T G T T A G C T G C A G C T G C A A A T G T C A G A G A G A A G G A A A T G A A C T C [A / G] CACACACCTCTTTTATGTTCTTCAGCTGTGTGTCTTAAGAACATTGAAGCTATCACAACT
Mt038	A T T G A G C T A C T G T T C A C C A T T G C T G T G T R G T T G C A G G T A G T G A C A C T G C A G G A G C T G T T C [A / G] TGGTCGACAGGTGAGTTGTAATAGGAATGAGTGAGCTGCGGGAGTAAGTGAGACGATGGA
Mt049	G C A G T C A T C A T C C A T T C T G C G G C T A C C T T C T G C A G G A A T A T G T G C C T T C T T A T A C W A G T T [A / T] TATTTTCCTTGCGCAGGAGATAATGGACTATTGAACCAAGAGGTTCGATCTGCTGCAGTA
Mt064	A C A T G A T S C A G G G C C A G A T T C A A G A T G A T A A A T C A C A T C T A A G A A T A G A T T A A T G C A T C A [C / G] TTGTTATTCATTTTATTTGCCTCTCTTTTGACATATTGCTCAAGGAATCTTCTTTAGGTT
Mt070	A T A C G T T C T G A A T C G T A G A C S C T C C G A C C G A G A T T A A A A G G A A A A C A A T G C T G C A G G A T A [C / G] ATGATTGAGAAACTGATAGGGGAGGAGAAGGAACTCAGAGGACCAGCTTCAACGAATCGA
Mt071	G C T G C A G C A T C A A T G C S T T T T T G A C T G C A G A A G C A C T T C T G G T C C T C A T G T C T T C C T G C A [C / G] CTCATCTCCTTCTCCTATGTTTCTGCCGAGGTCCAACAAAAAGATGGAGTGCCCCTCAAG
Mt075	G G T C A T T C G T A T T T G A G A T G C A T T T C T C G G G C S A C C A T G T C A T T G T T C T T G G C T G A C T T G [C / G] AGGAAGTTGGTTGACTCTAAATGACACTGTCCTCGACTGCAGGTCCAAGTTTTGAACTCT
Mt078	A G A T C C A T C C T A C T G G A C G T G C A G A A G A A A A A T A C T A G C T G C T T G T G G T C A A C C A A A A A T [C / G] TCTTGTATAACTGTTGGCAAAGACAATCAAAAGAAGATCTGTCGACTTTGTATAGTAATG
Mt080	T G A C C G T T T G C C C T T C T G T C A C A G A G A T G T C T A C C T T T G G T G A G T C T G C A G G A S C A T T A G [C / G] TTTGGCATCCTTGGTAGGTGACGATGGCTTTTTTCCTGCAGCTGCAGCGGCAGCACCATC
Mt087	T C G A A G A A A C A T T A A T A T C A T C C T A T T T T T T G T T T C A T T A T A T T C T G C A G A T A Y T T A A T T [C / T] CATTAATGCTTTTGTGGAAATTACTGAGACAAGTCGATGTCTTGCCTCCCAGTATCAAAA
Mt089	T G T G A C A T T C T T G T A A G T C C A T A T A T A T T G C A G C A A G A C T G C A G G C A G C A A G C C A T C A A A [C / T] ATATGAAATCCATGTATTTCAAAAAATTGTAACCGTACTATTATCTGTTCTTAATGGAAA
Mt092	T G T T T C T A C T G C A G G T T A A T G C T C T C T A T A G T A G A T A T G A T A A T T A T A T Y A A T T T T T G T T [C / T] ATAACCAATTTTTGATCCAAAAGAATTTTGAACTGATGCTTTCTGAGGTGGATTATCTTG
Mt097	T C T C T G T C A G C T C T A A G A G C A G A A A T T T C T G C A G C A A G G G A G T T A A T G A A T T T T G A C T C Y [C / T] CTCCTTTTACCCCAGCTCTGGCAGCTGCTTTCTTCACATCCTCGATGCCATCCATAATTT

Mt099	G G A G A C T G T G T G G C C T T T G C T G C A G A A C A T T T G G C T T Y G C C T G G G G C C T A A A T G A T G A T G [C / T] AGATGCAGGCATTTGCTTTCCTTGCGATGATACTAACGACTGCTGTGGCACAGACGATAT
Mt101	G C T T G G C T G A Y G A A C T T A A A G A G C C A C C G T T T T C T G A C C C A C T T C C A G G T C G G C A T C G G A [C / T] ATCTATATGTTTGGCTAATGGCTTTCCACTGGGAAATGGCGAGATCACAAAGGGAAAAAA
Mt102	A A A A G T A A T A G C A G A T G C A C A A C G A T G A C T T G T C C A G C T C A T G T T T C T C G G C T G C A G C G A [C / T] GCATATGCAAATTGTATGAATCGTTCGCTCATTTGACTTCCAAGATTACTTGCTGGTAGG
Mt105	T C A A G A A G A C A A A G C A T G T C A G C C A A G T K A A A G G A G A A G G A G A T G A G A T C T A A C A G G A G C [G / T] GATCAGATGCTTACTCCCAGGGAACATCACCGACCAACATCAAGTCTCCATCTTTGTCCT
Mt106	G C A G A T G A A T T G C T G C T A C A K A A A A C T G A A C A A A T G G A C T C G T T T G G A A T A T A A A A A A A G [G / T] ATGAACAAATATGACTACTGCATCTGAAAGTTGTTGCTGCAGGAAACTGAATAGATGGAC
Mt114	T A C T T A T C A A C T A C T C T T K C A A T C A C C A G C G T A G T T T T G T A T A T C C A A T T C C A C G T T G T C [G / T] AAAGAAAACTAGAAATGGGAGTCTCTGCAGAATTGTGCTTCTCTCACTCAACTCTCCGAA
Mt118	C A G A A G A C A T G T A C C T G A T C G C C A T T T A T T G A A C A C C A C A A T T G K T G A T C C T T T C A A T G C [G / T] CTGCAGTGGCATTGTGGGTAGGTAGTCTAGCTGTTGCCACAACCGTCTGCCATTTTCGAG
Mt130	C T T G T T C T A C T C C C A T C K G C T G C A G G A C C A T G C T T T C T T G C T T C C T C T C G A T G A A T T G C T [G / T] GTGGTGGTGGTGCTGCTGCATCAACTGGATCGGCCTCAGACGGTGGTCCGGCTGCCTCCC
Mt133	G A C A A T G A A T T G T G R A A G A T G G A A G G G A C G A G A G T C A C C T G C T G C T G C A G A A G A T A A C A T [G / A] CTGTAGCTGAGGCTGGGCTCCACCCCCAAGCTTATCTTCTGGATCAGAACATTATCTTTG
Mt140	A A R C A A G C A C A A C C A A G C C T G C A G C A C C C G C G G T G A A G G A C G A C G A C A T A G C G A G C G T C G [G / A] GGAAGCATTAGTTAGCATGGGCAGGAGGAGTCTTGTGGTGGTGGGAGAGTGCTCGGATAC
Mt146	C C K T G G C C G C T T G G C T C G A G C T G C A G C A T G G C C A C C A G T G C A C C A G T C C C C A G A G C A G G A [G / T] CATCAAGAGGAAGGAGCTCACGTTCCTCATTGCATGCAACACTCCCCTGAACCCATTGAA
Mt151	G C W A G G C C T G A T C T C A A G G T A A G T G G T A T A C A T G T G T G A A G C T G C A G C A C A G A G G A G G A G [A / T] TCTTAGTAGGATGTATGGTTTCTTGAACAGGTAGGGATATGTGGTGAGCATGGTGGCGAA
Mt155	C G G C T G C A G C A A T G G C A G C T G C A G T T S G A A G A G A A G A A G G A G A G G A A G A T G A G C A G G G G A [G / C] GAAGAAGAGGCTGCGGTCGTGGCTGAGGCTGCGACTGCAGCAGTGCAGCTGGGAAAGAAG
Mt157	A G T C A G C A T C G A G G G G T T T G A R A G C G G A T T G G A A A T G C T T C T T C A G G C A G C T G A T C C A A A [G / A] TCGAGTCTCTCTGCTCAACATGCTTAGCTCATGAAGAAACTCATCCTATCTCGTGCCCTG
Mt167	A T T A A G A A T G T C A C T G T C A A A A A A A G A T G C A A G T C T G C A G M A G G A A A T A C A A A T A T A A A T [A / C] TCTGAAAACAGAGCACTCTGTGTAACAAGCCTGCAGCAAAATTTATACCGAATATTTGTG
Mt172	G T C A T G T C A A G A G M T C A A C A A A G G A A A C T T G A G C A A G A T G G T C A A G A A A G A G T C A C T G C A [A / C] GAGATGATAAAGCTAAAGAACACTACAAGGATGAGGAAGAAAAGCTTTACAGTGTGAAGA
Mt173	G A G A T C C A T A T Y A A G C C G G A G A G G C A T A A T G T G A G T T C T G C A G T G G T A G A T G T T G G T A A T [C / T] ATGGAGTTGATAATTCTGATAAAGAAAAGATGCAGATTGTATTAGATGATGACCTACATG
Mt179	A G A G C A C T T A G C C A C T G C A G C T C T G G C A A C A C A A C C T T T T C G A G A T C T G G T C K A T C C T T C [G / T] TTCTCAGCTCTGTGCACTTGAGTGCCTTCTCAGCAAGCCGCTGAGCAGCCTCCACCGGCC
Mt184	C C A C T G C A G C C C A A C T A A T A A T C A T G T A C G A A A A T A C A C C A A A A A T G A T A A C C A A C C C A C [A / G] AACTTTATATCCGACTGTAGAAAATCATAATCATTTTCTACAACTGACTAACAAAGAGAA
Mt190	A T A T A G T T T A T C A T G A T T A A C T G C T G C A G A A T A A A T C G A G T T T M G C T C C C C T T T T C A T T A [A / C] GCACCTTGTAACTTTTCAAATCGCCTGAGTCTTTGACTTTCTGCAGCCTGACACTAAAAA
Mt200	A A T G G T G A A C T T T T T T A T C A A G G A T T A A A G C A T T G C C A T A C T G C A G C G A A T A C T G R A C T G [G / A] CATATGGCATCAGTGCTGCAAGACCCTTACAGGTTATTACAATTCATGCATGTGCCAGCA
Mt216	T A T A C T T T C T G A T A A T A T G T T T T C T G G A A C C R T A C C T T C C A T C A T A G G A A A G C T C T C T C A [G / A] TTGACAGAACTGCAGATGGGTGGTAATCAATTTTTTGGCACAATACCAAATGAATTGGGT
Mt217	G A A C A A C C T T T C T C G G G C T C C T T C T G C A G C T A C A G A A A A G G G A G G C A G C T T C C T T T C A T R [A / G] AAGACGACGCCACTGCCTTCGGCATAACAACCATGGCACAATCTACATGCCTGCAGCCAC
Mt236	C A A A A T G G A A C G T A C C T T A W T T G C C A A T C A T C A T C G G C C A T C T G C A G A T A A T T C A A C A A C [T / A] TTGTGCAGTCAGATGTTTGTTCATGGATGGCCTGCACAAGCTATGAGTTGATGTGCCCAT
Mt238	A A C T T T G T G A T G T T G T T C T G C A G T G C T G T K A C A T C T A T T A A C T A C T G C A G C A A C C T C G A C [T / G] TAACACCAGCACCTAACAGCTTATGCCTGCCTTCAATATATAAACATGGTAATAACATAT
Mt240	C C T C T G C A G C T T C C T G T G A G C C C G T G T A G C T A T T A C T C A T C A A T G A A G A G C G T T T G A T T C [G / A] CACTATTCCAAAAATTTCCCAACAACTTCGGATATCATAGCAAGGATGTAAGAAAATAAA
Mt243	G G A G A A G G C C T C T C G T T G C C A T C T C G C A C G T A G C G C G C A G G G A T C A C C R T C G G A T G A G A G [G / A] GCCGTAGCTCTTGAACGTCCTCGACGCGGCCTTCGATCACTGCAGTGCCGGAATTCTCCT
Mt244	T C A C A C A G C T T T A G T A T G A A A A T A A T T T T T C T A A A G G A C A G T T T T C T G C A G T C A T A G A A A [A / G] CAAGAACAAGTGAAAATATTTCTCCTGTATTACCTTCATTGAGAGGGACTTGATCTGTTT
Mt247	T G C A G C A G C T T A G C T A C C T A T T A T A C A G A G C G G C C A C A G T G G G T A T A C T G C A G C A C A C A R [A / G] CCTGATCGGATCAGTGTGAGGCCCTCTTGTTCCTTGAACAAAAAAACAATTGCTTGATCA
Mt264	C T T G T T C T A C T C C C A T C K G C T G C A G G A C C A T G C T T T C T T G C T T C C T C T C G A T G A A T T G C T [G / T] GTGGTGGTGGTGCTGCTGCATCAACTGGATCGGCCTCAGACGGTGGTCCGGCTGCCTCCC
Mt271	A C C A T T A A T A A G A A A A G G A C C T G C A G G T T T A A A T G T G T A A G T A C R G T T T T A C A T G C C A T G [A / G] GATAGAAATACTTGGTTATTTACTTGGGTTAATTATTTTTTGTTCTTGTAATTGCTGCAG

Mt276	T C A G K T T G A G T G A G C A A T A C A A C C T C A G A G A G G G G T C T G C A G A A A T G A T G A A T G T C A G C A [G / T] GACCACCGGCCATGGACAACTCAAATGGAAGGTGGAGGCTCATTGCGTTCCAACATCAAC
Mt282	T G G T C C A A G A A T A Y C A A A T T C A T G G G G C A T G T A A T C T T C A T T T G G A C T G T A G T A T T T G T C [T / C] AGAGAGTGCAAGCTCAAGTTTGTTACTTGTCTTGTGTATAAATAAGCAAACCTCTCAACC
Mt301	A A A T A T C R G T C T A A A T A T A C T G C A G G A C T G A T T T T A A T G A T C C A A A A C T A A A A C C A A T G G [G / A] ATATTAAAGGCAGTCAATAAAAAAATAATCCAAATTATGAAAATAGTTAAAATTAAAATT
Mt309	G T G C T T C T C T C C T G C C T G C A G A G T G T A G S T C G T T C T C A C T G T G A A T G C T G C A G A T G G T T C [G / C] ATTCTAATTTCATATCCTTTTTAATGCATGTTGTACACATATATATGACGTGAAATTTCG
Mt313	G T C C G C G A T T C T T T C C C T G C G A A G C T G C G A A C A G A A A C A A A G A A T A A T G G C Y G G G G G C A A [T / C] CGAACAAACAAAATGGAAACAGAGCAGAAGCTGCAGCACAACAAAGGACACGGAAGCTGC
Mt314	C C A G T C T T T T G A T C T T T T C C T A C T G A T T G C G C T G C A G T T T C G T A T C T A C K C C T G A C A A G G [T / G] AGCCAACAGCTACTATCTAGGTCCAGCTCCTCTCCAGGATATGGCTAGGTGAGAGACCGA
Mt326	G T C T G T T G C G A G G G A G T Y G A A C A C A A A T A T G G T A G G T G A A G A G G A C A G A A A A A T T A T G G T [T / C] GCTAGCAAAGGTCTGCAGCATTCTTTTTCATTTGAAAAAGCAGACAATGAACCACCCATC
Mt327	C T A A Y A A G A A A A T A C A G T A T A G T T G C T A G T A A A C A G T T A C A A T T C A G A T C A A A C T T A C G G [T / C] ACTTAGAGGCTGCAGGGTCATCTGAGCATAAACTTCATCTGTTTCCACATCTGCCTGCAA
Mt334	A T C A C C T G A A T C A T A T G C A G C A C A A C T C A G C C T T T T C T C C G C A T A A G C T T C T T C G G C T T C [C / A] ACCACTGCAGCCAATGTTCTATGTACAATAATGTCAGGATATCTTCTCAATGGAGAAGTG
Mt335	G A A C T A C T C C A C A C T A T T C A A A T G A A T G A G T G C A T G C T G C A T A G C A T T T T C C C C A T C C A A [C / T] TACTCTCACAATCTGGTTAAACACTTATCCATGTATACCTGCAATAATTTTTCTATCGGC
Mt336	C A C A A C C C A A G A C G T G C T A T C C A T G T T G T T G A T C T T A G C C A C C A Y C A T G T C T G C A G A G A A [C / T] TACATATCTTTGATCTTTAAGGCACCCATGGCTTCTCTGTTCAATGATAAAGAATGTCCA
Mt345	C T T G C A A C A T C G T T G A C A T C T T C T C T A T C T T G A A A A A C T T C T A G C T G C A G A C T A C A G A A A [A / G] CTTCTGAACTGAGTCCAGGAGGCTTATTGAAATATCGCACATCTTCATCTGTTTCAGCTG

Cultivars	Mt006	Mt010	Mt012	Mt020	Mt036	Mt038	Mt049	Mt064	Mt070	Mt071	Mt075	Mt078	Mt080	Mt087	Mt089	Mt092	Mt097	Mt099
Abuab Labo	C C	A A	C C	A C	A A	A A	A T	G G	G G	C C	C G	C G	C C	C T	C C	C T	C T	C T
Lacatan	C C	A A	A C	C C	A A	A G	A T	G G	0 0	C G	G G	C C	C C	0 0	C C	C T	C C	C T
Pisgan	C C	A A	A C	A C	A A	A A	T T	G G	G G	C C	C G	C C	C C	T T	T T	T T	C T	C T
Canton	A C	A C	C C	A A	A A	A A	T T	G G	G G	C C	G G	C C	C C	T T	C C	C T	C C	C T
Daratex	A C	A C	C C	A A	A A	A A	A T	G G	G G	C G	G G	C C	C C	C T	C C	T T	C T	C T
Hybrid 2	A C	A C	C C	A A	G G	G G	A T	G G	G G	C C	G G	C C	C C	T T	C T	C T	T T	C C
Hybrid I	A C	A A	C C	A C	G G	G G	A T	G G	G G	C G	G G	C C	C C	C T	C C	C T	C T	C C
Musa Tex 82	A C	A C	C C	A C	A A	A A	A T	G G	C C	G G	G G	C G	C C	C T	C C	C T	C C	C C
Mamakaw	A C	A A	C C	A C	A A	A A	A T	G G	C C	C C	G G	C C	C C	C C	C T	T T	T T	C C
Igit 1	C C	A A	C C	C C	A A	A A	T T	G G	0 0	C C	G G	C C	C C	T T	C C	C T	C T	C C
Igit 2	A C	A A	C C	C C	A A	A A	T T	C G	G G	C G	G G	C C	C C	C T	C C	T T	C T	T T
Puti 1	A C	A A	C C	A A	A A	A A	A T	C G	G G	C C	G G	C C	G G	C T	C T	T T	C C	C T
Puti 2	C C	A C	C C	A A	A A	A A	A T	G G	G G	C C	G G	C C	C C	T T	C T	T T	C T	T T

Table 3 Examples of DNA fingerprints based on the array of 60 SNPs for abaca cultivar identification (showing truncated profiles).

is presented in Table 4. The mean information index was 0.442, ranging from 0.143 to 0.693. The observed heterozygosity ranged from 0.016 to 1.00 with an average of 0.265, whereas the mean expected heterozygosity was 0.281ranging from 0.062 to 0.500. The minor allele frequencies of these 60 SNPs ranged from 0.032 to 0.500 with an average of 0.196 (Table 4).

The validation result demonstrated that the set of 60 SNP markers was effective for the assessment of genetic identity of abaca germplasm. All 62 abaca accessions can be clearly differentiated based on the 60 SNPs (Table 3). No duplicates were found in the present study. However, homonymous mislabeling was identified in two pairs of abaca cultivars. These accessions shared same name but were collected from different regions in the Philippines. Morphologically, it is difficult to differentiate them (Figure 1). In these cases, SNP genotyping provides clear evidence on which re-naming procedure can be taken for these accessions. The present result also showed that three inter-specific hybrids and one *M. balbisiana* sample were possibly mislabeled in terms of their pedigree and taxonomy status.

The approach of cross-species adaptation enabled us to generate high-quality SNP profiles for abaca cultivar identification. However, the overall success rate of using banana SNPs is relatively low. Out of the 384 validated SNPs, only 60 (15.6%) met the requirement as a genotyping panel. This was likely due to the large genetic difference between abaca and banana. The lacking of a draft genome in abaca is another hurdle to the effective development of SNP markers for abaca at the present time. For marker assisted breeding, many more SNP markers will be needed. The draft-genome of abaca is currently underway (Galvez, unpublished data), which will enable large

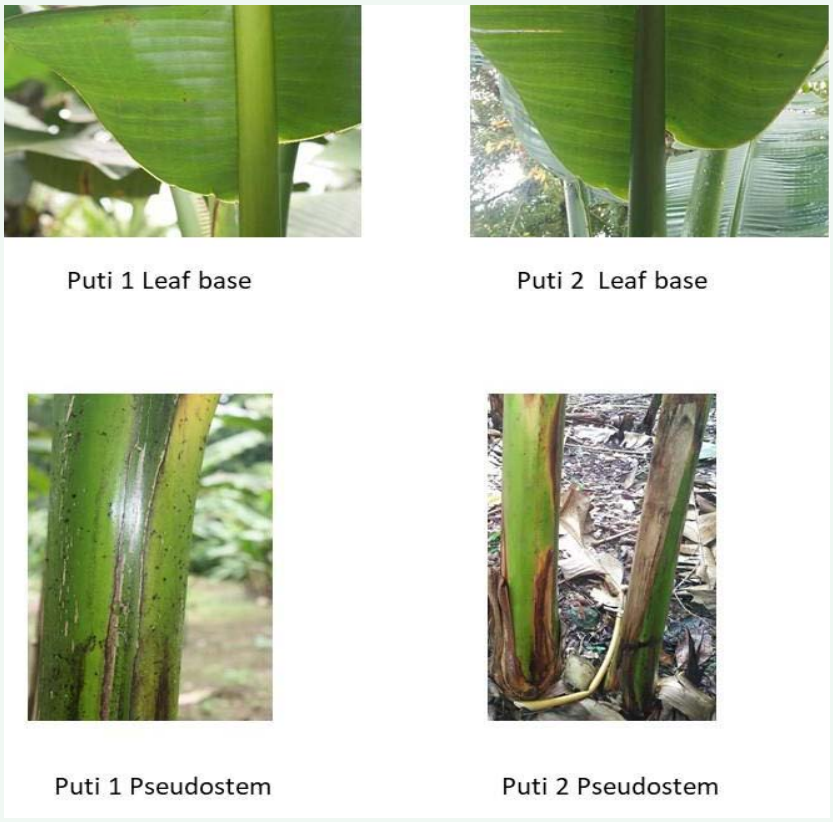

Figure 1 The homonymous pairs of abaca cultivar 'Puti', showing similar phenotypic characteristics.

Table 4: Information index, Observed heterozygosity, Expected heterozygosity and Minor allele frequency of the 60 SNP loci scored on 62 accessions of abaca and hybrids.

SNP ID	Information index	Observed heterozygosity	Expected heterozygosity	Minor allele frequency
Mt006	0.300	0.177	0.162	0.089
Mt010	0.408	0.283	0.243	0.142
Mt012	0.194	0.097	0.092	0.048
Mt020	0.655	0.403	0.462	0.363
Mt036	0.194	0.032	0.092	0.048
Mt038	0.455	0.125	0.282	0.170
Mt049	0.655	0.724	0.462	0.362
Mt064	0.558	0.356	0.371	0.246
Mt070	0.470	0.245	0.294	0.179
Mt071	0.507	0.279	0.326	0.205
Mt075	0.455	0.274	0.281	0.169
Mt078	0.245	0.133	0.124	0.067
Mt080	0.406	0.035	0.241	0.140
Mt087	0.476	0.333	0.299	0.183
Mt089	0.593	0.254	0.403	0.280
Mt092	0.389	0.262	0.228	0.131
Mt097	0.637	0.600	0.444	0.333
Mt099	0.690	0.383	0.497	0.458
Mt101	0.506	0.082	0.325	0.204
Mt102	0.581	0.500	0.392	0.268
Mt105	0.385	0.097	0.225	0.129
Mt106	0.284	0.131	0.150	0.082

Mt114	0.325	0.167	0.180	0.100
Mt118	0.143	0.032	0.062	0.032
Mt130	0.263	0.148	0.137	0.074
Mt133	0.317	0.193	0.174	0.096
Mt140	0.469	0.214	0.293	0.179
Mt146	0.681	0.847	0.488	0.424
Mt151	0.171	0.082	0.079	0.041
Mt155	0.693	1.000	0.500	0.500
Mt157	0.260	0.113	0.135	0.073
Mt167	0.194	0.097	0.092	0.048
Mt172	0.364	0.016	0.209	0.119
Mt173	0.685	0.217	0.491	0.435
Mt179	0.333	0.170	0.186	0.104
Mt184	0.276	0.123	0.145	0.079
Mt190	0.682	0.340	0.489	0.426
Mt200	0.321	0.118	0.177	0.098
Mt216	0.681	0.610	0.488	0.424
Mt217	0.592	0.557	0.402	0.279
Mt236	0.343	0.150	0.193	0.108
Mt238	0.373	0.246	0.216	0.123
Mt240	0.171	0.082	0.079	0.041
Mt243	0.451	0.255	0.278	0.167
Mt244	0.321	0.164	0.177	0.098
Mt247	0.692	0.952	0.499	0.476
Mt264	0.493	0.119	0.314	0.195
Mt271	0.220	0.115	0.108	0.057
Mt276	0.329	0.203	0.183	0.102
Mt282	0.287	0.133	0.153	0.083
Mt301	0.455	0.339	0.282	0.170
Mt309	0.691	0.241	0.498	0.466
Mt313	0.393	0.267	0.231	0.133
Mt314	0.660	0.271	0.468	0.373
Mt326	0.486	0.310	0.307	0.190
Mt327	0.493	0.153	0.314	0.195
Mt334	0.567	0.228	0.379	0.254
Mt335	0.632	0.241	0.441	0.328
Mt336	0.516	0.390	0.334	0.212
Mt345	0.481	0.176	0.303	0.186

scale development of SNP markers through NGS technology. Nonetheless, this validated set of SNPs is highly useful for abaca genotype identification, germplasm management and certification of planting materials, which will all contribute to more efficient crop improvement and crop production.

Genetic diversity and population structure in the abaca collection

The genetic relationship among the analysed abaca samples were presented in the principal coordinates analyses (PCoA) plots (Figure 2A-2B). The three main PCoA axes accounted for 26.3% of the total variation. Although the pattern of grouping was not apparent, it appeared that all the tested 62 accessions could be grouped into two types. The first type was comprised of most cultivars of *M. textilis* origin, both farmer cultivars and breeding lines. The second cluster is much smaller in size, including hybrids between *M. textlis* and *M. balbisiana* such as 'Canton', 'Daratex', 'Musa Tex 82', Hybrid 1, Hybrid 2 and 'Mamakaw'. However, there were three inter-specific hybrids ('Agpas', 'Mi-NC' and 'MTP') and one *M. balbisiana* sample ('Lacatan') grouped with the *M. textilis* accessions, suggesting possible mislabeling for these accessions.

The result of cluster analysis is fully consistent with that of PCoA. Two deeply separated clusters were revealed in the UPGMA dendrogram (Figure 3). Six accessions with known hybrid origin, including 'Canton', 'Daratex', 'Musa Tex 82', Hybrid 1, Hybrid 2 and 'Mamakaw', were grouped in a small cluster, demonstrating that they had different genetic background than the M. textilis. Again, the UPGMA did not separate the other three inter-specific hybrids ('Agpas', 'Mi-NC' and 'MTP') and one *M. balbisiana* sample ('Lacatan') from the rest of the *M. textilis* samples, which further support the possible mislabeling in this collection.

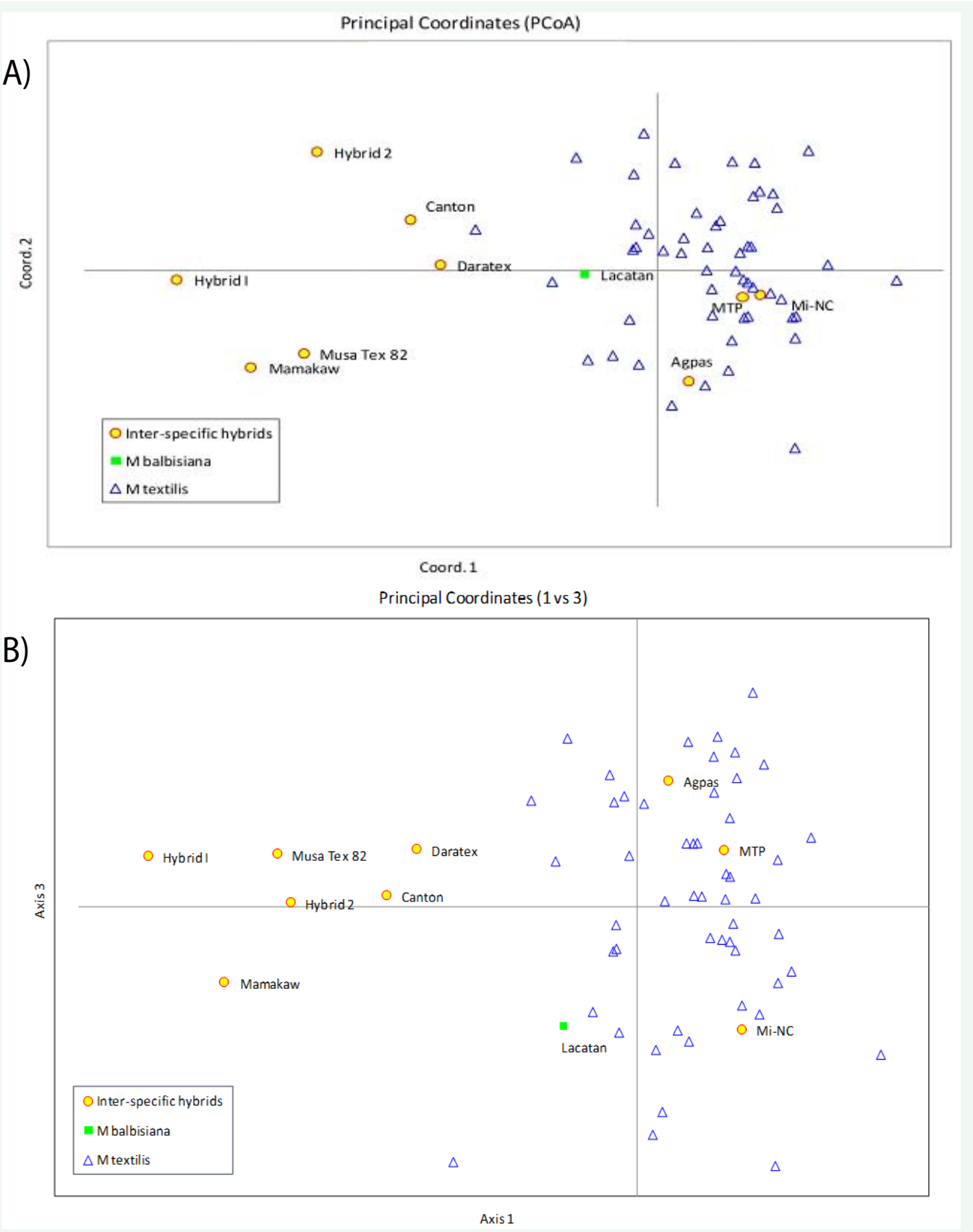

Figure 2 Principal coordinates analysis plots for the 62 abaca samples. The first axis represents 11.64% of the total information, the second 7.52% and the third 7.16%. (A) Axis 1 vs. 2; (B) Axis 1 vs. 3.

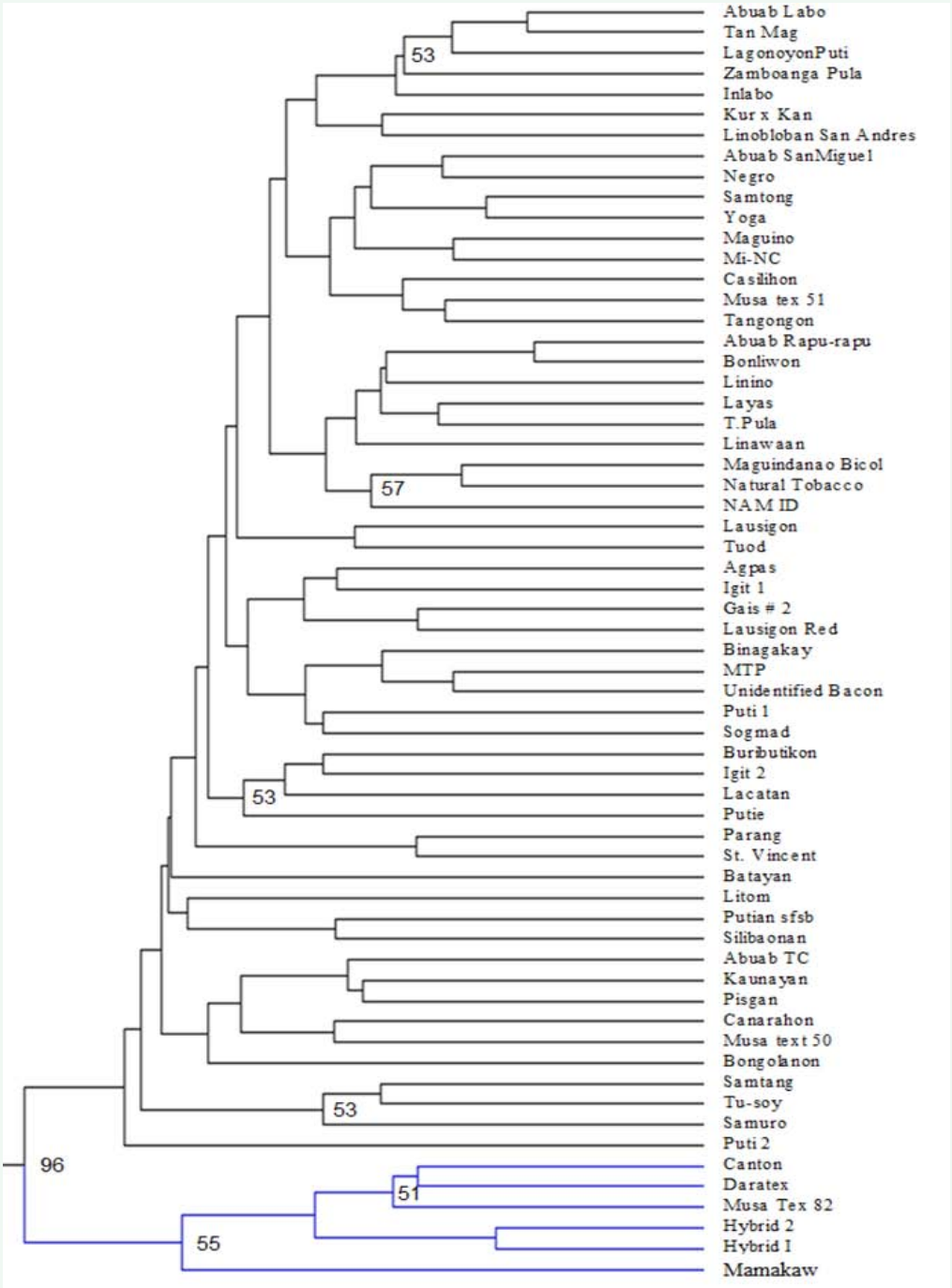

Figure 3 UPGMA (unweighted pair group method with arithmetic mean) dendrogram depicting the relationship among 62 abaca cultivars sampled from the abaca germplasm collection in the Philippines. Kinship coefficient was used as genetic distances. All cultivars correspond to the sample list in Table 1.

Population stratification of the 62 abaca accessions, based on Δ*K* value computed by STRUCTURE HARVESTER [37], revealed two clusters as the most probable number of *K* (Figure 3) and the partition was largely consistent with the principle coordinate analysis (Figure 2). Out of the 62 abaca accessions, five accessions, including 'Canton', 'Daratex', 'Hybrid 1', 'Hybrid 2' and 'Mamakaw' were differentiated from the rest of the accessions, demonstrating their exotic genetic background due to inter-specific hybridization. However, same as the result of PCoA, the additional three recorded inter-specific hybrids ('Agpas', 'Mi-NC' and MTP'), as well as one accession of *M. balbisiana* ('Lacatan') were not differentiated from the *M. textlis* cultivars, showing their mislabelled status. In addition, cultivar 'Binagakay', 'Canarahon' and 'Linono' were found having a partial pedigree contribution from hybrid parents, indicating they were backcrossed progeny of the inter-specific (*M. textilis* x *M. balbisiana*) hybrids.

Although the result is only based on 62 abaca accessions, the distance and model based analytical methods both clearly showed that the abaca genepool is heterogeneous. As shown in the PCoA (Figure 3), UPGMA (Figure 4) and Bayesian stratification (Figure 5), there were several 'outsiders', which did not belong to the core group of *M. textilis*. This heterogeneous structure appeared compatible with the breeding history of abaca in the Philippines. *Musa textilis* is indigenous to the Philippines and wild populations still exist in the highlands [1,2,8]. *M. balbisiana* is also widely distributed in the Philippines, although Philippines may not be the center of origin of this species [1, 40]. Nonetheless, 'Pacol' – a *M. balbisiana*-type has long been cultivated by subsistence farmers in the Philippines as source of food and fiber [2,8]. It is well documented that both natural hybridization occurred between 'Pacol' and *M. textilis* [1,2]. Despite of their pedigree from *M. balbisiana,* these inter-specific hybrids were often considered as 'abaca', of which 'Canton' is a well-known example [2]. Since 1920, numerous hybrids have been developed by various breeding programs through intra-and inter-specific hybridization, with the main objectives to increase productivity and resistance to bunchy top and mosaic viruses [8,13,17]. Therefore, the intensive genetic introgression from *M. balbisiana* (and possibly from other Musa species as well) explained the broad genetic diversity in the current genepool of abaca.

However, to accurately dissect the ancestries of current abaca germplasm, more SNP markers that can generate polymorphic profile across *M. textilis, M. balbisiana* and other related species need to be developed. This would require either screening for more SNPs using the present strategy of cross species adaptation or using the method of next generation sequencing (e.g. genotyping-by-sequencing). These general *Musa* SNP markers could enable effective genotyping of all possible ancestral species of abaca. In addition, SNP profile of M. *balbisiana* and other related species need to be established at population level, to better quantify the ancestral contribution of *M. balbisiana* (and possibly other Musa species) to the hybrid abaca germplasm.

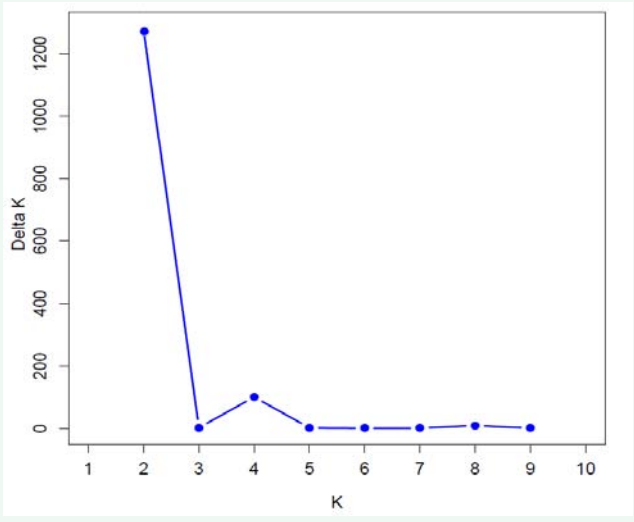

Figure 4 Plot of ΔK calculated as the mean of the second-order rate of change in likelihood of K divided by the standard deviation of the likelihood of K m|L''(K)|/s[L(K)].

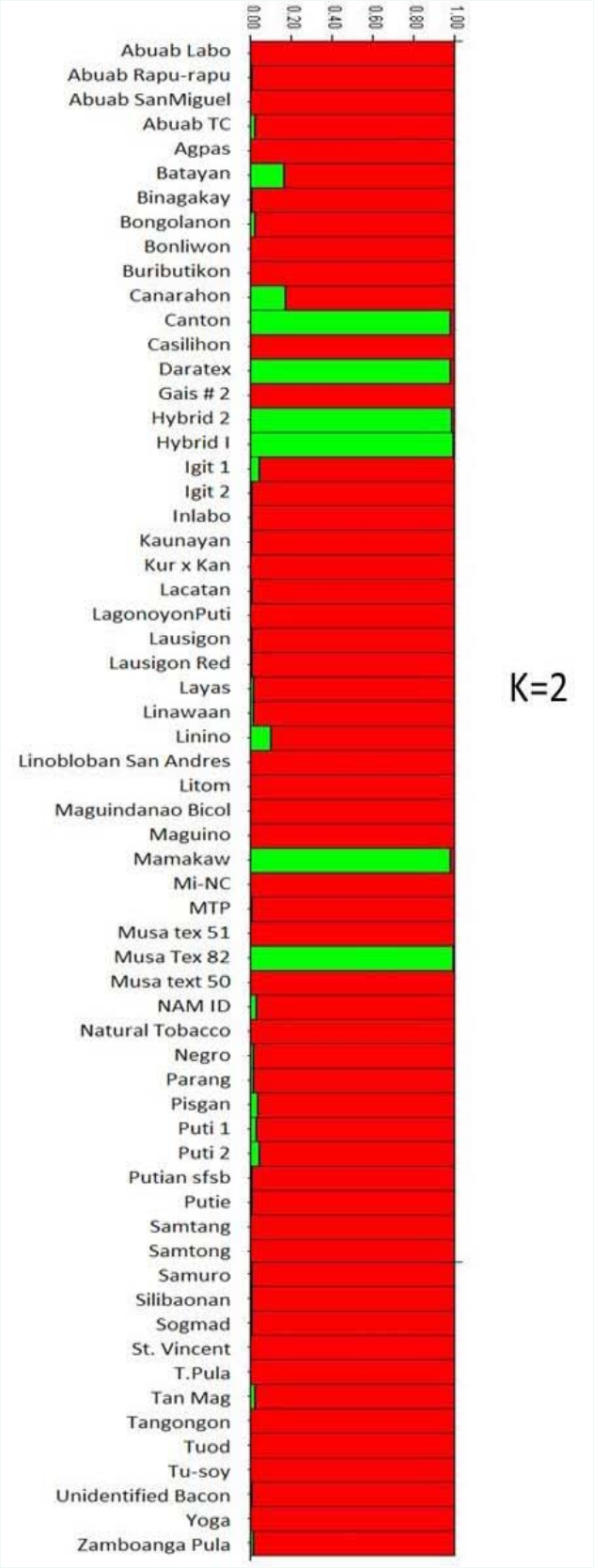

Figure 4 Plot of ΔK calculated as the mean of the second-order rate of change in likelihood of K divided by the standard deviation of the likelihood of K m|L''(K)|/s[L(K)].

Moreover, the national abaca germplasm collection in the Philippines maintained more than 200 abaca accessions and there are many uncollected landraces in farmer fields. The present study only analysed a small fraction of the accessions available in the genebank. The full spectrum of germplasm accessions need to be included to understand the population structure in the primary gene pool of abaca. Additional analytical approaches, such as discriminant analysis of principal components (DAPC) could be applied to provide insight that is complementary to the present study.

CONCLUSION

Despite the economic importance of abaca in the Philippines, research tool for germplasm management and genetic improvement of abaca is still in the infant stage. Lack of accurate information on genetic integrity is a primary concern for abaca researchers and growers. It's been difficult in determining a true-to-type cultivar solely based on phenotype, which causes confusion and uncertainty in the use of breeding materials. We conducted a pilot study to evaluate a set of banana SNP markers for abaca genotyping using a nanofluidic array. The cross-species transfer of nuclear SNP markers led to selection of 60 polymorphic SNPs suitable to abaca DNA fingerprinting. The generated SNPs profiles enabled accurate identification of all tested abaca cultivars and detection of homonymous naming mistakes. Cultivars with a background of inter-specific hybrid were differentiated using multi-variant analysis and Bayesian stratification. The result demonstrated that this approach could serve as a shortcut for SNP development in abaca. These selected SNPs are highly useful for downstream applications for abaca industry, including cultivar identification, nursery accreditation and protection of breeder's right.

ACKNOWLEDGEMENTS

We would like to give special thanks to Stephen Pinney for assisting with SNP genotyping using nanofluidic array, Sue Mischke for editing this manuscript and Brian Irish for valuable discussion. This work was partially supported by the Fulbright Scholar Program. References to a company and/or product by the USDA are only for the purposes of information and do not imply approval or recommendation of the product to the exclusion of others that may also be suitable.

REFERENCES

1. Halos S. The Abaca. Department of Agriculture. Quezon City. 2008; 188.
2. Valmayor RV, Espino RRC and Pascua OC. The wild and cultivated bananas of the Philippines. PAARFI, Los Baños, Philippine Agriculture and Resources Research Foundation, (Laguna), Philippines. 2002; 242.
3. Copeland EB. Nomenclature of the abaca plant. Philipp J Sci. 1927; 33:141-153.
4. Cheesman EE. Classification of the bananas. I. The genus Ensete Horan and the genus Musa L. Kew Bulletin 2; 1947; 97-117.
5. Argent GCG. Two interesting wild Musa species (Musaceae) from Sabah, Malaysia. Gardens' Bulletin Singapore. 2000; 52: 203-210.
6. Wong C, Kiew R, Ohn S, Lamb A, Lee SK, Gan LH. Sectional placement of three Bornean species of Musa (Musaceae) based on AFLP. Gardens' Bulletin Singapore. 2001; 53: 327-341.
7. Armecin RB, Sinon FG and Moreno LO, Abaca fiber: a renewable bio-resource for industrial uses and other applications. In Biomass and Bioenergy, edited by K.R. Hakeem, M. Jawaid and U. Rashid, Springer Nature. 2014; 108-116.
8. Lalusin AG and Villavicencio MLH. Abaca (Musa textilis Nee) Breeding in the Philippines. In: Cruz V.M.V, Dierig D.A. (eds) Industrial Crops. Handbook of Plant Breeding, Springer, New York, NY. 2015.
9. Torres JP and Garrido TG. Progress report on the breeding of abaca (Musa textilis Nee). The Philipp J Agric. 1939; 10: 211-230.
10. Dempsey JM. Long fiber development in South Vietnam and other Asian countries. US Department of Commerce, Washington DC. 1963; 1957-1962.
11. Wood FA, Roberts GAF, Prance S, Nesbitt M. The Cultural History of Plants. New York, NY: Routledge. 2005; 460.
12. PhilFIDA Report. Philippine Fiber Industry Development Authority Annual Report. Department of Agriculture. Diliman, Quezon City. 2018; 240.
13. Labrador AF. The abaca project of La Carlota Experiment Station. Philippine Agr Rev. 1928; 1: 3-19.
14. Altoveros NC and TH Borromeo. The state of plant genetic resources for food and agriculture in the Philippines, A country report, Bureau of Plant Industry, Department of Agriculture, Malate, Philipines. 2007; 19.
15. Villavicencio MLH, Borromeo TH, Altoveros NC and De la Cruz Jr. FS. Enhancing the capacity of stakeholders on in situ conservation and sustainable use of indigenous abaca (Musa textilis Nee). Terminal Report SFRT. 2007; 69.
16. Jeffries MJ. Biodiversity and Conservation. Routledge, London and New York. 1997; 208.
17. Boguero APB, Parducho MAL, Mendoza MR, Abustan MAM and Lalusin GG. Molecular screening of abaca (Musa textilis Nee) accessions using microsatellite markers associated with resistance to bunchy top virus. Philipp. J Crop Sci. 2016; 41: 2: 13-19.
18. Rafalski A. Applications of single nucleotide polymorphisms in crop genetics. Curr Opin Plant Biol. 2002; 5: 94-100.
19. Fang W, Meinhardt LW, Tan H, Zhou L, Mischke BS and Zhang D. Varietal identification of tea (Camellia sinensis) using nanofluidic array of single nucleotide polymorphism (SNP) markers. Hortic. Res. 2014; 1: 14035.
20. Wu B, Zhong G, Yue J. Identification of pummelo cultivars by using a panel of 25 selected SNPs and 12 DNA segments. PLoS ONE. 2014; 9:e94506.
21. Wang B, Tan H, Fang W, Meinhardt LW, Mischke BS, Matsumoto BTK, Zhang D. Developing Single Nucleotide Polymorphism (SNP) markers from transcriptome sequences for the identification of longan (Dimocarpus longan) germplasm. Hortic Res. 2015; 2: 14065.
22. Zhou L, Matsumoto BTK, Tan H, Meinhardt LW, Mischke BS, Wang B and Zhang D. Developing Single Nucleotide Polymorphism (SNP) markers for the identification of pineapple (Ananas comosus) germplasm. Hortic Res. 2015; 2: 15056.
23. Zhou L, Vega FE, Tan H, Ramirez LA, Meinhardt LW, Fang W, Zhang D. Developing Single Nucleotide Polymorphism markers for the identification of Coffee germplasm. Trop Plant Biol. 2016; 9: 82-95.
24. Davey MR, Gudimella JA, Harikrishna LW, Khalid SN, Keulemans J. A draft Musa balbisiana genome sequence for molecular genetics in polyploid, inter- and intra-specific Musa hybrids. BMC Genomics 2013; 14: 683.

25. D'Hont A, Denoeud F, Aury JM. Baurens FC, Rouard, M, Guignon V, et al. The banana (Musa acuminata) genome and the evolution of monocotyledonous plants. Nature. 2012; 488: 213-217.

26. Martin G, Baurens FC, Droc G, Rouard M, Cenci A, Kilian A, Hastie A, Françoise C, D'Hont A. Improvement of the banana 'Musa acuminata' reference sequence using NGS data and semi-automated bioinformatics methods. BMC Genomics. 2016; 17: 1-12.

27. Ruas M, Guignon V, Sempere G, Sardos J, Hueber Y, H. Duvergey, et al. MGIS: managing banana (Musa spp.) genetic resources information and high-throughput genotyping data. Database (Oxford University Press. 2017; 1-12.

28. Wang J, Lin M, Crenshaw A, Hutchinson A, Hicks B, Yeager M, Berndt S, et al. High-throughput single nucleotide polymorphism genotyping using nanofluidic Dynamic Arrays. BMC Genomics. 2009; 10: 561.

29. Fluidigm. Fluidigm SNP Genotyping User Guide Rev H1, PN 68000098. South San Francisco, CA: Fluidigm Corporation. 2011.

30. Peakall R, Smouse PE. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics. 2012; 8: 2537-2539.

31. Dieringer D and Schlötterer C. Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite datasets. Mol Ecol. Notes. 2003; 3: 167-169.

32. Felsenstein, J. PHYLIP—phylogeny inference package (version 3.2). Cladistics 1989; 5: 164-166.

33. Rambaut A. Molecular evolution, phylogenetics and epidemiology: FigTree. 2006-2009.

34. Pritchard JK, Stephens M and Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000; 155: 945-959.

35. Evanno G, Regnaut S and Goudet J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol. 2005; 14: 2611-2620.

36. Earl DA, vonHoldt BM. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 2012; 4: 359-361.

37. Jakobsson M. and Rosenberg NA. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics. 2007; 23: 1801-1806.

38. Rosenberg NA. DISTRUCT: a program for the graphical display of population structure. Mol Ecol 2004; 4: 137-138.

39. De Langhe E, Vrydaghs L, de Maret P, Perrier X and Denham T. Why Bananas Matter: An introduction to the history of banana domestication. Ethnobot. Res Appl. 2009; 7: 165-177.

Cite this article

Galvez LC, Meinhardt LW, Goenaga R, Zhang D (2021) Accurate Identification of Abaca (Musa textilis Née) Cultivars Using Single Nucleotide Polymorphisms (SNP) Markers Developed for Banana (Musa acuminata Colla). Int J Plant Biol Res 9(1): 1125.