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Assessment of genetic diversity among Thai banana cultivars (*Musa* spp.) based on RAPD and SRAP markers

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Abstract

This study was conducted to assess the genetic diversity and relationship of 19Thai *Musa* accessions of four (ABB, AAA, AA, and BB) genomes based on Random Amplified Polymorphic DNA (RAPD) and Sequence Related Amplified Polymorphism (SRAP) markers. The results revealed that thirteen RAPD and eight SRAP markers generated a total of 129 alleles (calculated from 1005 amplified fragments), with sizes ranging from 100 to 2100 bp long. Moreover, both markers provided high polymorphism with average PIC value of 0.75, which indicated a high level of genetic diversity among studied *Musa* accessions. The UPGMA approach based grouping showed two distinct clusters, which were cluster-I comprised of ABB, AA, and BB genomes and cluster-II consisted of only the AAA genome. Additionally, its analysis demonstrated high genetic relationship within the ABB genome, compared to other (AAA, AA, and BB) genomes. This study also confirmed that both RAPD and SRAP markers were proficient tools for rapidly accessing the genetic diversity within all the banana genomic groups.

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Introduction

Banana (*Musa* spp.) is the fourth world's most important food crop next to rice, wheat, and maize. They belong to the order Zingiberales, family Musaceae, and originate in Papua New Guinea and the Pacific Islands (Ploetz *et al.*, 2007; Singh *et al.*, 2014; Kiran*et al.*, 2010). Moreover, banana trends to be in high demand in global markets, expected to reach 18.1 million tons in 2017 (FAO, 2017).The edible and plantain banana cultivars resulted from hybridization of two wild cultivars of *M. acuminata* (AA genome) and *M. balbisiana* (BB genome). These cultivars are mostly seedless and classified into various polyploidy levels, according to their genome component; diploid (AA and BB), triploid (AAA, AAB, ABB, and BBB) and tetraploids(AAAA, AAAB, AABB, and ABBB) (Kiran *et al.*, 2015).Of these genomes, triploid genomes are widely cultivated and played a significant role in world economy and food security in the developing countries, especially in South East Asia, East and Central West Africa (Poerba and Ahmad, 2010; Opara *et al.*, 2010; Singh *et al.*, 2014).

Thailand is the third largest producer of bananas (especially ABB genome) in South East Asia and among the leading in the world. Most Thai banana products were used in its local markets and food industries. Some banana cultivars within ABB genome (such as Kluai Nam Wa) are suitably processed into various food products such as, banana chips, banana cookies and solar-dried banana (Ruangsuttapha *et al.,* 2007; Onguso *et al.,* 2012; Suvittawat *et al.,* 2014; Rotchanapreeda *et al.,* 2016). There were many cultivars within the ABB genome which have few morphological differences but significant genetic variations. However, there is still minimum genetic information among ABB genome, and this remains a challenge to be further studied.

One of the most powerful techniques which can be used for such studies is the molecular marker techniques. These techniques were vastly applied by various researchers for the same purpose of genetic characterization and can be employed to assess the genetic diversity among the banana cultivars. The essential molecular marker techniques applied were Polymerase Chain Reaction (PCR)-based such as; Simple Sequence Repeat (SSR) (Valquíria *et al.,* 2012; Rotchanapreeda *et al.*, 2016),Amplified Fragment Length Polymorphism (AFLP) (Wong *et al.,* 2001; Opara *et al.,*2010), Restriction Fragment Length Polymorphism (RFLP) (Ning *et al.,* 2007), Random Amplified Polymorphic DNA (RAPD) (Nsabimana *et al.*, 2007;Brown *et al.,*2009; Santos *et al.,* 2009; Želmíra *et al.,* 2016) and Sequence Related Amplified Polymorphism (SRAP) markers (Esposito *et al.*, 2007; Abdelhamid *et al.,* 2017; Bhatt *et al.,* 2017). Of these markers, the RAPD and the SRAP markers have been efficiently and successfully applied for identifying DNA polymorphism in various banana cultivars (Nsabimana and Staden, 2007; Masoud *et al.,*2008; Poerba *et al.,* 2010; Onguso *et al.,* 2004; Abdelhamid *et al.,* 2017;Bhatt *et al.,* 2017). Both markers can generate high polymorphism and were able to provide ample information to precisely establish the genetic diversity between and among banana cultivars (Phothipan *et al.,* 2005; *et al.,* 2006; Pankaj*et al.,* 2007; Brown *et al.,* 2009; Abdelhamid *et al.,* 2017).

Since *Musa* cultivars of the ABB genome are the most important group used in food industries in Phitsanulok province of Thailand; they have to be identified based on their genetic diversity in this province. Most studies and publications previously done on Thai banana genetic diversity were conducted outside Phitsanulok province (Phothipan *et al.,* 2005; Ruangsuttapha *et al.,* 2007). Therefore, the objective of the study was to assess the extent of genetic diversity among banana cultivars within ABB genome and other genomes using RAPD and SRAP markers.

Materials and methods

Nineteen Thai *Musa* accessions, consisting of four different (14 ABB, 2 AAA, 1 AA and2 BB) genomes (Table 1), were used in this study. Young fresh cigar leaves were harvested from banana plants at Phitsanulok Agricultural Extension and Development Center, Thailand, and stored in -20˚Cfor further genomic DNA extraction.

Genomic DNA isolation and quantification

Individual young banana leaf sample (approximately 2g) was ground to fine powder in liquid nitrogen and applied for isolating genomic DNA (gDNA) using the Innu PREP Plant DNA KIT (Analytik Jena, Germany) according to the manufacturer's instruction.

The gDNA was quantified and qualified by a UV-spectrophotometer at $OD_{260}$and OD_{280}nm (Microplate reader, synergy H1 BioTech, USA). Its integrity was examined by 1.0% (w/v) agarose gel electrophoresis, dissolved in 1X TAE and stained with0.5µg/ml ethidium bromide (EtBr) (Invitrogen™ UltraPure™, USA). The gel image was visualized by the gel documentation system (Thermo Fisher Scientific, USA). The gDNA was stored at -20˚C.

RAPD and SRAP-PCR amplification

Standard PCR with the OnePCR master mix (containing *Taq* DNA polymerase, PCR Buffer, dNTPs, gel loading dyes, and fluorescence dye) was applied to fingerprint 19banana cultivars according to the manufacturer's instruction (GeneDireX, USA). The PCR amplifications were performed by using sixty-three RAPD universal markers (10-mer oligonucleotide primers) (Operon Technologies Inc., Alameda, USA) (Table 2) and 1µl of gDNA template (25ng/µl).

The RAPD-PCR condition was performed with the initial denaturation at 95˚C for 5 minutes, followed by 35 cycles of 95˚C for 30 seconds, 25˚Cfor 30seconds, and 72˚Cfor 1 minute, and the final extension at 72˚C for 5 minutes in a thermal cycler (BIO-RAD T100™, USA).

Similarly, the PCR amplifications were performed by using eight SRAP marker pairs (Table 2) (Li and Quiros, 2001) and 1.0µl of gDNA template **(**25ng**/**µl**).** The SRAP-PCR condition was performed with the initial denaturation cycle of94˚C for 1 minute, and 5 cycles of 94˚Cfor 1 minute, 35˚C for 1 minute and 72˚C for 1 minute and followed by 35 cycles of 94˚C for 1 minute, 50˚C for 1 minute, 72˚C for 1 minute and final extension 72˚C for 5minutes.

Both RAPD- and SRAP-PCR amplicons were separated by 1.5% (w/v) agarose gel electrophoresis, dissolved in 1X TAE buffer, stained with 0.5µg/ml EtBr, and visualized under the gel documentation system (Thermo Fisher Scientific).

Data analysis

The PCR-amplicons generated by both RAPD and SRAP markers were considered as independent putative alleles. Distinct and clear bands were selected and scored for presence (1) or absence (0) of DNA fragments. A dendrogram based on hierarchical clusters was analyzed by the UPGMA (Unweight Pair Group Method with Arithmetic mean) approach using FreeTree and TreeView programversion1.6.6. Moreover, the scored data (1 or 0) were calculated for Polymorphic Information Content (PIC) value by using the formula $PIC = 1 - \Sigma p_i^2$, Where p_i is the frequency of the i^{th} allele for individual p, applied on excel spreadsheet as provided by CIMMYT Applied Biotechnology for genetic diversity research (Crossa and Warburton, 2002).

Results and discussion

RAPD and SRAP profiles

Sixty-three RAPD and eight SRAP markers were initially used to screen 19 *Musa* accessions (Table 1). Only 13 out of the 63 RAPD (approximately 21%) and 8 SRAP markers generated good quality PCR amplicons, and reproducible DNA-fingerprinting profiles with high consistency. Two randomly representatives of the RAPD markers (OPK07 and OPD08) (Figure 1) and two randomly representatives of SRAP markers (Me1/Em6 and Me1/Em8) (Figure 2) were the best informative markers with clear and distinct bands. A total of129 alleles (calculated from total 1005 amplified fragments) were generated by the RAPD and SRAP markers, out of which 79alleles (532 bands) were generated by RAPD markers and 50 alleles (473 bands) by SRAP markers with average number of bands of 40.92 and 59.13as generated by RAPD and SRAP, respectively (Table 2). Of these markers, OPD07 and OPK08 generated the highest number of alleles (9 alleles), and OPA05 generated the lowest number of alleles (2 alleles). All the alleles

generated 100% polymorphic fragments. Moreover, these PCR-amplicon sizes were ranged from 250 to 2100 bp as generated by RAPD markers (Table 2) and100 to 1700 bp generated by SRAP markers(Table 2). The RAPD and SRAP-marker amplified with a range from 2 to 9 alleles (an average 6.08 alleles per marker), and from 4 to 7 alleles (an average of 6.25 alleles per marker), respectively.

Table 1. Lists of *Musa* accessions used in this study.

No	Local name	Genome	Cultivar
1	Kluai Nam WaTha Yang	ABB	*Musa x paradisiaca*
2	Kluai Nam WaKabKhiao	ABB	*Musa x paradisiaca*
3	Kluai Nam Wa Mali-Ong	ABB	*Musa x paradisiaca*
4	KluaiHakMuk Thong	ABB	*Musa x paradisiaca*
5	KluaiHakMukNuan	ABB	*Musa x paradisiaca*
6	Kluai Nam Wa Dam	ABB	*Musa x paradisiaca*
7	Kluai Nam WaNgern	ABB	*Musa x paradisiaca*
8	Kluai Nam WaNuan Jan	ABB	*Musa x paradisiaca*
9	Kluai Nam WaKhiao	ABB	*Musa x paradisiaca*
10	Kluai Nam Wa U-bon	ABB	*Musa x paradisiaca*
11	Kluai Nam WaPhrarachthan	ABB	*Musa x paradisiaca*
12	Kluai Nom Mi	ABB	*Musa x paradisiaca*
13	KluaiHin	ABB	*Musa x paradisiaca*
14	Kluai Nam Wa Pak Chong 50	ABB	*Musa x paradisiaca*
15	KluaiHom Thong	AAA	*Musa x paradisiaca*
16	KluaiHomKhiao	AAA	*Musa x paradisiaca*
17	Kluai Nam Thai	AA	*Musa acuminata*
18	KluaiTani Dam	BB	*Musa balbisiana*
19	KluaiTani	BB	*Musa balbisiana*

Note: *Musa* × *paradisiaca* indicates hybrid cultivars used for cooking and dessert bananas.

These resultsindicated that both markers were able to generate an almost similar average number of alleles per marker, which agreed with a report from Ohzan *et al.,*2016,who found that the DNA extracted from *Cyclamen* were successfully amplified by using RAPD and SRAP markers, with a relatively similar average number of alleles per marker.

RAPD and SRAP polymorphism

The polymorphic information content (PIC) value was calculated among 19 *Musa* accessions using scored data from both molecular markers. The lowest and the highest PIC value was found in RAPD markers, which ranged from 0.44 (as observed in the OPK12) to 0.87 (as observed in the OPA05) with anaverageof0.74 (Table 2).

The SRAP markers exhibited moderately high PIC value among the cultivars which ranged from 0.63 (as observed in the Me2/Em1) to 0.84 (as seen in the Me1/Em4 markers) with an average of0.76 (Table 2).

This PIC value result indicated that both RAPD and SRAP markers were powerful tools in revealing genetic polymorphism in the banana accessions as reported earlier (Phothipan*et al.,* 2005; Pankaj *et al.,* 2007; Abdelhamid *et al.,* 2017; Dnyaneshwar*et al.,* 2006; Brown *et al.,* 2009).

The average PIC value generated by SRAP is comparably higher than RAPD indicating its efficiency for revealing genetic polymorphism in the banana accessions because it characteristically tends to be more specific than RAPD (Phothipan *et al.,* 2005; Bhatt *et al.,* 2017).

The SRAP markers effectiveness for detecting genetic diversity was also reported in *Ocimum*(Patel *et al.,* 2015) and *Cyclamen* (Ohzan *et al.,* 2016). However both markers were commended for high efficiency in generating high polymorphism in assessing genetic diversity in Banana cultivars.

Cluster analysis

The phylogenetic tree analysis among 19 *Musa* accessions was calculated by the UPGMA approach resulting in two significant clusters (I and II) (Fig. 3). Cluster-I comprised of all ABB, AA, and BB genomes,

while cluster-II consisted of only AAA genome. Additionally, this analysis demonstrated that there was high genetic relationship within the ABB genome, compared to AAA, AA and BB genomes. In cluster-I, the result was able to separate two sub-clusters, I-a and I-b distinctly.

Table 2. Features of RAPD- and SRAP- PCR amp icons used for assessing genetic diversity of the nineteen *Musa* accessions.

No	NM	Sequences (5´-3´)	NA	NB	PIC	PPA (%)	AS
1	OPA01	CAGGCCCTTC	4	69	0.79	100	900-2000
2	OPA02	TGCCGAGCTG	7	53	0.80	100	400-1800
3	OPA05	AGGGGTCTTG	2	63	0.87	100	850-1000
4	OPD07	TTGGCACGGG	9	54	0.83	100	500-1400
5	OPD08	GTGTGCCCCA	8	32	0.75	100	350-1400
6	OPE01	CCCAAGGTCC	6	33	0.55	100	600-2000
7	OPE16	GGTGACTGTG	5	54	0.83	100	600-1400
8	OPK01	CATTCGAGCC	5	56	0.82	100	1200-2100
9	OPK04	CCGCCCAAAC	6	29	0.72	100	250-1500
10	OPK07	AGCGAGCAAG	8	20	0.68	100	300-1300
11	OPK08	GAACACTGGG	9	27	0.74	100	300-1200
12	OPK11	AATGCCCCAG	6	30	0.85	100	520-1800
13	OPK12	TGGCCCTCAC	4	12	0.44	100	750-900
	Total		79.00	532	-	-	-
	Average		6.08	40.92	0.74	-	-
1	Me1	TGAGTCCAAACCGGATA	7	74	0.84	100	250-1400
	Em4	GACTGCGTACGAATTTGA					
2	Me1	TGAGTCCAAACCGGATA	6	47	0.74	100	250-1000
	Em6	GACTGCGTACGAATTGCA					
3	Me1	TGAGTCCAAACCGGATA	7	48	0.73	100	150-1000
	Em8	GACTGCGTACGAATTCTG					
4	Me2	TGAGTCCAAACCGGAGC	4	39	0.63	100	250-500
	Em1	GACTGCGTACGAATTAAT					
5	Me2	TGAGTCCAAACCGGAGC	6	67	0.78	100	200-1000
	Em5	GACTGCGTACGAATTAAC					
6	Me4	TGAGTCCAAACCGGACC	7	64	0.78	100	250-1500
	Em2	GACTGCGTACGAATTTGC					
7	Me1	TGAGTCCAAACCGGAAT	6	52	0.74	100	100-1400
	Em1	GACTGCGTACGAATTAAT					
8	Me3	TGAGTCCAAACCGGACC	7	80	0.83	100	150-1700
	Em8	GACTGCGTACGAATTCTG					
	Total		50	473	-	-	-
	Average		6.25	59.13	0.76	-	-
		Grand total	129	1005	-	-	-
		Cumulative average	-	-	0.75	-	-

Note: No. = Serial number, NM = Name of marker, NA = Number of alleles, NB = Number of bands, PPA = Percentage of polymorphic allele, AS = Amplicon size (bp).

The sub-cluster I-a contained only ABB genome of 14 *Musa* cultivars (KluaiHin, Nam WaNgern, Nam WaKabKhao, Mali Ong, Nam Wa Dam, Nam WaNuanJan, Nam WaPhrarachthan, Nam WaTha Yang, Nam WaKhiao, Nam WaUbon, PakChong50, HakMuk Thong, HakMukNuan, and Nom Mi).

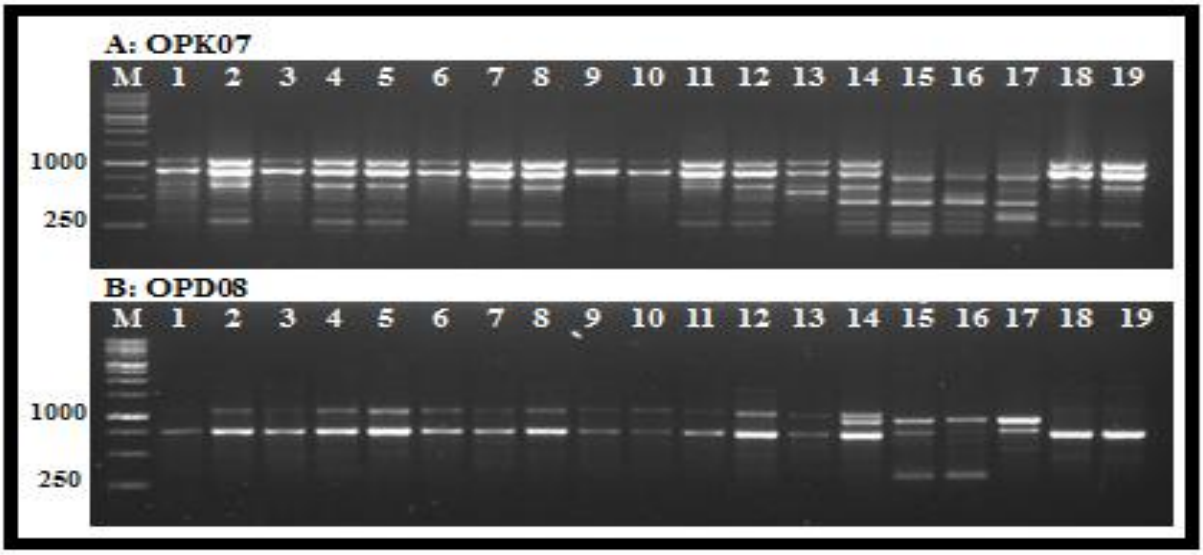

Fig.1.RAPD-PCR amplicons profiles of 19 *Musa* accessions usingOPK07 (A), OPD08 (B) markersNote: lane M indicates1kb DNA RTU Ladder (GeneDireX, USA) and lane 1-19 refers to the lists of *Musa* accessions in Table 1.

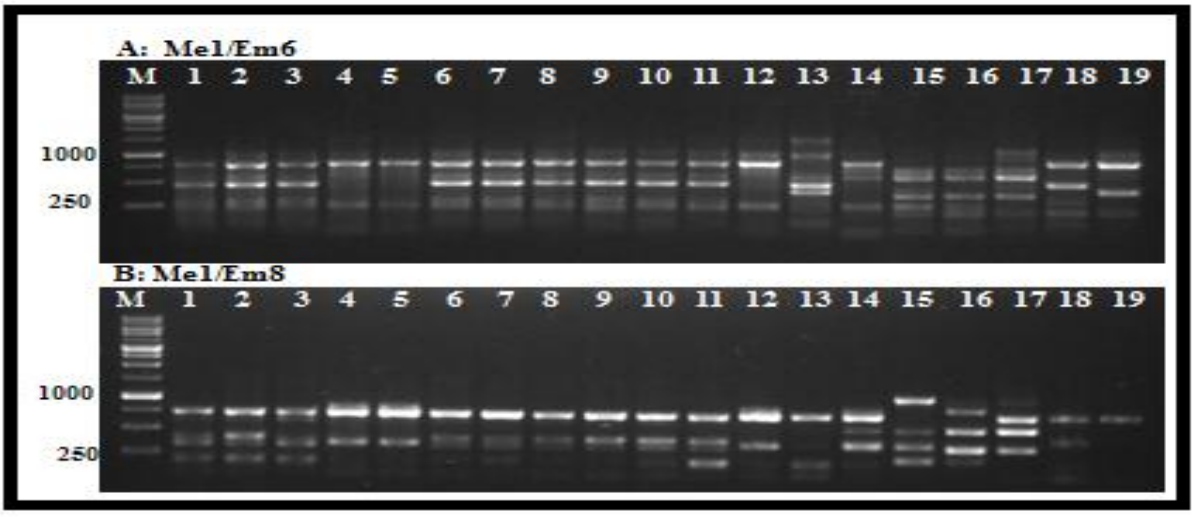

Fig.2. SRAP-PCR amplicons profiles of 19 *Musa* accessions using Me1/Em6 (A) and Me1/Em8 (B) markers
Note: lane M indicates 1kb DNA RTU Ladder (GeneDireX, USA), and lane 1-19 refers to the lists of *Musa* accessions in Table 1.

The sub-cluster I-b comprised of AA (Kluai Nam Thai) and BB (KluaiTaniand KluaiTani Dam) genomes of one and two *Musa* cultivars respectively. The cluster II consisted of only AAA genome of two *Musa* cultivars (HomKhiao and Hom Thong).

This result indicated that both markers were a useful tool for assessment of genetic diversity, which grouped the genomic groups into a high level in cluster-I (ABB, AA, and BB) a moderate level cluster-II (AAA) and a low level in sub-cluster I-a and sub-cluster I-b. Several publications reported that the RAPD and SRAP markers had high potential to provide polymorphism for genetic diversity of *Musa* cultivars (Phothipan *et al.,* 2005; Pankaj *et al.,* 2007; Masoud *et al.,* 2008; Peng *et al.,* 2014).

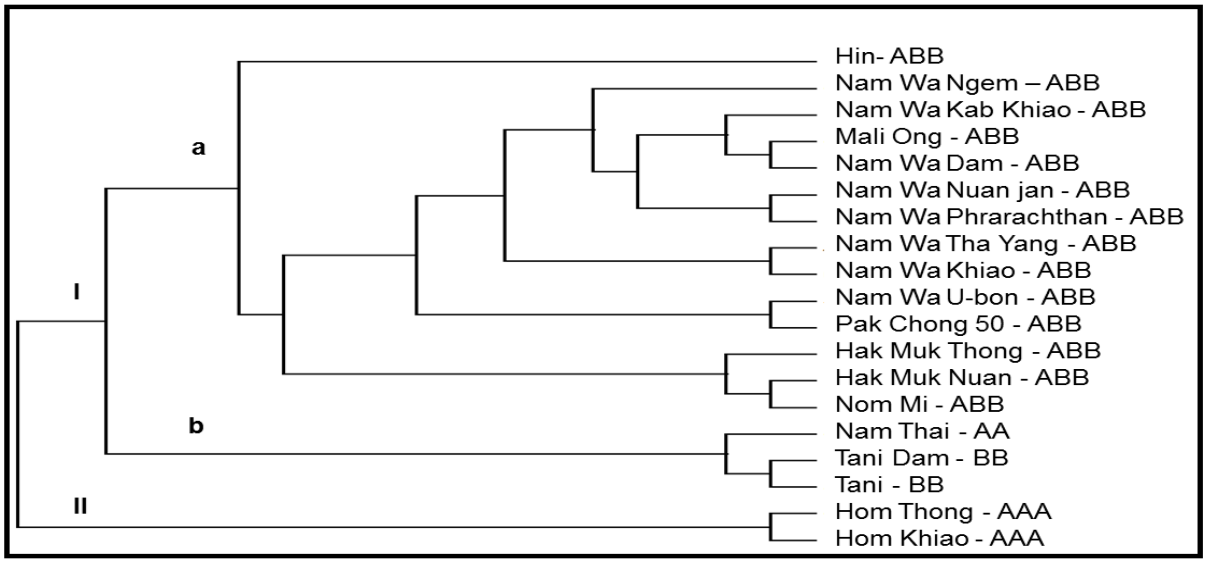

Fig.3. Dendrogram depicting genetic diversity among 19 *Musa* accessions based on RAPD- and SRAP-markers, using UPGMA cluster analysis approach from the Free Tree and Tree View software version 1.6.6. Note: All cultivars names starts with Kluai(Table 1).

Conclusion

This present study demonstrated that RAPD and SRAP markers were useful for classifying genetic relationship among the banana cultivars. These two sets of markers distinctively grouped the ABB genome separately from the AA and BB in the same cluster indication of closed genetic ancestry while AAA genome proved genetically of different parentage. It was also observed that selected RAPD markers (OPK07 and OPD08) (Fig. 1) and SRAP markers (Me1/Em6 and Me1/Em8) (Fig. 2) produced a maximum number of bright, distinct polymorphic and reproducible fragments. These markers were feasible to be used for the further specific molecular application such as the development of sequence-characterized amplified regions (SCAR) markers and marker-assisted selection (MAS) tool.

Conflicts of interest

The authors declare no conflicts of interest.

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