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Genetic variability among 18 cultivars of cooking bananas and plantains by RAPD and ISSR markers

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ABSTRACT

Poerba YS, Ahmad F (2010) Genetic variability among 18 cultivars of cooking bananas and plantains by RAPD and ISSR markers. Biodiversitas 11: 118-123. This study was done to assess the molecular diversity of 36 accessions (18 cultivars) of the plantain and cooking bananas (*Musa acuminata x M. balbisiana*, AAB, ABB subgroups) based on Random amplified polymorphic DNA (RAPD) and and Inter Simple Sequence Repeats (ISSR) markers and to determine genetic relationships in the bananas. RAPD and ISSR fingerprinting of these banana varieties was carried out by five primers of RAPDs and two primers of ISSRs. RAPD primers produced 63 amplified fragments varying from 250 to 2500 bp in size. 96.82% of the amplification bands were polymorphic. ISSR primers produced 26 amplified fragments varying from 350 bp to 2000 bp in size. The results showed that 92.86% of the amplification bands were polymorphic. The range of genetic distance of 18 cultivars was from 0.06-0.67.

Key words: RAPD analysis, *Musa acuminata, Musa balbisiana*, plantain, cooking bananas.

INTRODUCTION

Plantain and cooking bananas (*Musa acuminata x M. balbisiana*, AAB, ABB subgroups) are important components of food security in the tropics and they also provide income to the farming community through local trade (Crouch et al. 1998). The fruits are usually boiled, steamed, roasted or fried before consumption. The bananas are natural triploid (2n=3x=33) hybrids of two diploid species, *M. acuminata* Colla and *M. balbisiana* Colla, which contributed the A and B genomes, respectively (Swennen et al. 1995).

The bananas originated in South-east Asia (Ude et al. 2003; Simmonds and Shepherd 1955). The bananas are regarded as the most diverse of *Musa* subgroups among triploid *Musa*. These triploid genotypes are virtually or completely sterile and develop their fruit by vegetative parthenocarpy. The accumulation of recurrent somatic mutations followed by human selection for their tasty fruit led to great phenotypic diversity amongst plantain and cooking bananas in the region (De Langhe 1969).

Germplasm characterization and classification provide useful information for the genetic improvement of crops (Ortiz 1997). Morphological description has proven very useful for the identification of the large diversity of plantain and cooking banana cultivars that exist in the tropics (Jarret and Gawel 1995). However, close genetic relationships among cultivars as well as frequent somatic mutations and morphological changes due to environment which have resulted in large number of cultivars, are major obstacles that limit the use of this technique. Consequently, the use of only morphological parameters could result in over- or underestimations of the degree of relatedness among plantain cultivars (Kaemmer et al. 1992).

In addition to the use of morphological description in identification of specific banana cultivars, various DNA-based marker techniques are also been employed. These techniques can supply additional information which are not available from the examination of morphological characteristics alone (Jarret and Gawel 1995). The random amplified polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) are DNA-based marker techniques that has been successfully used to determine genetic diversity and relationships *Musa* germplasm (Kaemmer et al. 1992; Howell et al. 1994; Bhat and Jarret 1995; Crouch et al. 2000; Jain et al. 2007; Racharak and Eidathong 2007; Ruangsuttapha et al. 2007; Agoreyo et al. 2008; Brown et al. 2009) and for genome identification (Howell et al. 1994; Pillay et al. 2000), analysis of *Musa* breeding populations (Crouch et al. 1999), detection of somaclonal variants (Grajal-Martin et al. 1998), and genetic stability (Harirah and Khalid 2006; Ray et al. 2006; Lakshmanan et al. 2007; Venkatachalam et al. 2007). Using these techniques an unlimited number of polymorphic bands can be produced with relative ease from minute amounts of genomic DNA (Welsh and McClelland 1990; Godwin et al. 1997; Reddy et al. 2002) allowing simultaneous screening of a large number of accessions.

The present study reports the use of RAPD and ISSR analyses for the assessment of genetic variability among 36 accessions (18 cultivars) of plantains and cooking bananas collection of Cibinong Science Center (CSC).

MATERIALS AND METHODS

Materials

DNA materials consist of 36 accessions (18 cultivars) of *Musa acuminata x M. balbisiana*, AAB, ABB subgroup collected from different sources and grown at Cibinong Science Center, West Java. The materials were leaf samples dried with silica gel collected from those areas using method for DNA sampling (Widjaya and Poerba 2004).

Methods

DNA extraction

Total genomic DNA was isolated from dried silica leaves according to Delaporta et al. (1983) with the addition of RNase treatment (100 mg mL^{-1}). Isolated DNA was visualized for its quantity and quality by running them in 1% Agarose gel electrophoresis.

DNA amplification

DNA amplification was performed in Takara Thermocycler according to Williams et al. (1990) with total volume of PCR reaction of 15 µl consisting of 0.2 nM dNTPs; 1X reaction buffer; 2mM $MgCl_2$; 10 ng of DNA sample ; 0.5 pmole of single primer; and 1 unit of *Taq* DNA polymerase (Promega). Five arbitrary RAPD primers: OPA-18, OPA-13, OPD-08 OPN-06 dan OPN-12 (Operon Technology Ltd.) and two ISSR primers: UBC 834 dan UBC 828 (University of British Columbia, Canada) were used in the analyses. PCR reaction was conducted twice to ensure the reproducibility of RAPD. PCR products were visualized in 2% agarose gel electrophoresis for 60 min at 50 Volt. This was followed by EtBr staining (0.15 µl mL^{-1}) before photographed in gel documentation system (Atto Bioinstruments) and 100 bp ladder (Promega) was used as DNA marker.

Data scoring

Each band in the RAPD and ISSR fingerprint pattern will be considered as a separate putative locus. Only distinct, reproducible, well-resolved fragments were selected and scored for presence (1) and absence (0) of a band. The binary matrices of RAPD phenotypes will then be assembled for analyses. A similarity matrix was constructed and subjected to cluster analysis following the un-weighted pair group method with arithmetical averages (UPGMA) of the computer program NTSYS-pc version 1.8 (Rohlf 1993). Measurement of genetic distance for pair-wise accessions was based on Nei's unbiased genetic distances (Nei 1978) using POPGENE software (Yeh et al. 1999)

RESULTS AND DISCUSSION

RAPD profiles

Results of DNA amplification showed that the 36 accession of bananas produced a wide array of strong and weak bands. However, only distinct, reproducible, well-resolved fragments were scored as present or absent band for each of the RAPD primers with 36 accessions. Figure 1 and 2 illustrated the typical level of polymorphisms observed among the 36 banana accessions for primer OPA-18. For genetic identification purposes, primer used is important to be able to differentiate varieties or cultivars of the species. The DNA amplification produced 63 bands. The amplification products showed that 4.16% were monomorphic and 96.82% were polymorphic bands. Among the selected primers OPD-08 and OPN-06 produced maximum number of polymorphic 15 bands, while OPA-18 produced the minimum number of polymorphic 7 bands. Size of DNA bands varied from 250 bp to 2.5Kbp, OPA-13 being the highest range of DNA size (250bp-2.5 Kb) and OPA 18 is the lowest (250 bp-2.0 Kb) (Table 1).

Table 1. List of primers, their sequences, number of amplified fragments and number of polymorphic bands generated by PCR using five RAPD primers.

Primer code	Primer nucleotide sequence (5'-3')	Total bands	Poly-morphic bands	%	Size (bp)
OPA-13	CAGCACCCAC	13	12	92.31	250-2000
OPA-18	AGGTGACCGT	7	7	100	300-1200
OPD-08	GTGTGCCCCA	15	15	100	350-2000
OPN-06	GAACGGACTC	15	15	100	300-1800
OPN-12	CACAGACACC	13	12	92.31	300-1700
	Total	63	61	96.82	

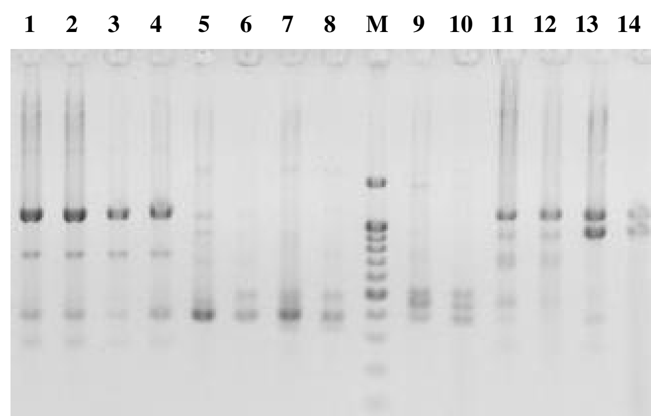

Figure 1. Random amplified polymorphic DNA profiles of 14 *Musa* accessions using primer OPA-18. Lanes 1–2 = Kepok kuning Jogya (ABB), 3-4 = Kepok Jember (ABB), 5-6 = Kepok OP (ABB), 7-8 = Siam (ABB), 9-10 = Raja sewu (AAB), 11-12 = Raja dengklek (AAB), 13-14 = Raja bulu (AAB), M = 100 bp DNA marker (Promega).

The RAPD profiles indicated that each primer could generate a polymorphism. Number of DNA amplification bands depended on how primer attached to its homolog at DNA template (Tingey et al. 1994). RAPD polymorphism are the result of either a nucleotide base change that alters the primer binding site, or an insertion or deletion within the amplified region (Williams et al. 1990), polymorphism usually noted by the presence or absence of an amplification product from a single locus (Tingey et al. 1994). The differences in polymorphism may be due to the

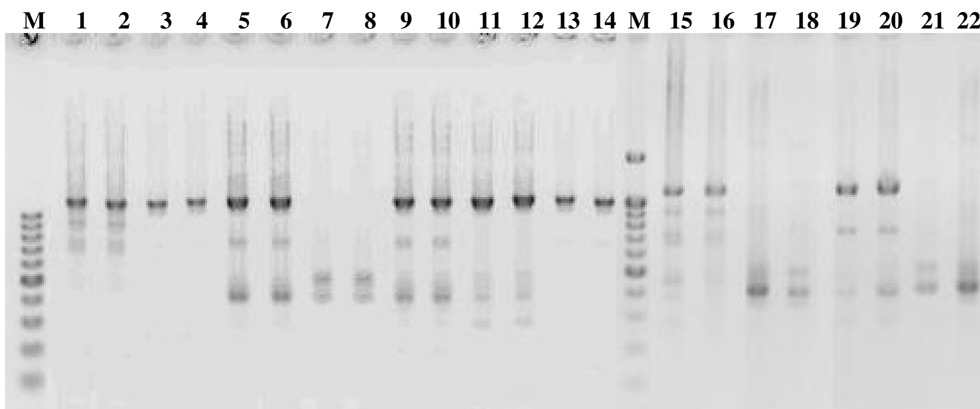

Figure 2. Random amplified polymorphic DNA profiles of 22 *Musa* accessions using primer OPA-18 Lanes 1-2 = Raja nangka (AAB), 3-4 = Tanduk Bawen (AAB), 5-6 = Pisang puju (ABB), 7-8 = Pisang awak (ABB), 9-10 =Tanduk byar (AAB), 11-12 = Budless Sulawesi (ABB), 13-14 = Raja Kristen (AAB), 15-16= Kepok Amorang (ABB), 17-18 = Raja Sere (AAB), 19-20 = Kepok SP (ABB), 21-22 = Raja Sewu (AAB), M = 100 bp DNA marker (Promega)

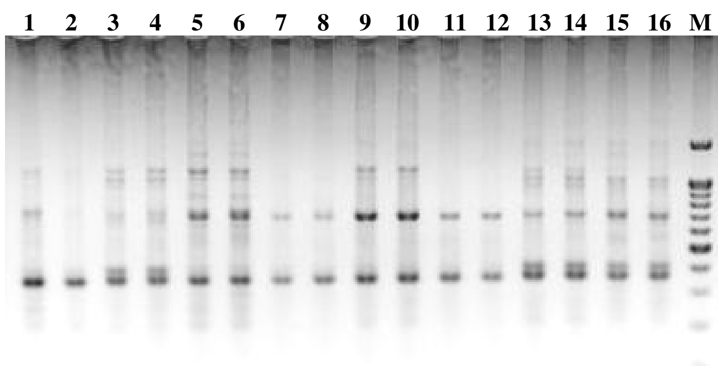

Figure 3. Inter Simple Sequence Repeats profiles for 16 *Musa* accessions using primer UBC-834. Lanes 1-2 = Raja Nangka, 3-4 = Raja Kristen, 5-6 = Tanduk Byar, 7-8 = Tanduk Bawen, 9-10 = Raja Sere, 11-12 = Raja Sewu, 13-14 = Taja Dengklek, 15-16 = Raja Bulu, M= 100 bp DNA marker (Promega)

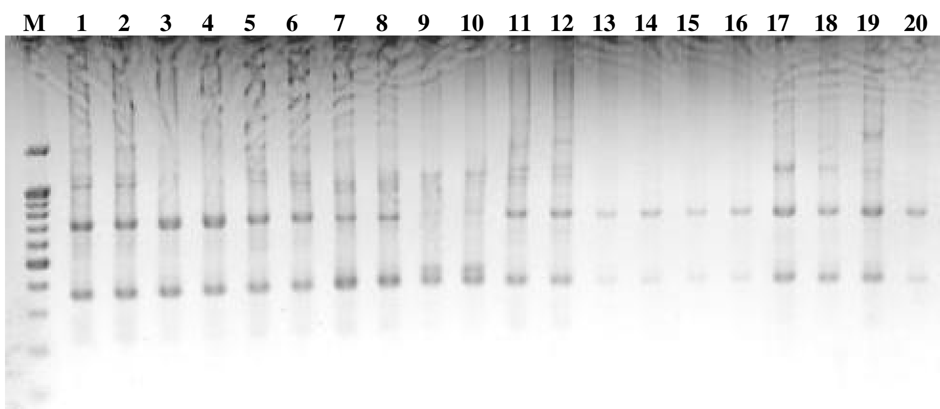

Figure 4. Inter Simple Sequence Repeats profiles for 20 *Musa* accessions using primer UBC-834. anes 1-2 = Pisang Puju, 3-4 = Pisang Awak, 5-6 = Kepok Alpha, 7-8 = Budless Sulawesi, 9-10 = Kepok SP, 11-12 = Kepok Amorang, 13-14 = Kepol Kuning Yogya, 15-16 = Kepok Jember, 17-18 = Kepok OP, 19-20 = Siam, M= 100 bp DNA marker (Promega)

differences in amount of genetic variation that exist among the different accessions.

ISSR Profiles

Results of DNA amplification showed that the 36 accession of bananas produced a wide array of strong and weak bands. However, only distinct, reproducible, well-resolved fragments were scored as present or absent band for each of the RAPD primers with 36 accessions. Figure 3 and 4 illustrated the typical level of polymorphisms observed among the 36 accessions for primer UBC-834.

The DNA amplification produced 28 bands, of which 8.14% were monomorphic, common to all the genotypes, and 92.86% were polymorphic bands. Among the selected primers UBC-826 produced the highest number of polymorphic 15 bands, while UBC-834 produced the lowest number of polymorphic 13 bands. Size of DNA bands varied from 350 bp to 2.0Kbp, UBC-834 being the highest range of DNA size (350bp-2.0 Kb) and UBC-826 is the lowest (Table 2).

The ISSR profiles indicated that each primer could generate polymorphisms among the accessions. The polymorphism may be due to mutation at priming sites and/or insertion/deletion event within the SSR region; and the extent of polymorphism also varies with the nature and the sequence repeat (motif) of the primer used (Reddy et al. 2002).

Table 2. List of primers, their sequences, number of amplified fragments and number of polymorphic bands generated by PCR using two ISSR primers.

Primer code	Primer nucleotide sequence (5'-3')	Total bands	Poly-morphic bands	%	Size (bp)
UBC-826	$(AC)_8C$	15	15	100	400-2000
UBC-834	$(AG)_8YT$	13	11	84.61	350-2000
Total		28	26	92.86	

Combined analyses of RAPD and ISSR

Cluster analysis performed from combining data of both RAPD and ISSR markers generated a dendrogram that separated the genotypes into two distinct clusters. First cluster is composed of all cultivars within ABB genome and second cluster is consist of all cultivars within AAB genome. Twenty accessions (10 cultivars of cooking banana) of *M. acuminata x M. balbisiana* (ABB) i.e Pisang Puju (1-2), Kepok Alpha (5-6), Pisang Awak (3-4), Budless Sulawesi (7-8), Kepok Siharangan Purba (9-10), Kepok Amorang (11-12), Kepok Kuning Yogya (13-14), Kepok Jember (15-16), Kepok OP (17-18), Siam (19-20) are clustered in the fist group. Second group was contained 16 accessions which were further divided into two subclusters. Among them, the first subcluster was consist of 8 accessions (4 cultivars of AAB genome) i.e, : Tanduk Byar (25-26), Raja Dengklek (33-34), Raja Bulu (35-36), and Raja Sere (29-30). Second subcluster was composed of 8 accessions (4 cultivars of AAN genome) i.e: Raja Nangka (21-22), Raja Kristen (23-24), Raja Sewu (31-32), dan Tanduk Byar (27-28) (Figure 3).

The conventional classification of banana genotypes into distinct genome combinations by Simmonds and Shepherd (1955) is based on their morphological similarity to *M. acuminata* Colla and *M. balbisiana* Colla. The cultivars examined in this study clustered accordingly to their hypothetical genetic homologies. These result agreed with Brown et al. (2009), for example cultivars designated having ABB genomic constituent (Pisang Puju, Pisang Awak, Kepok Alpha, Budless Sulawesi, Kepok SP, Kepok Amorang, Kepok Kuning Yogya, Kepok Jember, Kepok OP, and Pisang Siam) are clustered together (Fig 5).

The genetic distance values for the 36 accessions (18 cultivars of banana) ranged from 0.06 to 0.67 (Tabel 3). The lowest genetic distance (0.06) was observed between Pisang Puju (1) and Pisang Kepok Alpha (3), while the highest genetic distance (0.67) was detected between Kepok Amorang (6) and Pisang Raja Bulu (18) (Table 3).

Table 3. Genetic distance among 18 cultivars of triploid banana

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	-																	
2	0.14	-																
3	**0.06**	0.21	-															
4	0.24	0.22	0.32	-														
5	0.29	0.25	0.37	0.20	-													
6	0.26	0.37	0.28	0.37	0.47	-												
7	0.30	0.33	0.35	0.28	0.41	0.37	-											
8	0.32	0.32	0.37	0.36	0.43	0.40	0.10	-										
9	0.29	0.29	0.33	0.28	0.37	0.33	0.13	0.16	-									
10	0.29	0.19	0.36	0.26	0.33	0.37	0.15	0.15	0.11	-								
11	0.46	0.39	0.48	0.36	0.34	0.48	0.34	0.26	0.40	0.31	-							
12	0.58	0.40	0.60	0.47	0.36	0.59	0.53	0.46	0.50	0.41	0.22	-						
13	0.52	0.55	0.55	0.51	0.46	0.47	0.61	0.61	0.53	0.58	0.52	0.40	-					
14	0.35	0.29	0.40	0.32	0.20	0.44	0.34	0.30	0.39	0.28	0.18	0.24	0.45	-				
15	0.45	0.45	0.50	0.42	0.43	0.46	0.45	0.48	0.47	0.40	0.42	0.34	0.31	0.40	-			
16	0.46	0.39	0.48	0.36	0.34	0.48	0.34	0.26	0.40	0.31	0.02	0.22	0.52	0.18	0.42	-		
17	0.54	0.54	0.60	0.41	0.59	0.53	0.49	0.54	0.39	0.49	0.43	0.41	0.37	0.57	0.35	0.46	-	
18	0.60	0.60	0.66	0.42	0.53	**0.67**	0.62	0.66	0.51	0.63	0.50	0.48	0.40	0.58	0.40	0.54	0.10	-

Note: Lanes/columns 1 = Pisang Puju, 2 = Pisang Awak, 3 = Kepok Alpha, 4 = Budless Sulawesi, 5 = Kepok SP, 6 = Kepok Amorang , 7 = Kepok Kuning Yogya, 8 = Kepok Jember, 9 = Kepok OP, 10 = Pisang Siam, 11 = Raja Nangka, 12 = Raja Kristen, 13 = Tanduk Byar, 14 = Tanduk Bawen, 15 = Raja Sere, 16 = Raja Sewu, 17 = Raja Dengklek, 18 = Raja Bulu.

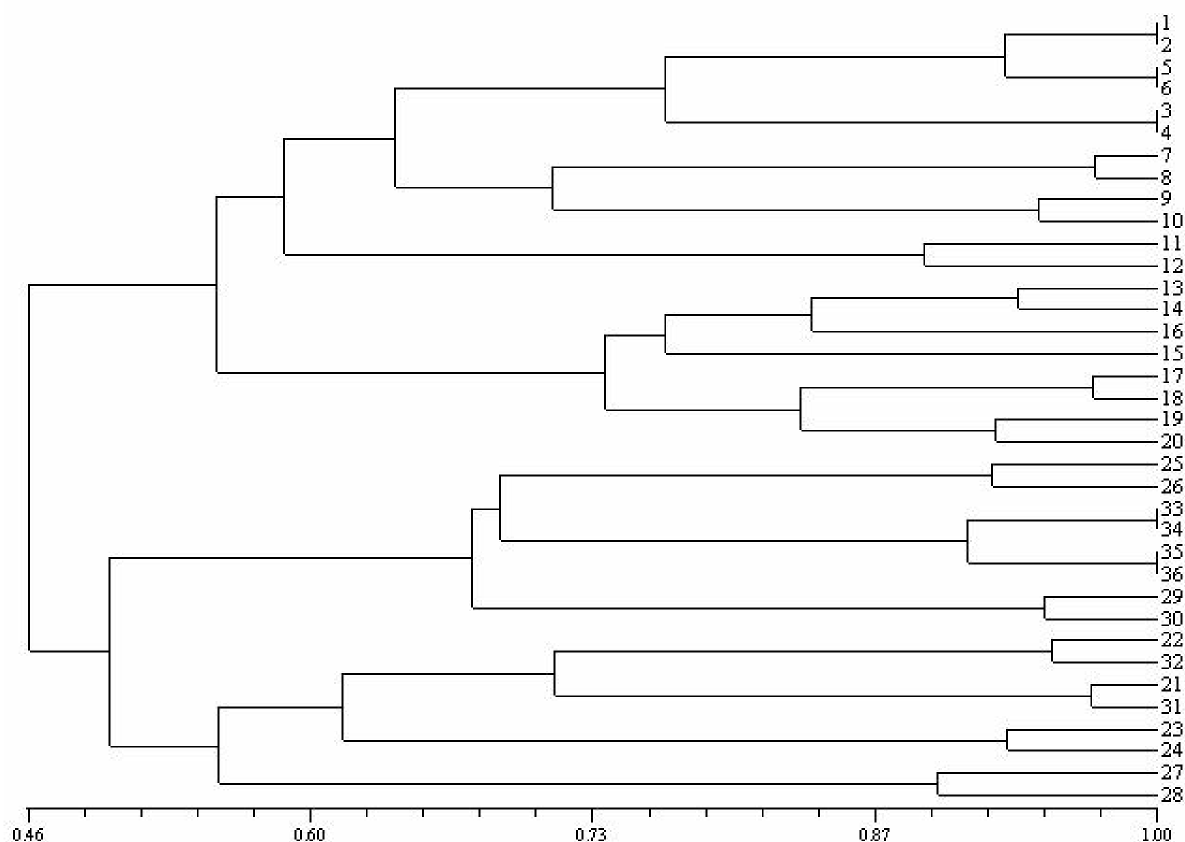

Figure 5. Dendrogram of 36 triploid banana accessions. Accessions 1-2 = Pisang Puju, 3-4 = Pisang Awak, 5-6 = Kepok Alpha, 7-8 = Budless Sulawesi, 9-10 = Kepok SP, 11-12 = Kepok Amorang , 13-14 = Kepok Kuning Yogya, 15-16 = Kepok Jember, 17-18 = Kepok OP, 19-20 = Pisang Siam, 21-22 = Raja Nangka, 23-24 = Raja Kristen, 25-26 = Tanduk Byar, 27-28 Tanduk Bawen, 29-30 = Raja Sere, 31-32 = Raja Sewu, 33-34 = Raja Dengklek, 35-36 = Raja Bulu

CONCLUSION

The five RAPD and two ISSR primers could be used to detect DNA polymorphism in 36 accessions of banana. RAPD primers produced 63 amplified fragments varying from 250 to 2500 bp in size. 96.82% of the amplification bands were polymorphic. OPD-08 and OPN generated the highest amplified bands (15). ISSR primers produced 26 amplified fragments varying from 350 bp to 2000 bp in size. 92.86% of the amplification bands were polymorphic. UBC-826 produced the highest number of polymorphic 15 bands. Cluster analysis performed from combining data of both RAPD and ISSR markers generated a dendrogram that separated the genotypes into two distinct clusters, according to genome constitution. The genetic distance values for the 36 accessions (18 cultivars of triploid bananas) ranged from 0.06 to 0.67. The lowest genetic distance (0.06) was observed between Pisang Puju (1) and Pisang Kepok Alpha (3), while the highest genetic distance (0.67) was detected between Kepok Amorang (6) and Pisang Raja Bulu (18). In conclusion, this research demonstrated RAPD and ISSR markers to be useful tool to detect DNA polymorphisms to examine genetic relationship in CSC banana germplasm.

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