J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 7(2): 39 - 47, 2016

GENETIC RELATIONSHIP AMONG *Musa balbisiana* ACCESSIONS AND IDENTIFICATION OF SRAP MARKERS LINKED TO *Musa* B GENOME

Youssef, M.[*1] and R. M. Escobedo-GraciaMedrano[2]
1-Genetics Department, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt
2-Unidad de Bioquímica y Biología Molecular de Plantas, Centro de Investigación Científica de Yucatán, A.C., Calle 43, # 130, Col. Chuburná de Hidalgo, CP. 97200 Mérida, Yucatán, México
*** Corresponding author: mkhirshy@aun.edu.eg**

ABSTRACT

The sequence related amplified polymorphism (SRAP) was used to assess genetic relationship among some *Musa balbisiana* related accessions including wild diploid, triploid cultivar, plantains, diploid and tetraploid hybrids. A total of 74 bands were generated, of which 65 bands (87.84%) were polymorphic among the tested accessions. The average of polymorphism information content (PIC) for all primers was 0.29, while the average of diversity index (DI) was 0.73. Results showed the SRAP efficiency in grouping the tested accessions and exposing minimum differences among genotypes. Interestingly, the wild diploid accessions were separated into two groups, in which BB-CICY, Tani, Cameron and Singapuri grouped together with the triploid cultivar "Lep Chang Kut", while the other diploid accessions, i.e. Butuhan, Pisang Batu and BB-545, formed the second group near to plantains and hybrids. These findings were subsequently supported by the identification of some specific bands generated by SRAP, which were existed in all B genome contained accessions with the exception of Butuhan, Pisang Batu and BB-545. The results suggested that these accessions may have recombinant chromosomes of A and B genomes or they are mislabeled. The identification of the specific bands for B genome found in this study provided markers linked to chromosomes 2, 8 and 11, which could be helpful in discriminating banana and plantain cultivars. Specific primers were designed from sequences of some specific bands, among which a 256bp fragment was successfully amplified, and was able to distinguish between *acuminata* and *balbisiana* accessions. Moreover, the information herein could be important in *Musa* basic breeding program, identification of mislabeled accessions and germplasm management and conservation.
Keywords: *Musa*, banana, SRAP, sequencing, specific markers, B genome

INTRODUCTION

Banana and plantain are belong to the genus *Musa* (family Musaceae), which was recently divided into two main sections, the first is Musa, which includes all species related to pre-classified Eumusa and Rhodochlamys, and the second section is Callimusa that includes species of the two other sections (Australimusa and Callimusa) (Häkkinen 2013). The world production of banana and plantain reaches approximately 105.96 and 37.88 million tons in 2013, respectively and are produced by about 120 countries (FAOSTAT 2015), which reflects their great importance worldwide. *Musa balbisiana* Colla is one progenitor of cultivated banana which contains B genome with a basic chromosome number of 2n = 22 (Davey *et al.* 2013). *M. balbisiana* is important for banana breeding program due to its valuable agronomic traits including vigor, resistance to pest, disease and drought (Ahmad *et al.* 2014). *M. balbisiana* and *M. acuminata* (A genome) formed plantains (AAB) and cooking banana (ABB), diploid (AB) and tetraploid hybrid (AAAB, AABB, ABBB) (Teo *et al.* 2005). Unlike the well understood *M. acuminata*, only few genetic diversity studies of *M. balbisiana* have been conducted (Ahmad *et al.* 2014). In addition, there is no record of subspecies classification of *M. balbisiana* (Ude et *al.* 2002).

Several molecular markers have been used previously with *Musa* spp., to assess genetic diversity and reveal relationship among *Musa* species and subspecies, including random amplified polymorphic DNA, RAPD (Ferreira *et al.* 2004), amplified fragment length polymorphism, AFLP (El-Khishin *et al.* 2009), inter simple sequence repeats, ISSR (Poerba *et al.* 2010), simple sequence repeats, SSR (Hippolyte *et al.* 2012), diversity array technology, DArT (Risterucci *et al.* 2009), restriction fragment length polymorphism, RFLP (Nwakanma *et al.* 2003), Variable number tandem repeat, VNTR (Crouch *et al.* 1999) and sequence related amplified polymorphism, SRAP (Youssef *et al.* 2011). Among these markers, SRAP has been confirmed to be more efficient and informative than other since it targets the open reading frames (Li and Quieros 2001). SRAP showed its excellency in exposing high level of polymorphism, several specific bands, clear relationship among *Musa* sections and high degree of differentiation between A and B genomes, as well as within plantains and cooking banana (Phothipan *et al.* 2005; Youssef *et al.* 2011; Valdez-Ojeda *et al.* 2014; Pinar *et al.* 2015). In addition, Youssef *et al.* (2011) indicated that SRAP was able to generate several unique and specific bands for *M. balbisiana* accessions which were existed in various genome compositions i.e. BB, AB, AAB, ABB, AABB and AAAB. These bands are of great importance to discriminate accessions contain B genome from other genotypes and successively can help in *Musa* breeding programs, germplasm management, as well as rapid identification of mislabeled accessions.

The present study is an extension to the previous work of Youssef *et al.* (2011) with more focus on *M. balbisiana* and B genome. Thus, in this study the number of *M. balbisiana* wild accessions was increased to be analyzed together with different accessions having B genome and to confirm the presence of the specific bands generated previously by SRAP. Identification of these bands by analysis of their sequences could assist

in focusing on the genome regions containing such markers especially after *Musa* genome sequence became publically available (D´Hont *et al.* 2012). Therefore, the present study aimed to assess the genetic relationship among *M. balbisiana* related accessions using SRAP markers and to identify the B specific bands sequences.

MATERIALS AND METHODS

Plant material

Thirteen accessions were used in this study, consisting of wild diploid of *Musa balbisiana*, triploid cultivar, plantains, diploid and tetraploid hybrids, as well as a double haploid *M. acuminata* as presented in Table 1. Young cigar leaves were collected from plants of the *Musa* germplasm collection of the Centro de Investigación Científica de Yucatán (CICY) held at the research station of the Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP) at Uxmal, Yucatán, México (20° 24' 40.10" Lat. N, and 89°45' 24.90" Long.W, 8.0 meters above sea level, m.a.s.l). All *Musa* accessions with an International Transit Code (ITC) number were provided by the Biodiversity International Centre for *Musa*, located at the Laboratory for Tropical Plant Improvement in the Catholic University of Leuven (Belgium), whereas accessions BB-CICY and Mal-CICY were collected at Teapa (Tabasco) in México.

Table 1. Accessions used in the present study.

No.	Species/hybrid	Subspecies/ subgroup	Genome	Name	Abbreviation	ITC[b]
1	*M. acuminata*	*malaccensis*	AA	CIRAD 930	CRD	1511
2	*M. balbisiana*	-	BB	Tani	TAN	1120
3	*M. balbisiana*	-	BB	Cameron	CAM	0246
4	*M. balbisiana*	-	BB	Butuhan	BUT	0565
5	*M. balbisiana*	-	BB	Balbisiana	B545	0545
6	*M. balbisiana*	-	BB	Balbisiana CICY	BB-CICY	*
7	*M. balbisiana*	-	BB	Singapuri	SIN	0248
8	*M. balbisiana*	-	BB	Pisang batu	PBT	1156
9	*M. a x M. b*	Ney Poovan	AB	Kunnan	KUN	1034
10	*M. balbisiana*	Lep Chang Kut	BBB	Lep Chang Kut	LCK	0647
11	Tetraploid hybrids	-	AAAB	FHIA-01	FHI	0504
12	Plantains	Pome	AAB	Prata ana	PRA	0962
13	Plantains	Plantain	AAB	Orishele	ORI	0517
14[a]	*M. acuminata*	*burmanicoides*	AA	Calcuta-IV	CAL	0249
15[a]	*M. acuminata*	*malaccensis*	AA	Malaccensis-399	Mal-399	0399
16[a]	*M. acuminata*	*malaccensis*	AA	Malaccensis-250	Mal-250	0250
17[a]	*M. acuminata*	*malaccensis*	AA	Malaccensis-CICY	Mal-CICY	*

[a] Accessions used for confirmation of specific bands, [b] International Transit Code, * Accessions from Teapa, Tabasco, Mexico.

DNA Extraction and SRAP amplification

Total genomic DNA from *Musa* accessions was extracted from 100 mg of frozen young cigar leaves following the protocol of Youssef *et al.* (2015). DNA quality and concentration were determined using a spectrophotometer according to Stulnig and Amberger (1994). The SRAP protocol was implemented using the tools of Khirshyat 1.0 (Youssef 2012). Five primer combinations were selected after Youssef *et al.* (2011) based on generation of specific bands for *Musa* accessions containing B genome as shown in Table 2. Each 20 µl SRAP amplification reaction consisted of 2 µl of 10× PCR buffer, 1.6 µl of 50 mM MgCl2, 1.6 µl of 10 µM of each forward and reverse primer, 2.5 µl of 2 mM dNTPs, 25 ng template DNA and 0.25 µl of 5U Taq-DNA polymerase (Invitrogen). The PCR was carried out with the initial cycle at 94°C for 2 min, 5 cycles of 94°C for 30 s, 35°C for 30 s and 72°C for 1 min, another 35 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 1 min, and the final extension at 72°C for 5 min. SRAP amplification products were visualized using ethidium bromide in 3% agarose gel.

Identification of specific bands for B genome

Four accessions were used for the recovery of specific bands i.e. BB-CICY, Singapuri, Tani and Prata ana. SRAP amplification products of these samples were separated on 6% polyacrylamide gel to insure clear separation. Specific bands for B genome were recovered from the gel and purified using ethanol precipitation. For each fragment, four samples were sequenced, each in forward and reverse sense at Macrogen, Inc. using a standard Sanger sequencing method. Sequences were automatically aligned using ClustalW in Sequencher 5.2.3 (Gene Codes) after removing the low-quality end regions (Q score <20). Subsequently, the Sequencher alignment file was exported in the FASTA format. Sequences were analyzed against NCBI database and Banana-Hub. Only important sequences according to the alignment of Banana-Hub were used for specific primer design (Table 2). For confirmation, four additional diploid accessions (AA) of *M. acuminata* ssp. *malaccensis* and ssp. *burmanicoides* were used (Table 1). PCR was performed for the amplification of the specific bands as 94°C for 2 min as initial denaturation followed by 30 cycles of 94°C for 1 min, 55°C for 1min and 72°C for 1min, and a final extension at 72°C for 3min. PCR products were visualized on 2% agarose using ethidium bromide.

Table 2. SRAP primer sequences used in the present study.

No.	Code	Forward primer (5´-3´)	Reverse primers (5´-3´)
1	Me1-Em1	GACTGCGTACGAATTAAT	TGAGTCCAAACCGGATA
2	Me2-Em1	GACTGCGTACGAATTAAT	TGAGTCCAAACCGGAGC
3	Me4-Em1	GACTGCGTACGAATTAAT	TGAGTCCAAACCGGACC
4	Me3-Em8	GACTGCGTACGAATTGAC	TGAGTCCAAACCGGTGT
5	Me4-Em8	GACTGCGTACGAATTAGC	TGAGTCCAAACCGGACC
6*	BB-246bp	ACATACCAGGGTTTACCGG	CGTCATTAGGGATACGTACG
7*	BB-256bp	TTGGGCTAAGGTACAACGG	TGGTTAGGCATGACTCAGC

*** Specific primers designed for specific bands amplification.**

Molecular data analysis

The presence (1) or the absence (0) of bands was made in a binary matrix from SRAP profiles. The percentage of polymorphism (%P) was calculated by dividing the number of polymorphic bands with the total number of generated bands. The polymorphism information content (PIC) was estimated for each marker using PIC*i* = $2f_i(1-f_i)$, where PIC*i* is the PIC of the marker *i*, f_i is the frequency of the amplified allele (band present), and (1-*fi*) is the frequency of the band absent (Roldan-Ruiz *et al.* 2000). The resolving power (R*p*) of each primer in all markers was calculated using the formula: , where , and *p* is the proportion of the accessions containing the I band (Prevost and Wilkinson, 1999). Diversity index (DI) was calculated using Nei's (1987) equation $DI = \frac{n(1-\sum[p_i^2])}{n-1}$, where n is the number of individuals analyzed and p_i is the frequency of the *i*th allele. The software NTSYSpc ver. 2.20s was used and genetic similarities were computed using Jaccard's coefficient (1908). Cluster analysis was carried out on similarity estimates using UPGMA. Statistical stability of the branches in the dendrogram was tested by bootstrap analysis with 1,000 replicates using the Free Tree 0.9.1.50 software program (Hampl *et al.* 2001).

RESULTS AND DISCUSSION

Assessment of genetic diversity and relationship helps in exposing the relatedness of genotypes and facilities the selection of useful genotypes in breeding and improvement programs. Most of the previous studies on *Musa* genetic diversity mainly focused on the diversity of *M. acuminata* and its related cultivars. However, the study of genetic variation in *M. balbisiana* and its hybrids is only in the beginning (Valdez-Ojeda *et al.* 2014).

In the present study, different accessions related to *M. balbisiana* were used to assess genetic relationship among B genome contained accessions and to identify some sequences of SRAP bands associated with B genome. SRAP primers were amplified successfully with all tested accessions (Fig. 1). The total number of generated bands was 74, of which 65 bands (87.84%) were polymorphic among the tested accessions. The number of amplified bands per primer ranged from 13 to 19 with an average of 14.8 bands. The highest percentage of polymorphism (%P) was 100% generated by Me1-Em1, while the lowest was 71.43% generated by Me4-Em1 as presented in Table 3. The high genetic variability found in this study within *M. balbisiana* accessions was in agreement with previous reports using AFLP markers (Ude *et al.* 2002; Wang *et al.* 2006) and the morphological variability (Shepherd 1988; Hari 1989; Sotto and Rabara 2000). In addition, polymorphism information content (PIC) was calculated for each primer in the present study. The averaged PIC for all primers was 0.29 and ranged from 0.20 to 0.37 for Me4-Em1 and Me4-Em8, respectively. Moreover, the averaged diversity index (DI) among the tested accessions was 0.73 and ranged from 0.50 to 0.83 for Me4-Em1 and Me1-Em1, respectively. The resolving power (R*p*) was calculated as well for each primer, where Me4-Em8 showed the highest R*p* (11.08) while Me4-Em1 showed the lowest R*p* (3.85) with an average of 6.52 for all primers as shown in Table 3.

Genetic relationship among M. balbisiana related accessions

SRAP showed its efficiency in revealing the genetic relationship among *M. balbisiana* related accessions. Cluster analysis based on UPGMA and Jaccard's coefficient was performed, the dendrogram showed a clear relationship among the tested accessions as presented in figure 2. In this regard, *M. acuminata* accession (CIRAD) was out of group and placed in a separated branch at similarity value of 0.36. While the other 12 accessions, which contain B genome, were divided into two main clusters. Interestingly SRAP separated the diploid accessions (BB) into two groups, the first main cluster gathered BB-CICY, Tani, Cameron and Singapuri with the triploid cultivar (BBB) "Lep Chang Kut" indicating their clear relatedness. While the other diploid accessions (i.e. Butuhan, Pisang batu and BB-545) were separated in the second main cluster (sub-cluster II-A) and assembled near to sub-cluster II-B, which contained *acuminata* × *balbisiana* hybrids (i.e. Kunnan, FHIA-01, Prata ana and Orishele). These findings are in agreement with the previous studies of Youssef *et al.* (2011) and Valdez-Ojeda *et al.* (2014). However, unlike our findings Pisang Batu and BB-CICY have been clustered together using fluorescent SRAP markers (Valdez-Ojeda *et al.* 2014). In addition, Pisang Batu has a parthenocarpic fruit and is a relative weak plant susceptible to Sigatoka, while BB-CICY is a fertile seedy and vigorous plant; these morphological differences between these accessions supported our findings that they are very divergent.

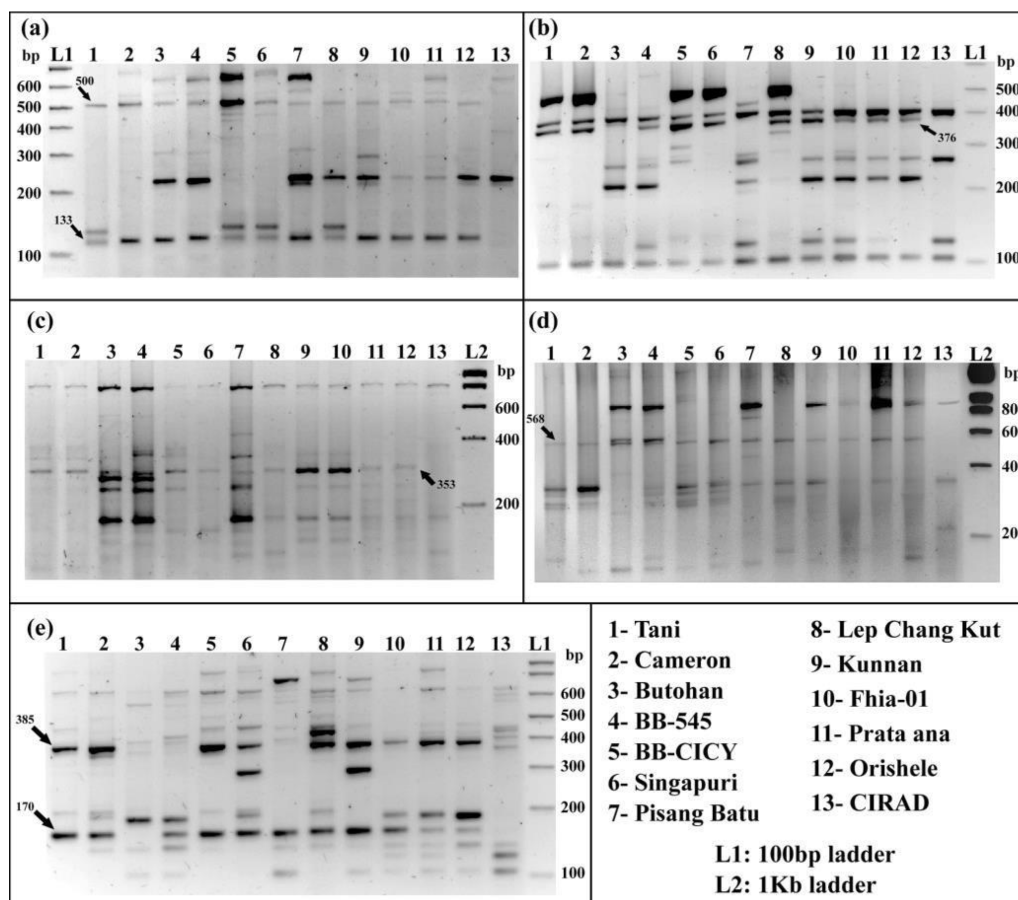

Figure 1. SRAP profiles generated by primers: (a) Me1-Em, (b) Me2-Em1, (c) Me4-Em1, (d) Me8-Em3 and (d) Me4-Em8. Arrows show specific bands for B genome contained accessions.

Table 3. Survey of the percentage of polymorphism, polymorphism information content, primer resolving power and diversity index of the used SRAP primers.

Primer	TNB	NPB	%P	PIC	R*p*	DI
Me1-Em1	13	13	100.00	0.34	6.31	0.83
Me2-Em1	14	12	85.71	0.30	6.15	0.75
Me4-Em1	14	10	71.43	0.20	3.85	0.50
Me8-Em3	14	13	92.86	0.26	5.23	0.79
Me4-Em8	19	17	89.47	0.37	11.08	0.77
Total	74	65	87.84	0.29	6.52	0.73

TNB: total number of bands, NPB: number of polymorphic bands, %P: percentage of polymorphism, PIC: polymorphism information content, R*p*: resolving power, DI: diversity index.

Genetic similarities (GS) among the tested accessions were calculated using Jaccard's coefficient as shown in Table 4. The *M. acuminata* accession "CIRAD" showed low GS (0.24 – 0.37) with most of the tested accessions; however it showed moderate GS (0.43) with both BB-545 and Prata ana (Table 4). Within *M. balbisiana* diploid accessions, the highest GS (0.69) was between Tani and both Cameron and BB-CICY, while the lowest (0.33) was between Tani and Butuhan. Additionally, the highest GS showed by the triploid cultivar "Lep Chang Kut" was 0.65 with both Tani and BB-CICY, while it showed lower GS (0.36) with Butuhan followed by 0.44 with Pisang batu. The diploid hybrid AB "Kunnan" used in this study showed highest GS (0.72) with FHIA-01 followed by 0.67 with Prata ana, while it showed lower GS (0.49) with BB-CICY. In addition, the two plantain accessions (Prata ana and Orishele) showed the highest GS (0.74) within the tested accessions (Table 4).

SRAP specific bands for B genome

The results of this investigation confirmed the previous finding of Youssef *et al.* (2011), which indicated that the specific bands were amplified with all B genome contained accessions used in the present study with some exceptions. For instance, as it was expected, the accession CIRAD (AA) failed to generate any of the specific bands for B genome. In addition, among the twelve *M. balbisiana* related accessions, three showed absence of some of the specific bands (i.e. Butuhan, BB-545 and Pisang Batu) as shown in Table 5. Regarding these accessions, in a previous study on genetic diversity in a set of *Musa* accessions using Ecotilling, Pisang Batu possessed a nucleotide polymorphism pattern of AA (*acuminata*) type form

(Till *et al.* 2010). In addition, Pisang Batu was reported as a mislabeled accession (Valdez-Ojeda *et al.* 2014), that it could be either a triploid plant of genome constitution AAB, or a diploid plant which may possess recombinant chromosomes with genome constitution A^bA or B^aB (B genome alleles superscripts indicate the possession of the A genome as background, and vice versa). In the present study, both BB-545 and Butuhan showed the same pattern of Pisang Batu in the absence of some specific bands. These accessions might have the same genome constitution of Pisang Batu or they have mutation in the binding sites of specific band generated SRAP primers. On the other hand, the fertile diploid accession BB-CICY was suggested that it probably has a B^aB constitution, as hypothesized by backcrossing involving primary AB or BA hybrids (Valdez-Ojeda *et al.* 2014). However, in this study BB-CICY was able to amplify all B specific bands and showed high genetic similarity with other diploid (BB) and triploid (BBB) accessions.

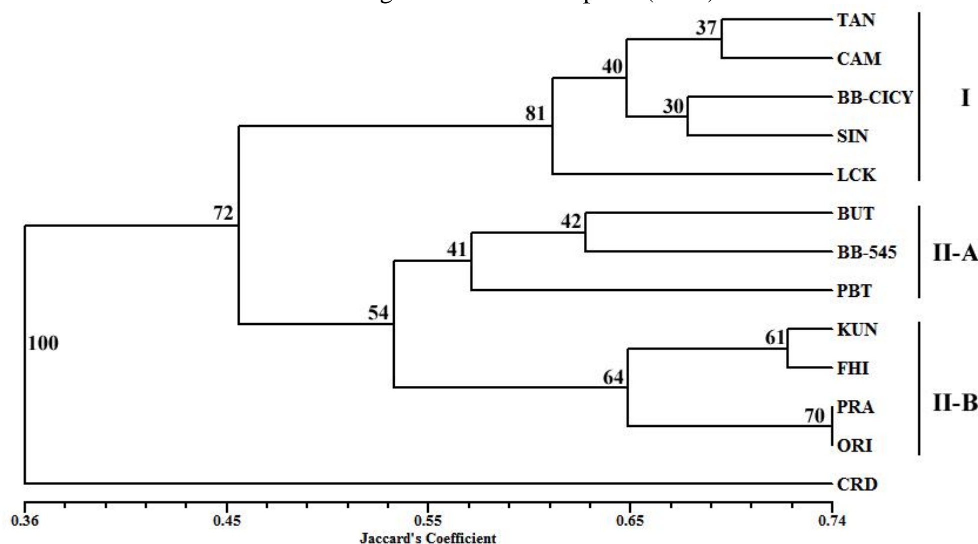

Figure 2. Dendrogram of the tested accessions using UPGMA cluster analysis based on SRAP data with Jaccard's coefficient, numbers over branches indicate the bootstrapping.

Table 4. Genetic similarities among the tested accessions generated by Jaccard's coefficient.

Accessions	TAN	CAM	BUT	BB545	BBCICY	SIN	PBT	LCK	KUN	FHI	PRA	ORI
CAM	0.69											
BUT	0.33	0.41										
B545	0.48	0.48	0.63									
BBCICY	0.69	0.61	0.38	0.49								
SIN	0.64	0.64	0.37	0.44	0.67							
PBT	0.38	0.34	0.52	0.62	0.40	0.35						
LCK	0.65	0.57	0.36	0.54	0.65	0.57	0.44					
KUN	0.55	0.51	0.47	0.60	0.49	0.50	0.57	0.54				
FHI	0.51	0.44	0.47	0.58	0.43	0.43	0.61	0.51	0.72			
PRA	0.46	0.46	0.51	0.55	0.44	0.48	0.51	0.55	0.67	0.68		
ORI	0.53	0.50	0.49	0.56	0.48	0.46	0.49	0.57	0.61	0.62	0.74	
CRD	0.24	0.31	0.41	0.43	0.30	0.27	0.41	0.33	0.36	0.41	0.43	0.37

Table 5. Presence and absence of unique and specific bands among the tested accessions.

Primer	bp	TAN	CAM	BUT	BB-545	BB-CICY	SIN	PBT	LCK	KUN	FHI	PRA	ORI	CRD
Me1-Em1	133	+	+	+	+	+	+	+	+	+	+	+	+	-
Me1-Em1	500	+	+	-	-	+	+	-	+	+	+	+	+	-
Me2-Em1	376	+	+	-	+	+	+	-	+	+	+	+	+	-
M4-Em1	353	+	+	+	+	+	+	+	+	+	+	+	+	-
Me8-Em3	568	+	+	+	+	+	+	+	+	+	+	+	+	-
Me4-Em8	170	+	+	-	+	+	+	+	+	+	+	+	+	-
Me4-Em8	385	+	+	-	-	+	+	-	+	+	+	+	+	-

Identification of specific bands for B genome

Out of the seven specific bands, five bands were sequenced and analyzed using sequencher software; those were M1 (133bp by Me1-Em1), M2 (500bp by Me1-Em1), M3 (376bp by Me2-Em1), M4 (170bp by Me4-Em8) and M5 (385bp by Me4-Em8). The consensus sequence of each band was generated from sequences of four accessions (i.e. Tani, Cameron, BB-CICY and Prata ana) after removing the low quality end regions. The software showed that only three sequences were clean and showed a good quality (i.e. M3, M4 and M5), which were used for NCBI and banana-Hub alignment using BLAST tool. Results showed that no significant alignment was found with NCBI; however banana-Hub BLAST tool gave good alignments with the specific sequences. In this regard, M3 sequence showed 100% identity (296/296) with E-value of e-166 when aligned with Pisang Klutuk Wulung (PKW) genome (BB-chromosome 11), while the same sequence showed lower identity (95%, 282/296) with E-value of e-130 when aligned with double haploid (DH) Pahang genome (AA-chromosome 11) in banana-Hub (Fig. 3). The M4 showed 100% identity with 8e-81 E-value with PKW genome (BB-chromosome 8) and 96% identity (147/152) with E-value of 8e-69 with DH-pahang genome (AA-chromosome 8) (Fig. 4). In addition, the M5 fragment showed 88% identity (273/309) and E-value of 2e-83 when aligned with PKW genome (BB-chromosome 2), while it gave 85% identity (258/302) with E-value of 8e-65 when aligned with DH-pahang genome (AA-chromosome 2) (Fig. 5).

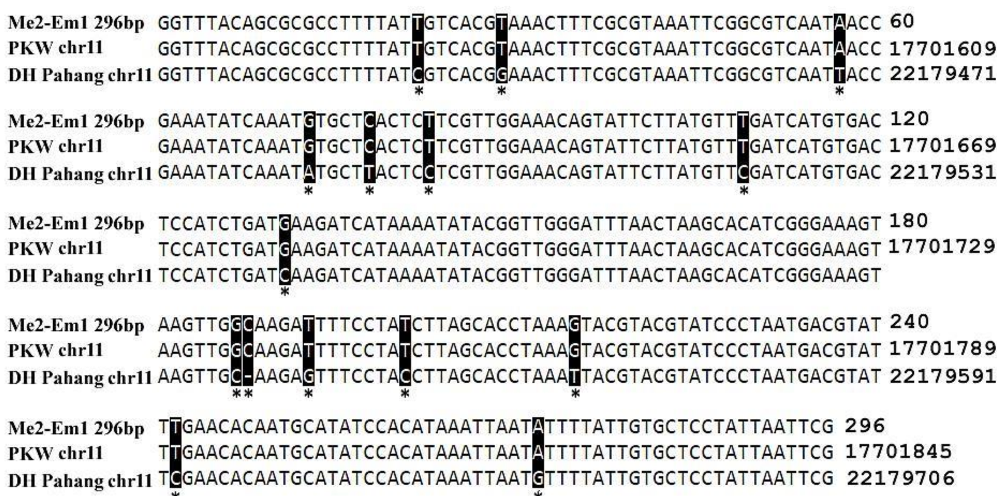

Figure 3. Sequence alignment of 296bp fragment (M3) generated by primer Me2-Em1 with *M. balbisiana* (Pisang Klutuk Wulung, PKW) and *M. acuminata* (Double haploid Pahang) genome sequences based on banana-Hub BLAST tool. Highlighted letters show base mismatch and stars show difference from *acuminata*.

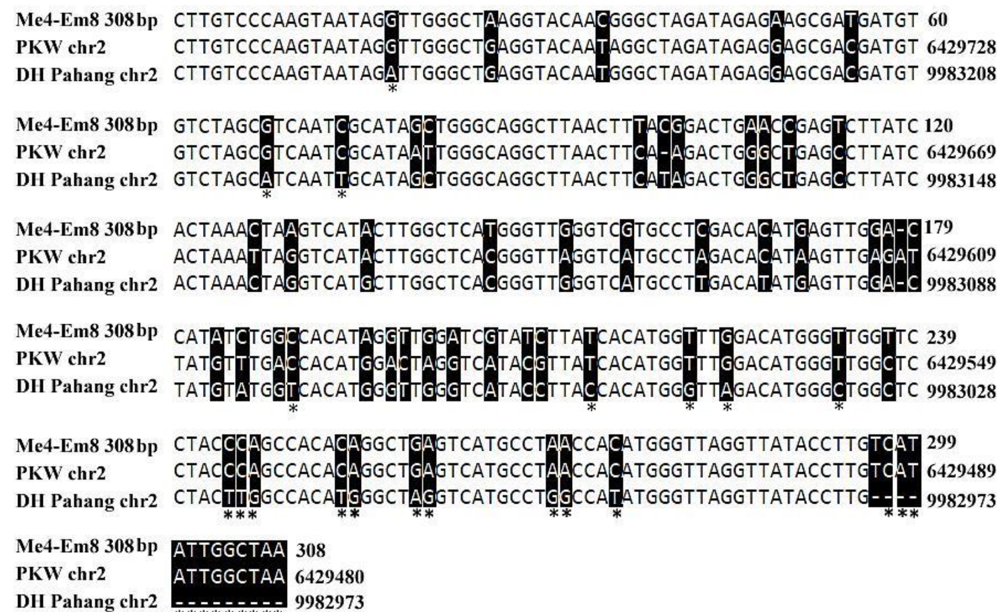

Figure 4. Sequence alignment of 308bp fragment (M4) generated by primer Me4-Em8 with *M. balbisiana* (Pisang Klutuk Wulung, PKW) and *M. acuminata* (Double haploid Pahang) genome sequences based on banana-Hub BLAST tool. Highlighted letters show base mismatch and stars show difference from *acuminata*.

```
Me4-Em8 152bp  CCAAACCAGACCGGCCGGCTTACCTGAGACAGCGTTCTCTACAAAGAGGTACGGATCTTT 60
PKW chr8       CCAAACCAGACCGGCCGGCTTACCTGAGACAGCGTTCTCTACAAAGAGGTACGGATCTTT 3876599
DH Pahang chr8 CCAAAGCAGACCGGCCGGCTTACCTGAGACAACATTCTCTACAAAGAGGTACGGATCTTT 4348457
                    *                         * *

Me4-Em8 152bp  CAGCCCTCCCTCCGGCCGTCGACTGCCTCTCGCCTTAACTTCTTCTTTGGTTCGTGATAG 120
PKW chr8       CAGCCCTCCCTCCGGCCGTCGACTGCCTCTCGCCTTAACTTCTTCTTTGGTTCGTGATAG 3876659
DH Pahang chr8 CAGCCGTCCCTCTGGCCGTCGACTGCCTCTCGCCTTAACTTCTTCTTTGGTTCGTGATAG 4348517
                    *      *

Me4-Em8 152bp  AACCGGTGAGCAATCGTCCTCGCTAATTCGTA 152
PKW chr8       AACCGGTGAGCAATCGTCCTCGCTAATTCGTA 3876691
DH Pahang chr8 AACCGGTGAGCAATCGTCCTCGCTAATTCGTA 4348549
```

Figure 5. Sequence alignment of 152bp fragment (M5) generated by primer Me4-Em8 with *M. balbisiana* (Pisang Klutuk Wulung, PKW) and *M. acuminata* (Double haploid Pahang) genome sequences based on banana-Hub BLAST tool. Highlighted letters show base mismatch and stars show difference from *acuminata*.

For confirmation of sequence specificity, M3 and M5 sequences were used for specific primer design to amplify fragments of 246 and 256bp in size, respectively. Results showed that, the first reaction failed to generate specific fragment. However, the second reaction was successful in generating a specific band of the target size (256bp) which was amplified in all the B-genome contained accessions with exception of Butuhan, BB-545 and Pisang batu. Moreover, for more confirmation, four accessions of *M. acuminata* "AA" (i.e. Calcuta-4, Mal-399, Mal-250 and Mal-CICY) were introduced in this test and none of them was able to amplify the specific band (Fig. 6). These results indicate the efficiency of SRAP markers to expose minimum differences between genotypes. Genetic clarification of this kind of genetic variation could be elucidated by in situ hybridization of A and B pairing (Valdez-Ojeda *et al.* 2014). This may imply that chromosome re-assortments and exchanges of chromosome segments, leading to unbalanced genome transmission with respect to the parental (Jeridi *et al.* 2011). The presence and absence of some of the specific band for B genome in this study was similar to what Nair *et al.* (2005) have found using IRAP markers, thus they suggested mislabeling or misidentification.

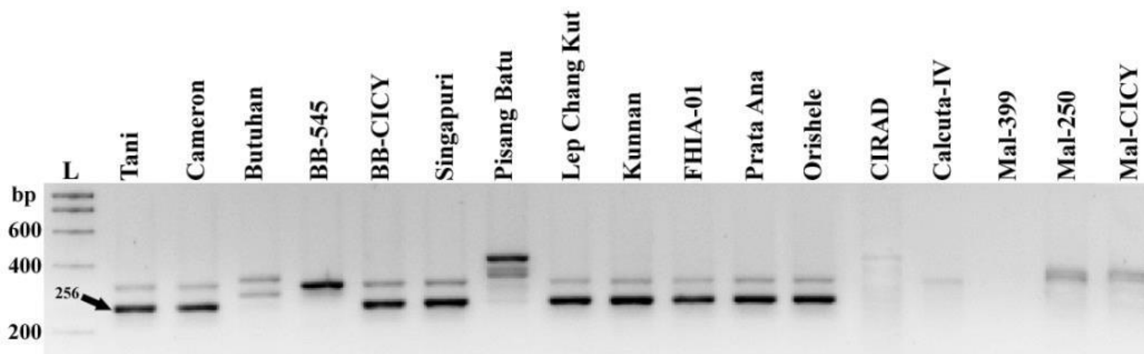

Figure 6. Amplification of a 256bp fragment (arrow) generated by specific designed primers, discriminating among *M. balbisiana* and *M. acuminata* related accessions.

In conclusion, molecular analysis performed in this study confirms the efficiency of SRAP markers in differentiation among *M. balbisiana* related accessions and indicates that SRAP is a suitable marker for genetic relationship and diversity assessment in *Musa* and other higher plants. The identification of the B genome specific bands generated by SRAP provided markers which could be used for identification of banana and plantain cultivars. This information is not only important in *Musa* basic breeding program but also in germplasm management and conservation as well as to authenticate mislabeled accessions.

ACKNOWLEDGMENT

This research work was funded by Secretaria de Agrícultura, Ganadería, Recursos Pesqueros y Alimentos (SAGARPA) and the Consejo Nacional de Ciencia y Tecnología (CONACyT) through the research grant 0048160 (RME). The first author (Dr. Youssef) is the recipient of a Postdoctoral fellowship from CONACyT-TWAS (The World Academy of Science) award # 3240267275. The authors acknowledge José Roberto Ku-Cauich for technical support.

REFERENCES

Ahmad, F.; R. Megia and Y.S. Poerba (2014) Genetic Diversity of *Musa balbisiana* Colla in Indonesia Based on AFLP Marker. HAYATI Journal of Biosciences, 21:(1) 39-47.

Crouch, J. H.; H. K. Crouch; H. Constandt; A. Van Gysel; P. Breyne M. Van Montagu; R.L. Jarret and R. Ortiz (1999). Comparison of PCR based molecular marker analyses of *Musa* breeding populations. Molecular Breeding, 5: 233–244.

D'Hont, A.; F. Denoeud; J.M. Aury *et al.* (2012) The banana (*Musa acuminata*) genome and the evolution of monocotyledonous plants. Nature, 488:213–21.

Davey, M.W.; R. Gudimella; J.A. Harikrishna; L.W. Sin; N. Khalid and J. Keulemans (2013) A draft *Musa balbisiana* genome sequence for molecular genetics in ploidy, inter- and intra- specific *Musa* hybrids. BMC Genomics, 14:683. http://dx.doi.org/10.1186/1471-2164-14-683.

El-Khishin, D. A.; E.L. Belatus; A. Abd El-Hamid and K.H. Radwan (2009) Molecular charactarization of banana cultivars (*Musa* spp.) from Egypt using AFLP. Research Journal of Agriculture and Biological Sciences, 5: 272–279.

FAOSTAT (2015) Food and Agriculture Organization of the United Nations. http://faostat.fao.org.

Ferreira, C. F.; S.O. Silva; N.P.D. Sobrinho; S.C.S. Damascena; F.S.A.O. Alves and O. Da Pereira Paz (2004). Molecular characterization of banana (AA) diploids with contrasting levels of black and yellow sigatoka resistance. American Journal of Applied Sciences, 1: 276–278.

Häkkinen, M. (2013) Reappraisal of sectional taxonomy in *Musa* (Musaceae). Taxon, 62 (4): 809–813.

Hampl, V.; A. Pavlícek and J. Flegr (2001) Construction and bootstrap analysis of DNA fingerprinting-based phylogenetic trees with the freeware program FreeTree: application to trichomonad parasites. Int J Syst Evol Micr, 51:731–735.

Hari, P.C. (1989) Morphological study of *Musa acuminata* ssp. *malaccensis* (Ridley) Simmonds to determine taxonomic characteristics. Saussurea, 19:187–217.

Hippolyte, I.; C. Jenny; L. Gardes; F. Bakry; R. Rivallan; V. Pomies; P. Cubry; K. Tomekpe; A. M. Risterucci; N. Roux; M. Rouard; E. Arnaud; M. Kolesnikova-Allen and X. Perrier (2012) Foundation characteristics of edible *Musa* triploids revealed from allelic distribution of SSR markers. Ann Bot-London, 109:937–951.

Jaccard, P. (1908) Nouvelles recherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat., 44: 223–270.

Jeridi, M.; F. Bakry; J. Escoute; E. Fondi; F. Carreel; A. Ferchichi; A. D'Hont and M. Rodier-Goud (2011) Homoeologous chromosome pairing between the A and B genomes of *Musa* spp. revealed by genomic *in situ* hybridization. Ann Bot-London, 108:975–981.

Li, G. and C.F. Quiros (2001) Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: Its application to mapping and gene tagging in Brassica. Theoretical and Applied Genetics, 103: 455–461.

Nair, A.S.; C.H. Teo; T. Schwarzacher and P.H. Harrison (2005) Genome classification of banana cultivars from South India using IRAP markers. Euphytica, 144: 285–290.

Nei, M. (1987) Molecular Evolutionary Genetics. Columbia University Press, New York.

Nwakanma, D.C.; M. Pillay and B.E. Okoli (2003) PCR-RFLP of the ribosomal DNA internal transcribed spacers (ITS) provides markers for the A and B genomes in *Musa* L. Theor App Genet, 108:154–159.

Phothipan, S.; B. Silayoi; K. Wanichkul and S. Apisitwanich (2005) Genetic relationship among banana in AA, AAB and B groups using random amplified polymorphic DNA (RAPD) and sequence related amplified polymorphism (SRAP) techniques. Kasetsart J. Nat. Sci. 39: 703–710.

Pinar, H.; A. Uzun; M. Unlu; M. Bircan; O. Gulsen; C. Yilmaz and H. Gubbuk (2015) Identification of Banana Accessions Sampled From Subtropical Region of Turkey Using SRAP Markers. Bulgarian Journal of Agricultural Science, 21 :(2) 270–276.

Poerba, Y. and A. Fajarudin (2010) Genetic variability among 18 cultivars of cooking bananas and plantains by RAPD and ISSR markers. Biodiversitas, 11:118–123.

Prevost, A. and M.J. Wilkinson (1999) A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor Appl Genet, 98:107-112.

Risterucci, A. M.; I. Hippolyte; X. Perrier; L. Xia; V. Caig; M. Evers; E. Huttner; A. Kilian; J.C.Glaszmann (2009) Development and assessment of diversity arrays technology for high-throughput DNA analyses in *Musa*. Theoretical and Applied Genetics, 119: 1093–1103. doi: 10.1007/s00122-009-1111-5.

Roldan-Ruiz, I.; J. Dendauw; E. VanBockstaele; A. Depicker and M. De Loose (2000) AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). Mol Breed, 6: 125–134.

Shepherd, K. (1988) Observation on *Musa* taxonomy. In: Identification of genetic diversity in the genus *Musa*. Proc Int Workshop held at Los Banos, Philippines 5–10 September 1988. INIBAP, Montpellier, France, pp 158–165.

Sotto, R.C. and R.C. Rabara (2000) Morphological diversity of *Musa balbisiana* Colla in the Philippines. Info Musa, 9:28–30.

Stulnig, T.M. and A. Amberger (1994) Exposing contaminating phenol in nucleic acid preparations. BioTechniques, 16: 403–404.

Teo, C.H.; S.H. Tan; C.H. Ho; Q.Z. Faridah; Y.R. Othman; J.S. Heslop-Harrison; R. Kalendar and A.H. Schulman (2005) Genome constitution and classification using retrotransposon based markers in the orphan crop banana. J Plant Biol, 48: 96–105.

Till, B.; J. Jankowicz-Cieslak; L. Sági; O.A. Huynh; H. Utsushi; R. Swennen; R. Terauchi; C. Mba (2010) Discovery of nucleotide polymorphisms in the *Musa* gene pool by Ecotilling. Theor Appl Genet, 121:1381–1389.

Ude, G.; M. Pillay; A. Nwakanma and A. Tenkouano (2002) Analysis of genetic diversity and selectional relationships in *Musa* using AFLP markers. Theor Appl Genet, 104: 1239–1245.

Valdez-Ojeda R.; A. James-Kay; J. Ku-Cauich and R.M. Escobedo-Gracia Medrano (2014) Genetic relationships among a collection of *Musa* germplasm by fluorescent-labeled SRAP. Tree Genetics and Genomes, 10: 465-476. DOI: 10.1007/s11295-013-0694-9.

Wang, X.; T. Chiang; N. Roux; G. Hao and X. Ge (2006) Genetic diversity of wild banana (*Musa balbisiana* Colla) in China as revealed by AFLP markers. Genet Resour Crop Evol, 54: 1125-1132.

Youssef, M. (2012) Khirshyat 1.0: a simple micro-program for some molecular biology protocols. Genes, genomes and genomics, 6:102-105.

Youssef, M.; A. James; R. Rivera-Madrid; R. Ortiz and R.M. Escobedo-Gracia Medrano (2011) *Musa* genetic diversity revealed by SRAP and AFLP. Mol Biotech, 47:189–199.

Youssef, M.; R. Valdez-Ojeda; J.R. Ku-Cauich and R.M. Escobedo-GraciaMedrano (2015) Enhanced Protocol for Isolation of Plant Genomic DNA. Journal of Agriculture and Environmental Sciences, 4:(2) 172-180. DOI: 10.15640/jaes.v4n2a20.

العلاقة الوراثية بين تراكيب وراثية لموز *Musa balbisiana* وتعريف الواسمات الجزيئية SRAP المرتبطة بجينوم B في الموز

محمد أحمد الملقب بالخرشي محمد يوسف[١] و روزا ماريا إيسكوبيدوجراسياميدرانو[٢]

١- قسم الوراثة – كلية الزراعة – جامعة أسيوط – مصر

٢- وحدة الكمياء الحيوية والبيولوجيا الجزيئية للنبات – مركز يوكاتان للبحوث العلمية – ميريدا – المكسيك

تم في هذه الدراسة استخدام الواسم الجزيئي SRAP لتقدير درجة العلاقة الوراثية بين مجموعة تراكيب وراثية لموز *M. balbisiana*، واشتملت هذه التراكيب على طرز برية ثنائية المجموعة الكروموسومية و صنف ثلاثي المجموعة الكروموسومية و بلانتين (هجين ثلاثي) و هجن ثنائية ورباعية. نتج من التحليل الجزيئي وجود ٧٤ حزمة كان من بينها ٦٥ حزمة (بنسبة ٨٧.٨٤%) متعددة الأشكال بين التراكيب المختبرة، وبلغ متوسط المحتوى المعلوماتي لتعدد الأشكال لكل البادئات المستخدمة قيمة ٠.٢٩، بينما كان متوسط دليل التنوع ٠.٧٣. وأظهرت النتائج كفاءة الواسم الجزيئي SRAP في تجميع التراكيب الوراثية المختبرة وإظهار أقل فروق بينها. كما قسَّم الواسم الجزيئي الطرز البرية الثنائية الى مجموعتين، حيث شملت المجموعة الأولى الطرز BB-CICY و Tani و Cameron و Singapuri وتجمعت مع الصنف ثلاثي المجموعة الكروموسومية "Lep Chang Kut" بينما ضمت المجموعة الثانية باقي الطرز البرية الثنائية (Butuhan و Pisang Batu و BB-545) وتجمعت بجوار الهجن. تم تدعيم هذه النتائج بتعريف وتحديد تتابع بعض الحزم الخاصة التي تم تخليقها بواسطة SRAP، والتي ظهرت في جميع التراكيب المحتوية على جينوم B باستثناء Butuhan و Pisang Batu و BB-545. ويقترح أن تكون هذه التراكيب محتوية على كروموسومات معادة الاتحاد من جينومي A و B، أو أنها تم تعريفها بشكل خاطئ (mislabeled). ولقد ساهم تعريف الحزم الخاصة بجينوم B في هذا البحث في تقديم واسمات جزيئية مرتبطة بكروموسومات ٢ و ٨ و ١١ في جينوم الموز والتي يمكن استخدامها في التمييز بين أصناف الموز التي تحتوي على جينوم A أو B. بالاضافة لذلك، فقد تم تصميم بادئات متخصصة من تتابعات بعض هذه الحزم و تم الحصول على شظية بطول ٢٥٦ زوج من القواعد، حيث باستخدامها تم التفرقة بين طرز *acuminata* وطرز *balbisiana*. وبذلك يمكن الاستعانة بالمعلومات المتحصل عليها في هذا البحث في برامج تربية الموز والتأكد من الطرز الوراثية التي سبق تعريفها بشكل خاطئ فضلاً عن حفظ وتداول الأصول الوراثية للموز.

الكلمات المفتاحية: الموز – SRAP – تحديد تتابع النيوكليوتيدات – الواسمات الجزيئية المتخصصة – جينوم B.