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Genetic Diversity of *Musa balbisiana* Colla in Indonesia Based on AFLP Marker

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Musa balbisiana **Colla, known as Pisang Klutuk/Pisang Batu, is important for banana breeding program due to its B genome and often considered to be resistant to pest, disease and drought. Banana is a big and tall herb up to 7-9 m in height.The study was conducted to assess genetic diversity of 21 accessions of *M. balbisiana* cultivars/ varieties from Indonesia based on Amplified Fragment Length Polymorphism (AFLP) markers in order to provide basic information to support banana breeding program. Analyses of similarity index of Nei and Li and clustering based on UPGMA was conducted using NTSYS program. Principal Component Analysis (PCA) was conducted by MINITAB 14. Twenty two primer pairs of AFLP markers produced 485 AFLP bands varying from 51-3206 bp in size and 46.18% of the AFLP bands were polymorphic. Genetic diversity among the 21 accessions of *M. balbisiana* was 13.8%. The genetic diversity of wild *M. balbisiana* was 12.9%, higher than the genetic diversity of cultivated *M. balbisiana* which was 11.5%. Cluster analysis based on UPGMA suggested that wild *M. balbisiana* and cultivated *M. balbisiana* could not be separated into different clusters. Both cluster analysis and PCA produced the same three groups of the accessions. The PCA analyses showed that 17 AFLP bands were responsible for the grouping.**

Keywords: amplified fragment length polymorphism (AFLP), *Musa balbisiana* Colla, genetic diversity, Indonesia

INTRODUCTION

Recent banana genetic diversity is a result of adaptation and domestication process during thousand of years. Center of origin and center of banana biodiversity is in South Asia and South East Asia. Indonesia, as one of the centers of banana biodiversity, holds precious source of genetic resources which is important for developing banana breeding program to fulfill food demand.

Musa balbisiana Colla is one progenitor of cultivated banana which contains B genome with a basic chromosome number is 2n = 22 (Davey *et al.* 2013). *M. balbisiana* and *M. acuminata* (A genome) formed triploid hybrid (AAB, ABB) and tetraploid hybrid (AAAB, AABB, ABBB) (Teo *et al.* 2005). B genome corresponds to abiotic stress or drought tolerant (Liu *et al.* 2010; Vanhove *et al.* 2012), vigour (Tripathi & Tripathi 2009) and have demonstrated resistance to *Xanthomonas* (Tripathi *et al.* 2007; Kumakech *et al.* 2013).

Taxonomically, *Musa balbisiana* Colla is classified into genus *Musa* under section *Musa* (Hakkinen 2013). In World Checklist of Selected Plant Families (http://apps.kew.org/wcsp/qsearch.do) *M. balbisiana* was classified into seven varieties. Unfortunately, there is no taxonomy study that classified Indonesian *M. balbisiana* into any variety. The variability of Indonesian *M. balbisiana* is mainly found on pigmentation of the pseudostem. 'Pisang Klutuk Wulung/Klutuk Hitam' has stronger pigmentation, with a black color pseudostem, than 'Pisang Klutuk Batu' and 'Pisang Klutuk Sukun'. Other variant is found on the shape and size of the bunch, such as 'Pisang Roti', and 'Pisang Pataga' which have a shorter and bigger bunch than 'Pisang Klutuk'.

In contrast to the well understood *M. acuminata,* only few genetic diversity study of *M. balbisiana* have been conducted. AFLP method has been performed to analyze *M. acuminata* and banana cultivars from Malaysia (Wong *et al.* 2001), Singapore (Loh *et al.* 2000), Egypt (El-Khishin *et al.* 2009), and Oman (Opara *et al.* 2010); *Musa* sections (Ude *et al.* 2002a); genetic diversity in *M. acuminata*, *M. balbisiana,* and their natural hybrids (Ude *et al.* 2002b); African plantains (Ude *et al.* 2003); and various wild and cultivated banana (Youssef *et al.* 2011). While for *M. balbisiana,* Wang *et al.* (2007) worked with wild population in China using AFLP marker and Uma *et al.* (2006) analyzed Indian collection using morphological and RAPD marker. In Indonesia only

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a few of *M. balbisiana* accessions had been analyzed using microsatelite markers (Retnoningsih *et al.* 2011) and genetic diversity of this group of banana had never been examined extensively. Therefore this study was conducted to assess genetic diversity level of *M. balbisiana* in Indonesia using Amplified Fragment Lenght Polymorphism (AFLP) method. AFLP is a dominant molecular marker (Wang 2005) with high reproducibility and produce great quantities of data compared to RAPD, RFLP or ISSR methods (Garcia *et al*. 2004). The AFLP method is based on restriction of DNA using an even and frequent restriction enzyme that will produce various DNA fragment length. The DNA fragments are then ligated with oligonucleotide adapters. Subsequently, the DNA fragment is amplified selectively using primer which is designed based on adapters sequence that extend into restriction fragments sequence, so that the reproducible DNA bands are produced (Vos *et al.* 1995). The results of the study would be important to support Indonesian banana breeding.

MATERIALS AND METHODS

Material Collection. Twenty one accessions of *M. balbisiana* was collected from Research Center for Biology Collection (Bogor), which consisted of seven accessions from West Java, two accessions from West Sumatra, two accessions from South Sulawesi, two accessions from North Sulawesi, six accessions from Yogyakarta and two accessions from Bogor Botanical Gardens (Table 1).

DNA Extraction. Total DNA was extracted from young leaves by Cetyltrimetylammonium bromide (CTAB) method (Syamkumar *et al.* 2003) with modification, i.e., using 4% CTAB and addition of 0.1 g Polyvinylpirrolidone (PVP) for each reaction.

AFLP Analysis. The analysis was conducted by AFLP method (Vos *et al*. 1995) with minor modification, i.e., without radioactive labeling of primer, but by fluourescence nucleic acid dye. Twenty six primer pairs for selective amplification were selected from previous studies (Ude *et al.* 2002a; El-Khisin *et al*. 2009; Opara *et al*. 2010; Wongniam *et al.* 2010). Restriction buffer, restriction enzyme, ligation buffer, adapter, pre-selective amplification primer, pre-selective amplification PCR reagent, and selective primers were the product of Invitrogen AFLP Analysis System I kit. Selective amplification PCR reagent was the product of Promega GoTaq® Flexy DNA Polymerase.

Approximately 14 ng/μL of DNA was digested by 2.5 unit *Eco* RI/*Mse* I in restriction buffer (10 mM Tris-HCl, 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mg/ml BSA, and 0.1% Triton® X-100) at 37 ^{o}C for 2 h. The restriction DNA fragments were ligated to *Eco* RI and *Mse* I adapters by adding 0.4 mM ATP, 10 mM Tris-HCl (pH 7.5), 10 mM Mg-acetate, 50 mM K-acetate, 4 μL/mL T4 DNA ligase, 1 mM DTT, 50 mM KCl, *Eco* RI adapter and *Mse* I adapter, the mixture were incubated at 20 ^{o}C for 2 h. The product of the reaction was diluted 1:10 by dissolving the ligation mix in TE buffer [10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA]. Pre-selective

Table 1. *Musa balbisiana* Colla accession used for the study

Accession	Cultivar/variety of *Musa balbisiana*	Origin	Note
KLT-JG1	Klutuk	Yogyakarta*	Cultivated
KLT-JG2	Klutuk	Yogyakarta*	Cultivated
KLT-JB	Klutuk	West Java	Cultivated
KS-JG1	Klutuk Sukun	Yogyakarta *	Cultivated
KS-JG2	Klutuk Sukun	Yogyakarta *	Cultivated
UB-SS1	Unti Batu	South Sulawesi	Cultivated
UB-SS2	Unti Batu	South Sulawesi	Cultivated
CM-JB1	Cau Manggala	West Java	Cultivated
CM-JB2	Cau Manggala	West Java	Cultivated
KW-JG1	Klutuk Wulung	Yogyakarta *	Cultivated
KW-JG2	Klutuk Wulung	Yogyakarta *	Cultivated
KW-JB1	Klutuk Wulung	West Java	Cultivated
KW-JB2	Klutuk Wulung	West Java	Cultivated
KH-JB1	Klutuk Hitam	West Java	Cultivated
KH-JB2	Klutuk Hitam	West Java	Cultivated
PR-SB1	Pisang Roti	West Sumatera	Wild
PR-SB2	Pisang Roti	West Sumatera	Wild
PAT-SU1	Pisang Pataga	North Sulawesi	Wild
PAT-SU2	Pisang Pataga	North Sulawesi	Wild
LIU-JP1	var. *liukiuensis* (Matsum.) Hakkinen	Japan **	Wild
LIU-JP2	var. *liukiuensis* (Matsum.) Hakkinen	Japan**	Wild

*Collection of Cibinong Science Center, **Collection of Bogor Botanical Garden.

amplification was prepared by mixing 2.5 µL diluted ligation mix, 20 µL pre-amplification primer mix (contain primers *Eco* RI+A and *Mse* I+C), 0.5 µL *Taq* DNA polymerase (5 unit/µL) and 2.5 µL 10x PCR buffer (200 mM Tris-HCl (pH 8.4), 15 mM $MgCl_2$, 500 mM KCl). PCR was conducted at 24 cycles at: 94 °C for 30 s, 56 °C for 60 s, and 72 °C for 60 s. The pre-selective amplification product was diluted 1:50 with TE buffer. Two and half micro liters pre-selective amplification product were used for selective amplification in a PCR tube containing 2.78 ng/µL selective primer *Eco* RI+NNN/*Mse* I+NNN primer, 20 mM Tris-HCl (pH 8.4), 1.5 mM $MgCl_2$, 50 mM KCl, 0.2 mM dNTP and 0.5 µL *Taq* polymerase (5 unit/µL). This reaction was done at three sections PCR condition. First section was done at one cycle at 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 60 s. Second section was done by lowering annealing temperature of 0.7 °C for each cycle during 12 cycles (a touchdown phase of 13 cycles). Last section was done at 23 cycles at 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 60 s.

Visualization of AFLP Bands. Electrophoresis was carried out on 3.5% non-denaturized acryl amide using ATTO mini gel apparatus.The electrophoresis was run at 85 volt for 80 minutes. AFLP band was stained using 1X Gel Red™ staining solution (Biotium) for 30 minutes. AFLP band was observed above UV light and photographed in a gel documentation system (ATTO).

Data Analysis. Only clear and visible AFLP bands were selected and scored for presence (1) and absence (0) of a band using densitograph ATTO CS Analyzer Ver. 2.08c in level 4 of sensitivity setting. Bands size was calculated based on 100 bp plus (Fermentas) DNA marker by spline curve of the densitograph. Clustering analysis was performed under unweighted pair group method with arithmetic averages (UPGMA) based on Nei and Li (1979), dissimilarity index using NTSYS (Numerical Taxonomy and Multivariate Analysis System) program version 2.02 (Rohlf 1998). Genetic distance was analyzed by Popgene 1.32 (Yeh *et al*. 1997) based on Nei's unbiased measures of genetic identity and genetic distance (Nei 1978). Genetic diversity value was calculated by the difference between the highest dissimilarity index and the lowest dissimilarity index. Principal Component Analysis was performed by Minitab 14 (Minitab 2003).

RESULTS

AFLP Bands. Twenty two out of 26 AFLP primer pairs produced clear AFLP bands and these were used to analyze the genetic diversity of 21 accessions of *M. balbisiana* (Table 2). A total of 485 AFLP bands size ranging from 51-3206 bp were scored and 224 bands (46.18%) were polymorphic. The highest number of AFLP band (31 bands) was produced by primer pair 13 (E-AGC/M-CAC), while the least amount of

Table 2. Total number and size of obtained AFLP band

Primer pair	Number of band	Polymorphic band	Polymorphic band (%)	Size (bp)
E-AAC/M-CTC[a]	29	18	62.07	153-3206
E-AAG/M-CTA[a]	29	20	68.97	70-3043
E-ACG/M-CAA[a]	22	12	54.55	110-2937
E-AGC/M-CTT[a]	19	6	31.58	85-1274
E-AGG/M-CTA[a]	21	6	28.57	110-1745
E-AAC/M-CAG[b]	25	16	64.00	92-1476
E-ACA/M-CTG[b]	15	12	80.00	61-2449
E-ACC/M-CTT[b]	20	14	70.00	55-1274
E-ACG/M-CTA[b]	16	9	56.25	71-1367
E-ACT/M-CAT[b]	19	9	47.37	56-1269
E-ACT/M-CTC[b]	14	7	50.00	57-537
E-ACT/M-CTG[c]	25	12	48.00	51-2860
E-AGC/M-CAC[c]	31	4	12.90	64-1544
E-AGC/M-CTT[c]	23	10	43.48	79-1236
E-ACC/M-CTA[d]	22	12	54.55	92-1259
E-ACG/M-CTC[d]	18	7	38.89	97-1342
E-ACT/M-CAT[d]	25	9	36.00	82-1478
E-ACC/M-CAT[b,c]	23	7	30.43	72-1200
E-AGG/M-CTT[c,d]	25	0	0.00	105-2804
E-AAC/M-CAA[b,c,d]	24	14	58.33	85-1385
E-AAG/M-CAC[b,c,d]	21	11	52.38	119-1308
E-ACA/M-CAG[a,b,c,d]	19	9	47.37	87-1137
Total	485	224	46.19	

[a]: Ude *et al*. (2002a), [b]: Opara *et al*. (2010), [c]: El-Khisin *et al*. (2009), [d]: Wongniam *et al*. (2010).

Table 3. Dissimilarity index of Nei and Li (1979) based on 22 AFLP primer pairs in 21 accessions of *M. balbisiana*

Accession code	KLT-JG1	KLT-JG2	KLT-JB	KS-JG1	KS-JG2	UB-SS1	UB-SS2	CM-JB1	CM-JB2	KW-JG1	KW-JG2	KW-JB1	KW-JB2	KH-JB1	KH-JB2	PR-SB1	PR-SB2	PAT-SU1	PAT-SU2	LIU-JP1	LIU-JP2
KLT-JG1	0																				
KLT-JG2	0.014	0																			
KLT-JB	0.033	0.035	0																		
KS-JG1	0.029	0.025	0.051	0																	
KS-JG2	0.033	0.029	0.052	0.017	0																
UB-SS1	0.061	0.057	0.065	0.056	0.06	0															
UB-SS2	0.080	0.078	0.082	0.077	0.074	0.036	0														
CM-JB1	0.048	0.043	0.056	0.040	0.037	0.047	0.061	0													
CM-JB2	0.040	0.036	0.058	0.038	0.030	0.051	0.063	0.026	0												
KW-JG1	0.047	0.045	0.062	0.038	0.041	0.053	0.075	0.038	0.033	0											
KW-JG2	0.053	0.049	0.064	0.048	0.050	0.055	0.069	0.034	0.034	0.022	0										
KW-JB1	0.126	0.118	0.123	0.129	0.124	0.098	0.099	0.096	0.108	0.113	0.102	0									
KW-JB2	0.113	0.113	0.113	0.119	0.118	0.093	0.101	0.099	0.105	0.108	0.109	0.070	0								
KH-JB1	0.049	0.044	0.064	0.043	0.038	0.048	0.062	0.037	0.029	0.036	0.042	0.107	0.102	0							
KH-JB2	0.055	0.050	0.072	0.043	0.044	0.044	0.058	0.043	0.045	0.047	0.046	0.109	0.104	0.027	0						
PR-SB1	0.145	0.141	0.138	0.149	0.148	0.132	0.130	0.129	0.133	0.143	0.132	0.142	0.152	0.134	0.131	0					
PR-SB2	0.102	0.100	0.100	0.101	0.096	0.090	0.093	0.086	0.090	0.097	0.101	0.129	0.121	0.089	0.088	0.066	0				
PAT-SU1	0.055	0.050	0.068	0.052	0.047	0.040	0.056	0.050	0.054	0.057	0.060	0.102	0.099	0.039	0.040	0.127	0.089	0			
PAT-SU2	0.051	0.044	0.064	0.044	0.045	0.048	0.057	0.051	0.048	0.050	0.045	0.105	0.114	0.038	0.034	0.124	0.092	0.023	0		
LIU-JP1	0.072	0.067	0.088	0.069	0.063	0.072	0.084	0.063	0.057	0.064	0.063	0.128	0.123	0.051	0.065	0.152	0.109	0.060	0.061	0	
LIU-JP2	0.078	0.076	0.079	0.082	0.074	0.070	0.078	0.066	0.060	0.072	0.074	0.112	0.109	0.067	0.073	0.144	0.104	0.074	0.072	0.042	0

bands (14 bands) was made by primer pair 11. The highest percentage (80%) of polymorphic band was formed by primer pair 7 (E-ACA/M-CTG), while primer pair 19 (E-AGG/M-CTT) did not show any polymorphic band.

Genetic Diversity of 21 *M. balbisiana* Accessions. Dissimilarity index (Nei & Li 1979) among the 21 accessions of *M. balbisiana* ranged from 0.014 to 0.152, with 13% genetic diversity. The lowest dissimilarity index was observed between KLT-JG1 and KLT-JG2 (cv. Klutuk from Yogyakarta), while the highest dissimilarity index was observed between PR-SB1 (Pisang Roti) and KW-JB2 (cv. Klutuk Wulung from West Java), and between PR-SB1 (Pisang Roti) and LIU-JP1 (var *liukiuensis*) (Table 3). Genetic diversity of wild *M. balbisiana* accessions were 12.9%, while for the cultivated one was 11.5%.

Cluster Analysis. Clustering performed by UPGMA analysis based on Nei and Li (1979) similarity index of 21 accessions of *M. balbisiana* generated three clusters at coefficient of 0.93 (Figure 1). Cluster I consists of cv. Klutuk from Yogyakarta and from West Java (KLT-JG1, KLT-JG2 and KLT-JB), cv. Klutuk Sukun-Yogyakarta (KS-JG1 and KS-JG2), cv. Unti Batu-South Sulawesi (UB-SS1 and UB-SS2), cv. Cau Manggala-West Java (CM-JB1 and CM-JB2), cv. Klutuk Wulung-Yogyakarta (KW-JG1 and KW-JG2), cv. Klutuk Hitam-West Java (KH-JB1 and KH-JB2), var. Pisang Pataga-North Sulawesi (PAT-SU1 and PAT-SU2) and var. *liukiuensis* from Bogor Botanical Garden (LIU-JP1 and LIU-JP2). Cluster II consists of cv. Klutuk Wulung-West Java (KW-JB1 and KW-JB2). Cluster III consists of Pisang Roti-West Sumatera (PR-SB1 and PR-SB2). Based on AFLP, the wild and cultivated accession of *M. balbisiana* did not separated into different clusters.

Principal Component Analysis. Pattern of grouping in Principal Component Analysis scatter plot was similar to the pattern of dendrogram from cluster analysis which consists of three groups, i.e., group A, B and C (Figure 2). Accessions of group A were the same to accessions in cluster II,

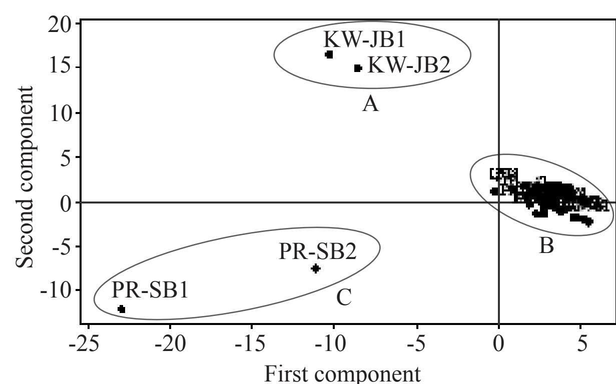

Figure 2. Scatter plot of principal component analysis of 21 accessions *M. balbisiana.*

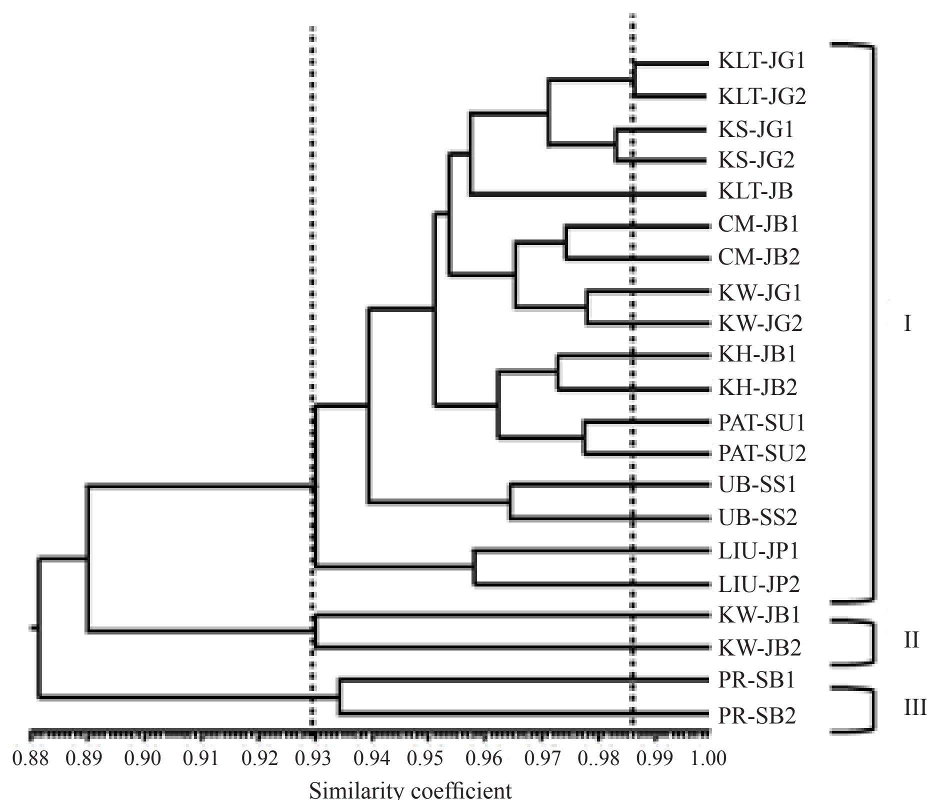

Figure 1. Dendrogram of UPGMA cluster analyses based on 22 AFLP primer pairs of 21 *Musa balbisiana* accessions.

Table 4. AFLP bands that play a role in principal component analysis grouping based on the first and second principal component's highest absolute value

Number of primer	Primer pairs	Size of band (bp)	Absolute value	
			First principal component	Second principal component
2	E-AAG/M-CTA	852	0.060	0.144
2	E-AAG/M-CTA	637	0.060	0.144
8	E-ACC/M-CTT	214	0.060	0.144
8	E-ACC/M-CTT	55	0.127	0.041
9	E-ACG/M-CTA	566	0.060	0.144
15	E-ACC/M-CTA	965	0.060	0.144
15	E-ACC/M-CTA	844	0.060	0.144
15	E-ACC/M-CTA	715	0.060	0.144
15	E-ACC/M-CTA	624	0.060	0.144
15	E-ACC/M-CTA	514	0.060	0.144
15	E-ACC/M-CTA	241	0.127	0.041
15	E-ACC/M-CTA	133	0.060	0.144
15	E-ACC/M-CTA	107	0.060	0.144
15	E-ACC/M-CTA	92	0.060	0.144
20	E-AAC/M-CAA	610	0.060	0.144
22	E-ACA/M-CAG	1066	0.060	0.144
22	E-ACA/M-CAG	756	0.127	0.041
Variance (%)			23.1	16.3
Cumulative of variance (%)			23.1	39.4

accessions of group B were the same to accessions in Cluster I and accessions in group C were the same to accessions in Cluster III.

Two principal components (PC I and PC II) consisted of 3 and 14 bands, respectively (Table 4), affected the pattern of grouping that grouped the accessions into three main clusters. PC I showed that three AFLP bands were responsible in separating Pisang Roti (PR-SB1 and PR-SB2) and cv. Klutuk Wulung from West Java (KW-JB1 and KW-JB2) from the rest of 17 *M. balbisiana* accessions into group C. These bands were 55 bp of primer pair 8 (E-ACC/M-CTT), 241 bp of primer pair15 (E-ACC/M-CTA) and 756 bp of primer pair 22 (E-ACA/M-CAG) (Table 4). PC II was component that grouped cv. Klutuk Wulung from West Java (KW-JB1 and KW-JB2) into group A. Fourteen AFLP bands are responsible for the grouping (Table 4). An AFLP band 852 bp of primer pair 2 (E-AAG/M-CTA) was possessed only by cv. Klutuk Wulung from West Java while 13 other bands were possesed by all accessions of *M. balbisiana* except for this cultivar.

DISCUSSION

In this study, 22 selective AFLP primer pairs produced various sizes of AFLP bands at different polymorphic levels of 21 accessions of *M. balbisiana*. The different number of AFLP bands produced by each pair of primers was due to different combinations of three adjacent bases at either 5'- or 3'-end. The highest number of band (31 bands) resulted from primer pair 13 and the lowest number of band resulted from primer pair 11 which produced 14 bands (Table 2).

Polymorphism in AFLP occurs because DNA fragments with the same combination of three bases at 3'-end have different length compared to other accessions. In this study, primer pair 7 (E-ACA/M-CTG) was the highest polymorphic band producer (80%) while primer pair 19 (E-AGG/M-CTT) did not produce any polymorphic band.

The level of AFLP bands polymorphism in this study (13.8%) was much lower than the genetic diversity of *M. acuminata* in Malaysia which was 93% (Wong *et al*. 2001). El-Khishin *et al*. (2009) using 11 banana cultivars from Egypt detected 91.6% of polymorphism level while Opara *et al*. (2010) who analyzed seven cultivars in Oman using 12 primer pairs obtained 92.5% of polymorphism. The study of Youssef *et al.* (2011) on 40 wild and cultivated in genus *Musa* yielded 94% polymorphic AFLP bands. The higher polymorphism level of AFLP of banana cultivars or hybrids might occur because the banana have genomes combination of *M. acuminata* and/or with *M. balbisiana* genomes (i. e. AA, AAA, AAB, ABB, AAAB, AABB, or ABBB) (Teo *et al.* 2005), the structure of the genotype is more heterogeneous. Therefore, the possibility of restriction sites in the cultivars or hybrids genome was more varied.

Genetic diversity of 21 accessions *M. balbisiana* from Indonesia was 13.8% which was much lower compared to genetic diversity of *M. balbisiana* from

India (Uma *et al*. 2006) and Thailand (Wongniam *et al*. 2010). Genetic diversity of 16 accessions of *M. balbisiana* in India was 32% (Uma *et al.* 2006) while genetic diversity of 19 Thailand accessions was 33% (Wongniam *et al*. 2010). This result may support the hypothesis that the center of origin and center of diversity of *M. balbisiana* might came from South Asia and Indochina (de Langhe 2009; Aung *et al*. 2010).

Based on AFLP markers, wild *M. balbisiana* has slightly higher genetic diversity than the cultivated one. Although cultivated *M. balbisiana* has ability to produce seeds, people dominate in vegetative propagation of this banana. Vegetative propagation will influence the low level of genetic diversity (Paredas *et al*. 2007; Nemati *et al*. 2012). Selections in domesticated banana, on morphological traits, by people decrease the genetic diversity of domesticated bananas (Li *et al.* 2013). This explained the low dissimilarity index among the same cultivars from the same region (Tabel 3). In contrast, wild *M. balbisiana,* such as two accessions of Pisang Roti, which vegetatively propagated (by suckers) and generatively propagated (by seeds), have the highest dissimilarity index (0.066).

AFLP markers in this study showed genetic diversity of cultivated *M. balbisiana*. Genetic diversity among vegetatively propagated banana might happen because of mutation or the existence of transposon, and more than a third of open reading frame (ORF) in the genome are related to transposon (Heslop-Horrison & Schwarzacher 2007). Transposon will led genetic diversity without gamete recombination (Bennetzen 2005; Morgante *et al.* 2005) because of the ability of transposon in moving and inserting in chromosome.

Klutuk accessions from Yogyakarta (KLT-JG1 and KLT-JG2) had the lowest dissimilarity index (0.014) (Table 3). This could be explained because the cultivars were from the same origin (Yogyakarta) and vegetatively propagated. On the other hand, the highest dissimilarity index (0.152) was observed between wild *M. balbisiana* Pisang Roti (PR-SB1) from Sumatra and cv. Klutuk Wulung (KW-JB2) from West Java, and between wild *M. balbisiana* Pisang Roti (PR-SB1) from Sumatra and *M. balbisiana* var. *liukiuensis* (LIU-JP1) (introduced to Bogor Botanic Garden from Japan, and cultivated for more than 20 years). The pattern of clustering between accessions from same origin is clustered together (Lyngdoh *et al.* 2011).

Cluster analysis did not separate wild *M. balbisiana* and cultivated *M. balbisiana* accessions into different clusters (Figure 1). Pisang Pataga (PAT-SU1 and PAT-SU2) and var. *liukiunensis* (LIU-JP1 and LIU-JP2) mixed with other cultivated accessions in cluster I. Meanwhile cv. Klutuk Wulung from West Java (KW-JB1 and KW-JB2) formed cluster II. The pattern of dendrogram was formed because there were no specific AFLP bands for wild or cultivated *M. balbisiana.* The absence of specific bands for wild and cultivated *M.balbisiana* probably because there has been hybridization between both *M. balbisiana*. This opinion agrees to de Langhe (2009) hypothesis who mentioned that recent *M. balbisiana* in South Asia is the result of hybridization among *M. balbisiana*.

M. balbisiana cv. Klutuk Wulung in Indonesia seems to be the most varied genetically. The cultivar from West Java (KW-JB1 and KW-JB2) was separated from Cluster I, a group of Klutuk Wulung from Yogyakarta (KW-JG1 and KW-JG2) and 15 other accessions, at a coefficent similarity of 0.93. An AFLP band of 852 bp at primer pair 2 (E-AAG/M-CTA) was the unique band possessed only by Klutuk Wulung from West Java. On the other hand, 13 other bands, possesed by Klutuk Wulung-Yogyakarta (including by all other accessions of *M. balbisiana* in cluster I), were absent in Klutuk Wulung accessions from West Java.

The pattern of grouping based on PCA was similar to the pattern based on UPGMA clustering. While PCA is unfavorable for grouping of closely related accessions, because PCA create group based by some selected character meanwhile UPGMA as hierarchical analysis (Matsen & Evans 2013) which considered to similarity of all character among each accession, the grouping using UPGMA showed more detail clustering. For example, at similarity coefficient of 0.98 (Figure 1) cv. Klutuk from Yogyakarta (KLT-JG1 and KLT-JG2) formed a sub-cluster despite of their slightly difference of AFLP banding characters. In PCA, these accessions were hardly grouping, instead of mixing with the other 15 accessions (Figure 2).

A benefit of PCA method is that it is the easy way to find important characters from bulky data by compressing data to smaller data that represent the grouping (Ilin & Raiko 2010), such as AFLP bands which are responsible for grouping from bulky data. This benefit was not obtained by UPGMA method. Seventeen AFLP bands that have the most important role in grouping of 21 accessions *M. balbisiana* were generated by PCA. The primer pair 15 (E-ACC/M-CTA) was the most important one since it constitutes 9 out of 17 AFLP bands of first and second principal components (Table 4). Together with primer pair 8 (E-ACC/M-CTA) and primer pair 22 (E-ACA/M-

CAG) as part of the first principal component, they contribute the most in the grouping.

The study conclude that AFLP is a good method to evaluate the genetic diversity of *M. balbisiana*. Although genetic diversity of banana in Indonesia is not observed as high as in its center of diversity in South Asia, it shows that banana still develop naturally. By studying more *M. balbisiana* accessions from all parts of Indonesia it could certainly increase our knowledge about genetic diversity of this germplasm in the country. The information will not only be important in basic breeding program but also in germplasm management and conservation.

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