GENETIC VARIATION OF WILD *Musa acuminata* COLLA FROM INDONESIA

YUYU SURYASARI POERBA*, DIYAH MARTANTI AND FAJARUDIN AHMAD

Research Center for Biology, Indonesian Institute of Sciences, Bogor 16911, Indonesia

Received 25 August 2017 / Accepted 11 May 2018

ABSTRACT

Indonesia is the center of origin and diversity of *Musa acuminata* Colla, one of the progenitors of cultivated bananas today. However, the genetic variation of wild *M. acuminata* has not been studied extensively, thus this study aimed to assess the genetic variation of the Indonesian wild *M. acuminata* based on 10 RAPD (Random Amplified Polymorphic DNA) and 10 ISSR (Inter Simple Sequence Repeats) markers. The genetic properties of 17 populations of wild *M. acuminata* were analyzed by Popgene 1.32 based on Nei's unbiased measures of genetic identity and genetic distance. Of the 443 DNA bands produced, 425 (95.94%) were polymorphic. Cluster analysis of the combined data of RAPD and ISSR produced a dendrogram which separated the population of *M. acuminata* (A genome) from *M. balbisiana* (B genome), but not from *M. schizocarpa* (S genome). Nei's genetic distance of the 17 populations of wild *M. acuminata* ranged from 0.3676 to 0.1634. The highest genetic distance was observed between *M. acuminata* var *rutilifes* (from East Java) and *M. acuminata* var *sumatrana* (from West Sumatra). The percentages of polymorphic loci among the 17 populations of *M. acuminata* ranged from 9.93% to 39.73%. Nei's gene diversity (h) ranged from 0.041 to 0.1418. *M. acuminata* var *malaccensis* population was the most diverse among the researched 17 *M. acuminata*. The high level of genetic diversity of the wild *M. acuminata* from Indonesia emphasizes the need for conservation and preservation of the natural population and its use in the banana breeding program.

Keywords: genetic variation, Indonesia, ISSR, RAPD, wild *Musa acuminata*

INTRODUCTION

Bananas (*Musa* spp., family *Musaceae,* order Zingiberales) are important crops in Indonesia and the world. These are essential components of the diet and important sources of income for about 400 million people in over 120 countries in the tropical and sub-tropical zones (Jones 2000). The *Musa* genus*,* together with other genera (*Ensete* and *Musella*), is an herbaceous monocot plant that has two generic sections, namely: *Callimusa* and *Musa*. Section *Callimusa* is a combination of three former sections, i.e. *Australimusa* (x=10), *Ingentimusa* (x=7) and *Callimusa* (x=10), while section *Musa* is formerly *Eumusa* (x=11) and *Rhodochlamys* (x=11) (Häkkinen 2013). Genus *Musa* carries A genome (*M. acuminata* Colla), B genome (*M. balbisiana* Colla), S genome (*M. schizocarpa* Simmonds), and T genome (*M. textilis* Née). There is no observed natural hybridization between B, T, or S genome, but *M. acuminata* hybridizes with *M. balbisiana, M. schizocarpa,* and *M. textilis*. Although there are few cultivated bananas with S and T genomes, only two species are regarded as the ancestors of cultivated bananas, i.e., *M. acuminata* and *M. balbisiana* (Simmond & Shepherd 1955). Their fruits contain many seeds with a small amount of edible pulp. The wild-seeded bananas in the genus *Musa* represent some of the best sources of genetic diversity that can be used in the breeding of new edible bananas (Häkkinen & Wallace 2011).

Musa acuminata is a complex species that is divided into at least 7 subspecies with different geographic distributions (Simmonds & Shepherd 1955; Perrier *et al.* 2011). The 7 subspecies are *M. acuminata* subsp. *acuminata, M. acuminata* subsp. *errans* (Blanco) RV Valmayor, *M. acuminata* subsp. *halabanensis* (Meijer) M

*Corresponding author: yyspoerba@yahoo.com

Hotta, *M. acuminata* subsp. *malaccensis* (Ridl.) NW Simmonds, *M. acuminata* subsp. *microcarpa* (Becc.) NW Simmonds, *M. acuminata* subsp. *siamea* NW Simmonds, and *M. acuminata* subsp. *truncata* (Ridl.). Three of the 15 varieties of *Musa acuminata* from Indonesia (*M. acuminata* var. *alasensis, M. acuminata* var. *nakaii,* and *M. acuminata* var. *rutilifes*) described by Nasution (1991) based on their morphology, are classified as rare plants of Indonesia (Mogea *et al.* 2001). The wild *M. acuminata* serves an important role in banana breeding, specifically in providing genetic resources for disease resistance (Javed *et al.* 2004; Uma *et al.* 2006; Sutanto *et al.* 2014; Fraser-Smith *et al.* 2016).

Assessment of the genetic variation is a representative tool for the management of genetic resources and plant breeding program. Although genetic diversity of wild *M. acuminata* has been studied extensively in other reports (Wong *et al.* 2001; Wong *et al.* 2002; Bartos *et al.* 2005; Raboin *et al.* 2005; Li *et al.* 2010; Liu *et al.* 2010; Christelová *et al.* 2011; Perrier *et al.* 2011; D'Hont *et al.* 2012; Mukunthakumar *et al.* 2013; Čížková *et al.* 2015; Martanti *et al.* 2015; Sardos *et al.* 2016), only a small amount of the material used for these studies has originated from Indonesia. Thus, this study aimed to analyze the genetic variation of wild bananas within *Musa acuminata* coming from Indonesia by using the Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) markers.

RAPD has been used as a cost-effective method for analyzing genetic variation (Williams *et al.* 1990; Welsh & McClelland 1990). Although it has been reported for its limited reproducible results for DNA amplification, RAPD has been used for analyzing banana genetic diversity (Kaemmer *et al.* 1992; Howell *et al.* 1994; Bhat & Jarret 1995; Uma *et al.* 2006; Jain *et al.* 2007; Poerba & Ahmad 2010a, 2010b; Poerba *et al.* 2012; Poerba & Ahmad 2013; Faure et al. 1993; Pillay *et al.* 2000, 2006; Lamare & Rao 2015). Similarly, Inter Simple Sequence Repeats (ISSR) is a cost-effective method for studying genetic variation (with no need of previous genome sequence), fast, and is a dominant marker (Zietkiewicz *et al.* 1994). The use of dominant marker for assessing the genetic variation within individual genotype and population is promising because many polymorphic loci could be generated with ease at a short time even without information from the previous genome sequence (Nybom & Bartish 2000; Nybom 2004). Polymorphisms within individual genotype are mainly caused by different sequences in one or two primer binding sites and could indicate the presence or absence of the amplified products (Sperisen & Bucher 1998).

MATERIALS AND METHODS

Samples

A total of 209 samples (from 19 populations) of wild bananas, *M. acuminata* (A genome), *M. balbisiana* (B genome) and *M. schizocarpa* NW Simmonds (S genome) collected from different locations in Indonesia were used in this study (Table 1). Descriptions of all accessions were based on morphology as described by Nasution (1991) and IPGRI-INIBAB/CIRAD (1996).

Table 1 List of samples used in the study

No	Coll. number	Scientific name	Local name	Origin
1	PAN01	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Yaki, Pisang Wolai	Sulawesi Utara
2	PAN08	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Yaki, Pisang Wolai	Sulawesi Utara
3	PAN09	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Yaki, Pisang Wolai	Sulawesi Utara
4	A12X	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Yaki, Pisang Wolai	Sulawesi Utara
5	A42X	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Yaki, Pisang Wolai	Sulawesi Utara
6	A62X	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Yaki, Pisang Wolai	Sulawesi Utara
7	PA 01	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
8	PA 02	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
9	PA 03	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
10	PA 04	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
11	PA 07	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
12	PA 08	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
13	PA 09	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
14	PA 10	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
15	PA 11	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
16	PA 13	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua

17	PA 14	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
18	PA 15	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
19	PA 16	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
20	PA 17	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
21	PA 18	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
22	PA 19	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
23	PA 21	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
24	PA 23	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
25	PA 24	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
26	PA 25	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
27	PA 26	*Musa acuminata* Colla subsp. *banksii* (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
28	PA61	*Musa acuminata* Colla var. *acuminata*	Pisang Hutan	Sentani, Papua
29	PA79a	*Musa acuminata* Colla var. *acuminata*	Pisang Hutan	Manokwari, Papua Barat
30	PA79b	*Musa acuminata* Colla var. *acuminata*	Pisang Hutan	Manokwari, Papua Barat
31	PA84	*Musa acuminata* Colla var. *acuminata*	Pisang Hutan	Manokwari, Papua Barat
32	PA85	*Musa acuminata* Colla var. *acuminata*	Pisang Hutan	Manokwari, Papua Barat
33	PA86	*Musa acuminata* Colla var. *acuminata*	Pisang Hutan	Manokwari, Papua Barat
34	PA89	*Musa acuminata* Colla var. *acuminata*	Pisang Hutan	Manokwari, Papua Barat
35	PA90	*Musa acuminata* Colla var. *acuminata*	Pisang Hutan	Manokwari, Papua Barat
36	PA91	*Musa acuminata* Colla var. *acuminata*	Pisang Hutan	Manokwari, Papua Barat
37	PA96	*Musa acuminata* Colla var. *acuminata*	Pisang Hutan	Manokwari, Papua Barat
38	PN 07	*Musa acuminata* Colla var. *alasensis* Nasution	Pisang Runcim	Aceh Tenggara
39	PN 11	*Musa acuminata* Colla var. *alasensis* Nasution	Pisang Keken	Aceh Tenggara
40	PN 18	*Musa acuminata* Colla var. *alasensis* Nasution	Pisang Keken	Aceh Tenggara
41	PN 20	*Musa acuminata* Colla var. *alasensis* Nasution	Pisang Keken	Aceh Tenggara
42	PN 22	*Musa acuminata* Colla var. *alasensis* Nasution	Pisang Keken	Aceh Tenggara
43	PN 23	*Musa acuminata* Colla var. *alasensis* Nasution	Pisang Keken	Aceh Tenggara
44	PN 24	*Musa acuminata* Colla var. *alasensis* Nasution	Pisang Keken	Aceh Tenggara
45	PN 25	*Musa acuminata* Colla var. *alasensis* Nasution	Galuh Rincim	Aceh Tenggara
46	PN 27	*Musa acuminata* Colla var. *alasensis* Nasution	Galuh Rincim	Aceh Tenggara
47	PN 28	*Musa acuminata* Colla var. *alasensis* Nasution	Galuh Rincim	Aceh Tenggara
48	II 23B#3	*Musa acuminata* Colla var. *bantamensis* Nasution	Cau Kole	Jawa Barat
49	II23B#4	*Musa acuminata* Colla var. *bantamensis* Nasution	Cau Kole	Jawa Barat
50	II 23B#5	*Musa acuminata* Colla var. *bantamensis* Nasution	Cau Kole	Jawa Barat
51	APH 192	*Musa acuminata* Colla var. *bantamensis* Nasution	Cau Kole	Jawa Barat
52	APH 193	*Musa acuminata* Colla var. *bantamensis* Nasution	Cau Kole	Jawa Barat
53	APH 194	*Musa acuminata* Colla var. *bantamensis* Nasution	Cau Kole	Jawa Barat
54	APH 195	*Musa acuminata* Colla var. *bantamensis* Nasution	Cau Kole	Jawa Barat
55	APH 196	*Musa acuminata* Colla var. *bantamensis* Nasution	Cau Kole	Jawa Barat
56	IV2B#1	*Musa acuminata* Colla var. *breviformis* Nasution	Cau Kole	Jawa Barat
57	IV2B#2	*Musa acuminata* Colla var. *breviformis* Nasution	Cau Kole	Jawa Barat
58	IV2B#3	*Musa acuminata* Colla var. *breviformis* Nasution	Cau Kole	Jawa Barat
59	IV2B#4	*Musa acuminata* Colla var. *breviformis* Nasution	Cau Kole	Jawa Barat
60	IV2B#5	*Musa acuminata* Colla var. *breviformis* Nasution	Cau Kole	Jawa Barat
61	PHD25	*Musa acuminata* Colla var. *breviformis* Nasution	Cau Kole	Jawa Barat
62	PHD26	*Musa acuminata* Colla var. *breviformis* Nasution	Cau Kole	Jawa Barat
63	PSNA02	*Musa acuminata* Colla var. *cerifera* Nasution	Cau Kole	Jawa Barat
64	PSNA03	*Musa acuminata* Colla var. *cerifera* Nasution	Cau Kole	Jawa Barat
65	PSNA04	*Musa acuminata* Colla var. *cerifera* Nasution	Cau Kole	Jawa Barat
66	PSNA05	*Musa acuminata* Colla var. *cerifera* Nasution	Cau Kole	Jawa Barat
67	PSNA07	*Musa acuminata* Colla var. *cerifera* Nasution	Cau Kole	Jawa Barat
68	PSNA09	*Musa acuminata* Colla var. *cerifera* Nasution	Cau Kole	Jawa Barat
69	PNK18b	*Musa acuminata* Colla var. *flava* (Ridl.) Nasution	Pisang Hutan/Pisang Monyet	Kalimantan Tengah
70	PNK18c	*Musa acuminata* Colla var. *flava* (Ridl.) Nasution	Pisang Hutan/Pisang Monyet	Kalimantan Tengah
71	PNK19b	*Musa acuminata* Colla var. *flava* (Ridl.) Nasution	Pisang Hutan/Pisang Monyet	Kalimantan Tengah
72	PNK26	*Musa acuminata* Colla var. *flava* (Ridl.) Nasution	Pisang Hutan/Pisang Monyet	Kalimantan Tengah
73	PNk27b	*Musa acuminata* Colla var. *flava* (Ridl.) Nasution	Pisang Hutan/Pisang Monyet	Kalimantan Tengah
74	PNK28a	*Musa acuminata* Colla var. *flava* (Ridl.) Nasution	Pisang Hutan/Pisang Monyet	Kalimantan Tengah
75	PNK42a	*Musa acuminata* Colla var. *flava* (Ridl.) Nasution	Pisang Hutan/Pisang Monyet	Kalimantan Tengah
76	PNK43a	*Musa acuminata* Colla var. *flava* (Ridl.) Nasution	Pisang Hutan/Pisang Monyet	Kalimantan Tengah
77	PNK43b	*Musa acuminata* Colla var. *flava* (Ridl.) Nasution	Pisang Hutan/Pisang Monyet	Kalimantan Tengah
78	PNK46	*Musa acuminata* Colla var. *flava* (Ridl.) Nasution	Pisang Hutan/Pisang Monyet	Kalimantan Tengah
79	PNK48	*Musa acuminata* Colla var. *flava* (Ridl.) Nasution	Pisang Hutan/Pisang Monyet	Kalimantan Tengah
80	PNK51	*Musa acuminata* Colla var. *flava* (Ridl.) Nasution	Pisang Hutan/Pisang Monyet	Kalimantan Tengah
81	PNK84a	*Musa acuminata* Colla var. *flava* (Ridl.) Nasution	Pisang Hutan/Pisang Monyet	Kalimantan Tengah
82	PS 104	*Musa acuminata* Colla var. *halabanensis* (Meijer) Nasution	Pisang Hutan	Sumatera Selatan
83	PS 105	*Musa acuminata* Colla var. *halabanensis* (Meijer) Nasution	Pisang Hutan	Sumatera Selatan
84	PS 107	*Musa acuminata* Colla var. *halabanensis* (Meijer) Nasution	Pisang Hutan	Sumatera Selatan
85	PS 108	*Musa acuminata* Colla var. *halabanensis* (Meijer) Nasution	Pisang Hutan	Sumatera Selatan
86	PS 109	*Musa acuminata* Colla var. *halabanensis* (Meijer) Nasution	Pisang Hutan	Sumatera Selatan
87	PS 121	*Musa acuminata* Colla var. *halabanensis* (Meijer) Nasution	Pisang Hutan	Sumatera Selatan
88	PS 122	*Musa acuminata* Colla var. *halabanensis* (Meijer) Nasution	Pisang Hutan	Sumatera Selatan
89	PS 125	*Musa acuminata* Colla var. *halabanensis* (Meijer) Nasution	Pisang Hutan	Sumatera Selatan
90	PS 129	*Musa acuminata* Colla var. *halabanensis* (Meijer) Nasution	Pisang Hutan	Sumatera Selatan
91	PS 130	*Musa acuminata* Colla var. *halabanensis* (Meijer) Nasution	Pisang Hutan	Sumatera Selatan
92	PS02	*Musa acuminata* Colla var. *longipetiolata* Nasution	Pisang Tengkayak	Sumatera Selatan
93	PS04	*Musa acuminata* Colla var. *longipetiolata* Nasution	Pisang Tengkayak	Sumatera Selatan
94	PS06	*Musa acuminata* Colla var. *longipetiolata* Nasution	Pisang Tengkayak	Sumatera Selatan

95	PS07	*Musa acuminata* Colla var. *longipetiolata* Nasution	Pisang Tengkayak	Sumatera Selatan
96	PS08	*Musa acuminata* Colla var. *longipetiolata* Nasution	Pisang Tengkayak	Sumatera Selatan
97	PS10	*Musa acuminata* Colla var. *longipetiolata* Nasution	Pisang Tengkayak	Sumatera Selatan
98	PS11	*Musa acuminata* Colla var. *longipetiolata* Nasution	Pisang Tengkayak	Sumatera Selatan
99	PS12	*Musa acuminata* Colla var. *longipetiolata* Nasution	Pisang Tengkayak	Sumatera Selatan
100	PS21	*Musa acuminata* Colla var. *longipetiolata* Nasution	Pisang Tengkayak	Sumatera Selatan
101	PS25	*Musa acuminata* Colla var. *longipetiolata* Nasution	Pisang Tengkayak	Sumatera Selatan
102	PS26	*Musa acuminata* Colla var. *longipetiolata* Nasution	Pisang Tengkayak	Sumatera Selatan
103	PS27	*Musa acuminata* Colla var. *longipetiolata* Nasution	Pisang Tengkayak	Sumatera Selatan
104	PS28	*Musa acuminata* Colla var. *longipetiolata* Nasution	Pisang Tengkayak	Sumatera Selatan
105	PS29	*Musa acuminata* Colla var. *longipetiolata* Nasution	Pisang Tengkayak	Sumatera Selatan
106	APH 40	*Musa acuminata* Colla var. *malaccensis* (Ridl.) Nasution	Cau Kole	Jawa Barat
107	APH 41	*Musa acuminata* Colla var. *malaccensis* (Ridl.) Nasution	Cau Kole	Jawa Barat
108	APH 42	*Musa acuminata* Colla var. *malaccensis* (Ridl.) Nasution	Cau Kole	Jawa Barat
109	APH 43	*Musa acuminata* Colla var. *malaccensis* (Ridl.) Nasution	Cau Kole	Jawa Barat
110	APH 258	*Musa acuminata* Colla var. *malaccensis* (Ridl.) Nasution	Cau Kole	Jawa Barat
111	APH 259	*Musa acuminata* Colla var. *malaccensis* (Ridl.) Nasution	Cau Kole	Jawa Barat
112	APH 260	*Musa acuminata* Colla var. *malaccensis* (Ridl.) Nasution	Cau Kole	Jawa Barat
113	APH 266	*Musa acuminata* Colla var. *malaccensis* (Ridl.) Nasution	Cau Kole	Jawa Barat
114	APH 301	*Musa acuminata* Colla var. *malaccensis* (Ridl.) Nasution	Cau Kole	Jawa Barat
115	APH 302	*Musa acuminata* Colla var. *malaccensis* (Ridl.) Nasution	Cau Kole	Jawa Barat
116	APH 303	*Musa acuminata* Colla var. *malaccensis* (Ridl.) Nasution	Cau Kole	Jawa Barat
117	PHD-124	*Musa acuminata* Colla var. *malaccensis* (Ridl.) Nasution	Cau Kole	Jawa Barat
118	II 17B #1	*Musa acuminata* Colla var. *malaccensis* (Ridl.) Nasution	Cau Kole	Jawa Barat
119	II 17B #2	*Musa acuminata* Colla var. *malaccensis* (Ridl.) Nasution	Cau Kole	Jawa Barat
120	II 17B #4	*Musa acuminata* Colla var. *malaccensis* (Ridl.) Nasution	Cau Kole	Jawa Barat
121	II 17B #5	*Musa acuminata* Colla var. *malaccensis* (Ridl.) Nasution	Cau Kole	Jawa Barat
122	APH 416	*Musa acuminata* Colla var. *malaccensis* (Ridl.) Nasution	Cau Kole	Jawa Barat
123	APH 419	*Musa acuminata* Colla var. *malaccensis* (Ridl.) Nasution	Cau Kole	Jawa Barat
124	APH 420	*Musa acuminata* Colla var. *malaccensis* (Ridl.) Nasution	Cau Kole	Jawa Barat
125	PNK07	*Musa acuminata* Colla var. *microcarpa* (Becc.) Nasution	Pisang Saluku	Kalimantan Tengah
126	PNK08a	*Musa acuminata* Colla var. *microcarpa* (Becc.) Nasution	Pisang Saluku	Kalimantan Tengah
127	PNK08b	*Musa acuminata* Colla var. *microcarpa* (Becc.) Nasution	Pisang Saluku	Kalimantan Tengah
128	PNK13a	*Musa acuminata* Colla var. *microcarpa* (Becc.) Nasution	Pisang Saluku	Kalimantan Tengah
129	PNK13b	*Musa acuminata* Colla var. *microcarpa* (Becc.) Nasution	Pisang Saluku	Kalimantan Tengah
130	PNK32a	*Musa acuminata* Colla var. *microcarpa* (Becc.) Nasution	Pisang Saluku	Kalimantan Tengah
131	PNK32b	*Musa acuminata* Colla var. *microcarpa* (Becc.) Nasution	Pisang Saluku	Kalimantan Tengah
132	PNK14a	*Musa acuminata* Colla var. *microcarpa* (Becc.) Nasution	Pisang Saluku	Kalimantan Tengah
133	PNK14b	*Musa acuminata* Colla var. *microcarpa* (Becc.) Nasution	Pisang Saluku	Kalimantan Tengah
134	APH235	*Musa acuminata* Colla var. *nakaii* Nasution	Cau Kole Beureum	Jawa Barat
135	APH236	*Musa acuminata* Colla var. *nakaii* Nasution	Cau Kole Beureum	Jawa Barat
136	APH314	*Musa acuminata* Colla var. *nakaii* Nasution	Cau Kole Beureum	Jawa Barat
137	PSNAH1a	*Musa acuminata* Colla var. *nakaii* Nasution	Cau Kole Beureum	Jawa Barat
138	PSNAH 06	*Musa acuminata* Colla var. *nakaii* Nasution	Cau Kole Beureum	Jawa Barat
139	PSNAH 14	*Musa acuminata* Colla var. *nakaii* Nasution	Cau Kole Beureum	Jawa Barat
140	PSNAH 15	*Musa acuminata* Colla var. *nakaii* Nasution	Cau Kole Beureum	Jawa Barat
141	PAA32	*Musa acuminata* Colla var. *sumatrana* (Becc.) Nasution	Pisang Karuak	Sumatera Barat
142	PAA48	*Musa acuminata* Colla var. *sumatrana* (Becc.) Nasution	Pisang Karuak	Sumatera Barat
143	PAA49	*Musa acuminata* Colla var. *sumatrana* (Becc.) Nasution	Pisang Karuak	Sumatera Barat
144	PAA50	*Musa acuminata* Colla var. *sumatrana* (Becc.) Nasution	Pisang Karuak	Sumatera Barat
145	PAA69	*Musa acuminata* Colla var. *sumatrana* (Becc.) Nasution	Pisang Karuak	Sumatera Barat
146	PAA70	*Musa acuminata* Colla var. *sumatrana* (Becc.) Nasution	Pisang Karuak	Sumatera Barat
147	PAA94	*Musa acuminata* Colla var. *sumatrana* (Becc.) Nasution	Pisang Palapah	Sumatera Barat
148	PAA95	*Musa acuminata* Colla var. *sumatrana* (Becc.) Nasution	Pisang Palapah	Sumatera Barat
149	PAA96	*Musa acuminata* Colla var. *sumatrana* (Becc.) Nasution	Pisang Palapah	Sumatera Barat
150	PAA107	*Musa acuminata* Colla var. *sumatrana* (Becc.) Nasution	Pisang Palapah	Sumatera Barat
151	PAA108	*Musa acuminata* Colla var. *sumatrana* (Becc.) Nasution	Pisang Palapah	Sumatera Barat
152	PAA110	*Musa acuminata* Colla var. *sumatrana* (Becc.) Nasution	Pisang Palapah	Sumatera Barat
153	PAA190	*Musa acuminata* Colla var. *sumatrana* (Becc.) Nasution	Pisang Palapah	Sumatera Barat
154	PAA200	*Musa acuminata* Colla var. *sumatrana* (Becc.) Nasution	Pisang Palapah	Sumatera Barat
155	PH 04	*Musa acuminata* Colla var. *rutilifes* Nasution	Pisang Cici	Jawa Timur
156	PH 05	*Musa acuminata* Colla var. *rutilifes* Nasution	Pisang Cici	Jawa Timur
157	PH 06	*Musa acuminata* Colla var. *rutilifes* Nasution	Pisang Cici	Jawa Timur
158	PH 07	*Musa acuminata* Colla var. *rutilifes* Nasution	Pisang Cici	Jawa Timur
159	PH 08	*Musa acuminata* Colla var. *rutilifes* Nasution	Pisang Cici	Jawa Timur
160	PH 09	*Musa acuminata* Colla var. *rutilifes* Nasution	Pisang Cici	Jawa Timur
161	PH 12	*Musa acuminata* Colla var. *rutilifes* Nasution	Pisang Cici	Jawa Timur
162	PH 15	*Musa acuminata* Colla var. *rutilifes* Nasution	Pisang Cici	Jawa Timur
163	PH 16	*Musa acuminata* Colla var. *rutilifes* Nasution	Pisang Cici	Jawa Timur
164	PH 19	*Musa acuminata* Colla var. *rutilifes* Nasution	Pisang Cici	Jawa Timur
165	PAR 72	*Musa acuminata* Colla var. *tomentosa* (K.Sch.) Nasution	Unti Darek	Sulawesi Selatan
166	PAR 73	*Musa acuminata* Colla var. *tomentosa* (K.Sch.) Nasution	Unti Darek	Sulawesi Selatan
167	PAR 74	*Musa acuminata* Colla var. *tomentosa* (K.Sch.) Nasution	Unti Darek	Sulawesi Selatan
168	PAR 76	*Musa acuminata* Colla var. *tomentosa* (K.Sch.) Nasution	Unti Darek	Sulawesi Selatan
169	PAR 78	*Musa acuminata* Colla var. *tomentosa* (K.Sch.) Nasution	Unti Darek	Sulawesi Selatan
170	PAR 93	*Musa acuminata* Colla var. *tomentosa* (K.Sch.) Nasution	Unti Darek	Sulawesi Selatan
171	PAR 94	*Musa acuminata* Colla var. *tomentosa* (K.Sch.) Nasution	Unti Darek	Sulawesi Selatan
172	PAR 100	*Musa acuminata* Colla var. *tomentosa* (K.Sch.) Nasution	Unti Darek	Sulawesi Selatan

173	PAR 101	*Musa acuminata* Colla var. *tomentosa* (K.Sch.) Nasution	Unti Darek	Sulawesi Selatan
174	PAR 103	*Musa acuminata* Colla var. *tomentosa* (K.Sch.) Nasution	Unti Darek	Sulawesi Selatan
175	PAR 106	*Musa acuminata* Colla var. *tomentosa* (K.Sch.) Nasution	Unti Darek	Sulawesi Selatan
176	III 21A#1	*Musa acuminata* Colla var. *zebrina* (v. Houtte) Nasution	Cau Kole Beureum	Jawa Barat
177	III 21A#3	*Musa acuminata* Colla var. *zebrina* (v. Houtte) Nasution	Cau Kole Beureum	Jawa Barat
178	III 21A#4	*Musa acuminata* Colla var. *zebrina* (v. Houtte) Nasution	Cau Kole Beureum	Jawa Barat
179	III 21A#5	*Musa acuminata* Colla var. *zebrina* (v. Houtte) Nasution	Cau Kole Beureum	Jawa Barat
180	III20G#1	*Musa acuminata* Colla var. *zebrina* (v. Houtte) Nasution	Cau Kole Beureum	Jawa Barat
181	III20G#2	*Musa acuminata* Colla var. *zebrina* (v. Houtte) Nasution	Cau Kole Beureum	Jawa Barat
182	III20G#3	*Musa acuminata* Colla var. *zebrina* (v. Houtte) Nasution	Cau Kole Beureum	Jawa Barat
183	III20G#5	*Musa acuminata* Colla var. *zebrina* (v. Houtte) Nasution	Cau Kole Beureum	Jawa Barat
184	II23A#3	*Musa acuminata* Colla var. *zebrina* (v. Houtte) Nasution	Cau Kole Beureum	Jawa Barat
185	II23B#1	*Musa acuminata* Colla var. *zebrina* (v. Houtte) Nasution	Cau Kole Beureum	Jawa Barat
186	II23B#2	*Musa acuminata* Colla var. *zebrina* (v. Houtte) Nasution	Cau Kole Beureum	Jawa Barat
187	PA 64	*Musa schizocarpa* NW Simmonds	Pisang Hutan	Papua
188	PA 66	*Musa schizocarpa* NW Simmonds	Pisang Hutan	Papua
189	PA 67	*Musa schizocarpa* NW Simmonds	Pisang Hutan	Papua
190	PA 68	*Musa schizocarpa* NW Simmonds	Pisang Hutan	Papua
191	PA 69	*Musa schizocarpa* NW Simmonds	Pisang Hutan	Papua
192	PA 76	*Musa schizocarpa* NW Simmonds	Pisang Hutan	Papua
193	PA 87	*Musa schizocarpa* NW Simmonds	Pisang Hutan	Papua
194	PA 92	*Musa schizocarpa* NW Simmonds	Pisang Hutan	Papua
195	PA 93	*Musa schizocarpa* NW Simmonds	Pisang Hutan	Papua
196	PA 95	*Musa schizocarpa* NW Simmonds	Pisang Hutan	Papua
197	PA 103	*Musa schizocarpa* NW Simmonds	Pisang Hutan	Papua
198	II20C#1	*Musa balbisiana* Colla	Pisang Klutuk	Jawa Barat
199	II20C#2	*Musa balbisiana* Colla	Pisang Klutuk	Jawa Barat
200	PHD22	*Musa balbisiana* Colla	Pisang Klutuk	Jawa Barat
201	PHD97	*Musa balbisiana* Colla	Pisang Klutuk	Jawa Barat
202	PHD101	*Musa balbisiana* Colla	Pisang Klutuk	Jawa Barat
203	PHD106	*Musa balbisiana* Colla	Pisang Klutuk	Jawa Barat
204	P001	*Musa balbisiana* Colla	Pisang Klutuk	Bali
205	PAR62	*Musa balbisiana* Colla	Pisang Klutuk	Sulawesi Selatan
206	PAR63	*Musa balbisiana* Colla	Pisang Klutuk	Sulawesi Selatan
207	II18C#1	*Musa balbisiana* Colla	Pisang Klutuk Sukun	Yogyakarta
208	II18C#3	*Musa balbisiana* Colla	Pisang Klutuk Sukun	Yogyakarta
209	I3C#1	*Musa balbisiana* Colla	Pisang Klutuk Wulung	Yogyakarta

DNA Extraction and Primers

The total DNA was extracted from young leaves by Cetyltrimetylammonium bromide (CTAB) method (Syamkumar *et al.* 2003) with modification, i.e., using 4% CTAB and an addition of 0.1 g Polyvinylpyrrolidone (PVP) for each reaction. The analyses were conducted with the RAPD method (Williams *et al.* 1990). Ten selected RAPD primers (OPA-02, OPA-07, OPA-13, OPA-18, OPB-07, OPB-18, OPN-06, OPN-12, OPN-14, OPU-06 (Operon Technology Ltd., USA) and ten selected ISSR primers (University of British Columbia, Canada), i.e. UBC-811, UBC-814, UBC-815, UBC-822, UBC-823, UBC-826, UBC-834, UBC-835, UBC-843 and UBC-844, were used in this study.

DNA Amplification

PCR reactions for RAPD analyses were conducted at a total volume of 15 µL, containing 0.2 nM dNTPs; 1X reaction buffer; 2 mM $MgCl_2$; 25 ng DNA sample; 1 pmole single primer; and 1 unit Taq DNA polymerase (Promega Go Taq Flexy DNA Polymerase) using Thermocycler (Takara Bio Inc., Japan, Model TP600/TP650) for 45 cycles. The first heating was at a temperature of 94 °C for 2 minutes, then followed by 45 cycles which consisted of 1 minute of denaturation at 94 °C, 1 minute of annealing at 36 °C, and 2 minutes of extension at 72 °C. When the 45 cycles ended, a 5-minute DNA extension at 72 °C and cooling at 25 °C followed. PCR reactions for ISSR analyses were conducted as follows: 5 minutes of denaturation at 94 °C, followed by 30 cycles of 1 minute of denaturation at 94 °C, 45 seconds of annealing at 50 °C, and 2 minutes of DNA extensions at 72 °C. When the 30 cycles were finished, the reaction was terminated at 5 minutes of extension at 72 °C.

Visualization of RAPD and ISSR Bands

Electrophoresis was carried out on 2.0% agarose gel using ATTO mini gel apparatus and was run at 100 volts for 50 minutes. RAPD and ISSR bands were stained using 1X Gel RedTM staining solution (Biotium) for 30 minutes. The bands were then observed under UV light and photographed using a gel documentation system (ATTO).

Data Analysis

Only the clear and visible RAPD and ISSR bands were selected and scored for the presence (1) and absence (0) of a band. Band's size was calculated based on 100 bp plus (Fermentas) DNA marker. Genetic distance was analyzed by Popgene 1.32 (Yeh *et al.* 1997) based on Nei's (1978) unbiased measures of genetic identity and genetic distance. Cluster analysis was performed with the unweighted pair group method with arithmetic averages (UPGMA) based on Nei's (1972) genetic distance using Popgene 1.32 (Yeh *et al.* 1997). The dendrograms produced were viewed with Treeview (Page 1998).

RESULTS AND DISCUSSION

RAPD and ISSR Bands

From a total of 443 RAPD and ISSR bands with sizes ranging from 100-2600 bp, 425 polymorphic bands (95.95%) were produced. The highest number of bands (27 bands) was produced by OPN-12, while the least number of bands (15 bands) was made by UBC-843. The primers of OPU-06, UBC-826, UBC-835, UBC-843, and UBC-844 generated the highest (100%) polymorphic bands. Each primer produced 22.15 bands, with an average of 21.15 polymorphic bands (Table 2). Primer OPN-14 produced the lowest number of polymorphic bands (90.91%). These results suggested that each wild banana genotype observed had DNA variations.

In this study, more polymorphic bands were produced than those of Mukunthakumar *et al.* (2013) which produced 87.5% RAPD polymorphism bands, Das *et al.* (2018) which produced 53.83% ISSR polymorphic bands, and Lamere and Rao (2015) which produced 85.09% and 90.06% for RAPD and ISSR polymorphic bands, respectively. RAPD is easy, fast and affordable and is widely used for banana diversity studies (Kaemmer *et al.* 1992; Howell *et al.* 1994; Bhat & Jarret 1995; Uma *et al.* 2006; Jain *et al.* 2007; Poerba & Ahmad, 2010a; Poerba *et al.* 2012; Poerba & Ahmad 2013; Faure *et al.* 1993; Pillay *et al.* 2000, 2006; Lamare & Rao 2015), however, ISSR provide higher reproducibility (Bornet & Branchard 2001).

The polymorphic level of arbitrary markers, RAPD and ISSR, is based on the primer sequences and either the position or number of annealing regions in the template sequences (William *et al.* 1990). Therefore, analyses of different genetic properties and markers result in the diversity of polymorphic content. The combined markers of RAPD and ISSR and the more genotypes used in this study were probably the most influential factors that contributed to the high polymorphism. This result showed that the 20 primers were effective in bringing out differences among the wild *Musa* genotypes.

Table 2 Number of amplified DNA bands using 20 primers of RAPD and ISSR of wild *Musa acuminata* from Indonesia

No	Primer code	Nucleotide sequence (5' - 3')	∑DNA bands	∑Polymorphic bands	(%)	Size (bp)
1	OPA-02	TGCCGAGCTG	26	25	96.15	100-1500
2	OPA-07	GAAACGGGTG	22	21	95.45	200-2500
3	OPA-13	CAGCACCCAC	22	21	95.45	250-2200
4	OPA-18	AGGTGACCGT	23	22	95.65	250-1600
5	OPB-07	GGTGACGCAG	21	20	95.24	300-1600
6	OPB-18	CCACAGCAGT	25	23	92	250-2200
7	OPN-06	GAGACGCACA	24	23	95.83	200-2400
8	OPN-12	CACAGACACC	27	26	96.3	200-2600
9	OPN-14	TCGTGCGGGT	22	20	90.91	300-3000
10	OPU-06	ACCTTTGCGG	23	23	100	150-2200
11	UBC-811	GAG AGA GAG AGA GAG AC	22	21	95.45	300-2200
12	UBC-814	CTC TCT CTC TCT CTC TA	23	22	95.65	350-2000
13	UBC-815	CTC TCT CTC TCT CTC TG	22	21	95.45	250-2200
14	UBC-822	TCT CTC TCT CTC TCT CA	22	20	90.91	250-2000
15	UBC-823	TCT CTC TCT CTC TCT CC	18	17	94.44	300-2000
16	UBC-826	ACA CAC ACA CAC ACA CC	22	22	100	300-2200
17	UBC-834	AGA GAG AGA GAG AGA GYT	23	22	95.65	200-1800
18	UBC-835	AGA GAG AGA GAG AGA GYC	22	22	100	250-2000
19	UBC-843	CTC TCT CTC TCT CTC TRA	15	15	100	250-2200
20	UBC-844	CTC TCT CTC TCT CTC TRC	19	19	100	250-2200
		Total	443	425		

Population Genetic Diversity of *M. acuminata*

Nei's (1973) genetic distances of the 17 populations of *M. acuminata* ranged from 0.3676 to 0.1634, with 20.42% genetic diversity. The lowest genetic distance was observed between *M. acuminata* subsp. *banksii* (from Papua) and *M. acuminata* var *acuminata* (from Papua), and between *M. acuminata* var. *flava* (from Kalimantan) and *M. acuminata* var. *microcarpa* (from Kalimantan), while the highest genetic distance was observed between *M. acuminata* var *rutilifes* (from East Java) and *M. acuminata* var *sumatrana* (from West Sumatra) (Table 3). *Musa acuminata* ssp. *banksii* and *M. acuminata* var. *acuminata* both originating from Papua are geographically isolated from the other subspecies or varieties, and *M. acuminata* subsp. *banksii* is a preferential autogamous (Carreel *et al.* 2002). Similarly, *M. acuminata* var. *flava* and *M. acuminata* var. *microcarpa* that both originated from Kalimantan are isolated from other subspecies or varieties, yet they constitute the same species according to De Langhe *et al.* (2009). The lower genetic distance among populations in the same geographic region is probably because the isolation drives the evolution of a particular trait with similar genetic properties, in contrast to the distinct environment or geographical condition that resulted in other adaptation patterns and genetic properties.

The 17 populations of *M. acuminata* exhibited varied genetic properties. The polymorphic loci ranged from 9.93% to 39.73%. It means that genetic variation within each population of *M. acuminata* varies from the lowest 9.93% (within *M. acuminata* var. *acuminata*) to the highest 39.73% (within *M. acuminata* var. *malaccensis*). Nei's (1973) gene diversity (h) ranged from 0.041 to 0.1418. *Musa acuminata* var *malaccensis* had the highest percentages (39.73%) of polymorphic loci and Nei's (1973) gene diversity (0.2106) among the 17 population of wild *M. acuminata*; while *M. acuminata* var. *acuminata* had the lowest (9.93%) (Table 4). *M. balbisiana* had the lowest (7.67%) polymorphic loci among 19 populations. The fact that *M. balbisiana* did not originate from Indonesia but was introduced and naturalized (De Langhe *et al.* 2009), probably explains why its genetic variation in Indonesia was the lowest. All the 209 samples had na, ne, Nei's gene diversity, and Shannon Information Index values of 1.9594, 1.5277, 0.3126, and 0.4727, respectively (Table 4).

Table 3 Nei's (1973) genetic identity and genetic distance of 19 populations of *Musa* spp.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	****	0.7744	0.7766	0.6773	0.7369	0.7204	0.7032	0.7509	0.6841	0.7262	0.7616	0.7478	0.7028	0.6738	0.6731	0.7014	0.7127	0.6876	0.6611
2	0.2557	****	**0.8366**	0.6818	0.7502	0.6872	0.7109	0.7575	0.6933	0.7411	0.7286	0,7650	0.7019	0.7011	0.6906	0.7649	0.7367	0.6982	0.6684
3	0.2528	**0.1784**	****	0.6587	0.7516	0.7002	0.6765	0.7626	0.6773	0.7037	0.7447	0.7199	0.7127	0.6581	0.6546	0.7482	0.7536	0.6682	0.6481
4	0.3897	0.3831	0.4175	****	0.7094	0.6533	0.6863	0.7035	0.7054	0.6921	0.7198	0.6819	0.7126	0.6571	0.6802	0.6582	0.6836	0.7102	0.6502
5	0.3053	0.2874	0.2856	0.3434	****	0.7564	0.7587	0.7493	0.7272	0.7718	0.7823	0.7257	7.7381	0.7533	0.6966	0.7196	0,8080	0,7230	0.6854
6	0,3280	0.3571	0.3564	0.4258	0.2792	****	0.7059	0.7011	0.6794	0,6870	0.7577	0.6777	0.6793	0.6658	0.6731	0,6840	0.7097	0.6821	0.6423
7	0.3521	0.3413	0.3909	0.3765	0.2762	0.3483	****	0.6955	0.7042	0.7257	0.7662	0.7024	0.7397	0.7062	0.6745	0,6620	0.7648	0.7087	0.6607
8	0.2864	0.2778	0,2710	0.3517	0.2887	0.3552	0.3631	****	0.7056	0.7169	0.7758	**0.8306**	0.7016	0.7311	0,7110	0.7451	0.7348	0.7362	0.6828
9	0.3797	0.3663	0.3896	0,3490	0.3186	0.3865	0.3507	0.3487	****	0.7296	0.7823	0.6827	0.6658	0.6794	0.6589	0.6986	0.7253	0.6947	0.6527
10	0,3200	0.2997	0.3514	0,3680	0,2590	0.3755	0.3206	0.3328	0.3153	****	0.7458	0.6971	0.6972	0.6606	0.6994	0.6725	0.7376	0.7146	0.6427
11	0.2724	0.3166	0.2948	0.3288	0.2456	0.2775	0.2664	0.2538	0.2455	0.2933	****	0.7496	0.7327	0.7126	0.7268	0.7454	0.7672	0.7702	0.7024
12	0.2906	0.2679	0.3287	0.3829	0.3206	0.3891	0.3533	**0.1857**	0.3817	0.3609	0.2883	****	0.6969	0.7261	0,7020	0.7425	0.7355	0.7066	0.6804
13	0.3527	0,3540	0.3387	0.3388	0.3036	0.3868	0.3015	0.3543	0.4068	0.3607	0,3110	0.3611	****	0.6954	0.6729	0.6815	0.7442	0.7048	0,6490
14	0.3948	0.3551	0.4184	0,4200	0.2833	0.4067	0.3478	0.3132	0.3866	0.4146	0.3388	0,3200	0.3633	****	**0.6313**	0.6869	0.7279	0.6663	0.6657
15	0.3958	0.3702	0.4237	0.3854	0.3616	0.3958	0.3938	0.3411	0.4172	0.3575	0.3191	0.3538	0.3962	**0.4599**	****	0.6735	0,6700	0.6885	0.6651
16	0.3547	0,2680	0.2901	0.4183	0.3291	0.3798	0.4125	0.2943	0.3587	0.3967	0.2939	0.2977	0.3834	0.3755	0.3952	****	0.7266	0,6820	0,6240
17	0.3387	0.3055	0.2829	0.3804	0.2132	0.3429	0.2681	0.3081	0.3211	0.3044	0.2651	0.3071	0.2955	0.3176	0.4005	0.3194	****	0,7140	0.6668
18	0.3746	0.3593	0.4031	0.3422	0.3244	0.3826	0.3443	0.3062	0.3642	0,3360	0.2611	0.3473	0.3499	0,4060	0.3733	0.3827	0.3368	****	0.6917
19	0.4139	0.4028	0.4337	0.4304	0.3778	0.4427	0.4144	0.3815	0.4267	0,4420	0.3533	0,3850	0.4324	0,4070	0.4078	0.4716	0.4052	0.3686	****

Notes: Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

1 = *Musa acuminata* Colla subsp. *banksii* (F. Muell.) NW Simmonds (Sulawesi Utara), 2 = *Musa acuminata* Colla subsp. *banksii* (F. Muell.) NW Simmonds (Papua), 3 = *M. acuminata* Colla var. *acuminata*, 4 = *M. acuminata* Colla var. *alasensis* Nasution, 5= *M. acuminata* Colla var. *bantamensis* Nasution, 6 = *M. acuminata* Colla var. *breviformis* Nasution, 7 = *M. acuminata* Colla var. *cerifera* (Backer) Nasution, 8 = *M. acuminata* Colla var. *flava* (Ridl.) Nasution, 9 = *M. acuminata* Colla var. *halabanensis* (Meijer) Nasution, 10 = *M. acuminata* Colla var. *longipetiolata* Nasution, 11 = *M. acuminata* Colla var. *malaccensis* (Ridl.) Nasution, 12 = *M. acuminata* Colla var. *microcarpa* (Becc.) Nasution, 13 = *M. acuminata* Colla var. *nakaii* Nasution, 14 = *M. acuminata* Colla var. *rutilifes* Nasution, 15 = *M. acuminata* Colla var. *sumatrana* (Becc.) Nasution, 16 = *M. acuminata* Colla var. *tomentosa* (K.Sch.) Nasution, 17 = *M. acuminata* Colla var. *zebrina* (v. Houtte) Nasution, 18 = *M. schizocarpa* NW Simmonds, and 19 = *M. balbisiana* Colla.

Table 4 Genetic properties of 19 populations of *Musa* spp. from Indonesia

No	Population	Sample size	na	ne	h	I	Number of polymorphic loci	(%)
1	*M. acuminata* subsp. *banksii* (Sulawesi)	6	1.2099	1.1557	0.0855	0.1239	93	20.99%
2	*M. acuminata* subsp. *banksii* (Papua)	21	1.2777	1.0511	0.0387	0.0714	123	27.77%
3	*M. acuminata* var. *acuminata* (Papua)	10	1.0993	1.0725	0.041	0.0596	44	9.93%
4	*M. acuminata* var. *alasensis*	10	1.1174	1.0749	0.043	0.0637	52	11.74%
5	*M. acuminata* var. *bantamensis*	8	1.2054	1.1463	0.082	0.1196	91	20.54%
6	*M. acuminata* var. *breviformis*	7	1.14	1.1127	0.0603	0.0863	62	14.00%
7	*M. acuminata* var. *cerifera*	6	1.1693	1.1147	0.0645	0.0948	75	16.93%
8	*M. acuminata* var. *flava*	13	1.2754	1.1773	0.1024	0.1516	122	27.54%
9	*M. acuminata* var. *halabanensis*	10	1.1332	1.084	0.0484	0.0719	59	13.32%
10	*M. acuminata* var. *longipetiolata*	14	1.1558	1.1061	0.0608	0.0894	69	15.58%
11	*M. acuminata* var. *malaccensis*	19	1.3973	1.2458	0.1418	0.2106	176	39.73%
12	*M. acuminata* var. *microcarpa*	9	1.1287	1.0884	0.0502	0.0736	57	12.87%
13	*M. acuminata* var. *nakaii*	7	1.1986	1.1476	0.0816	0.1183	88	19.86%
14	*M. acuminata* var. *rutilifes*	10	1.1174	1.0984	0.0533	0.0758	52	11.74%
15	*M. acuminata* var. *sumatrana*	14	1.1219	1.0813	0.0471	0.0693	54	12.19%
16	*M. acuminata* var. *tomentosa*	11	1.1309	1.0725	0.0432	0.0657	58	13.09%
17	*M. acuminata* var. *zebrina*	11	1.2483	1.1139	0.0719	0.1129	110	24.83%
18	*M. schizocarpa*	11	1.1174	1.05	0.0321	0.051	52	11.74%
19	*M. balbisiana*	12	1.0767	1.0436	0.0257	0.0389	34	7.67%
		209	1.9594	1.5277	0.3126	0.4727	425	95.94%

Notes: * na = Observed number of alleles
* ne = Effective number of alleles (Kimura & Crow 1964)
* h = Nei's (1973) gene diversity
* I = Shannon's Information index (Lewontin 1972)

Population diversity indexes such as allele frequencies (na and ne), Nei's gene diversity (h), and Shannon's information index (I) define the genome composition of a population. In this study, the observed number of alleles (na) and the effective number of alleles (ne) from combined RAPD and ISSR data were lower than those of other studies (Lamare & Rao 2015; Mukunthakumar *et al.* 2013). The combination of different markers and genotypes produced an observed diversity at a certain level of alleles; thus, these results are different from those of previous studies. In this study, na and ne values ranged from 1.0767 to 1.3973 and from 1.0436 to 1.072, respectively. Lamare and Rao (2015) used more markers (58) on 25 different genotypes. Nei's (1973) gene diversity within population ranged from 0.057 to 0.1418. Total Nei's (1973) gene diversity was 0.3126. *Musa acuminata* var *malaccensis* had the highest percentage of polymorphic loci and Nei's (1973) gene diversity among the 17 populations of wild *M. acuminata*, with the values of na, ne, h, I and percentage of polymorphism at 1.3973, 1.2458, 0.1418, 0.2106 and 39.73%, respectively. *M. acuminata* var. *acuminata* had the lowest percentage of polymorphic loci and Nei's (1973) gene diversity, with the values of na, ne, h, I, and percentage of polymorphism at 1.0993, 1.0725, 0.0596 and 9.95%, respectively.

Cluster Analysis

Cluster analysis of the combined data of RAPD and ISSR produced a dendrogram which separated the population of *M. acuminata* (1-17) from *M. balbisiana* (19), but not from *M. schizocarpa* (18) (Fig. 1). However, *M. acuminata* (carrying A genome) and *M. schizocarpa* (carrying S genome) were in the same cluster. This finding may need further study, specifically to identify the marker specified for each genome with more stringent markers such as AFLP (Wong *et al.* 2001; Opara *et al.* 2010), SSR (Christelová *et al.* 2017) or specific locus markers (Volkaert 2011) to resolve the clustering between *M. acuminata* and *M. schizocarpa*.

Cluster 1 consisted of 6 populations, namely: 1 *M. acuminata* subsp *banksii* (North Sulawesi), 2 (*M. acuminata* subsp *banksii* (Papua), 3 *M. acuminata* var *acuminata* (Papua), 8 *M. acuminata* var *flava* (Central Kalimantan), 12 *M. acuminata* var *microcrapa* (Central Kalimantan) and 16 *M. acuminata* var *tomentosa* (North Sulawesi). Cluster 2 consisted of 6 populations of 5 *M. acuminata* var *bantamensis*, 17 *M. acuminata* var *zebrina*, 11 *M. acuminata* var *malaccensis*, 7 *M. acuminata* var *cerifera*, 10 *M. acuminata* var *longipetiolata*, and *M. acuminata* var *nakaii*. The other 6 populations (14 *M. acuminata* var *rutilifes*, 18 *M. schizocarpa*, 6 *M. acuminata* var *breviformis*, 9 *M. acuminata* var *halabanensis*, 15 *M. acuminata* var *sumatrana*, 4 *M. acuminata* var *alasensis*) were separated within cluster of *M. acuminata–M schizocarpa* (Fig. 1). The results were different from those of Nasution's study (1991) using morphological characters. Based on morphological characters, *M. acuminata* was divided into two groups. Group 1 consisted of varieties of *alasensis, halabanensis, acuminata, tomentosa, flava, and sumatrana.* The other group was varieties of *nakaii, zebrina, cerifera, longipetiolata, bantamensis, rutilifes, breviformis, malaccensis, and microcarpa.* Some of the varieties, such as *flava* and *microcarpa,* which were in different morphological groups were in the same cluster (1). Similarly, varieties of *halabanensis* and *rutilifes,* which were in different morphological group were also in the same cluster (3).

The different genetic properties observed in wild *M. acuminata* from Indonesia confirmed that the species is genetically diverse. The wild *M. acuminata* collection in this study showed a highly valuable material that needs further studies specifically, on population genetic diversity and trait variation, with subsequent studies on combination with cultivated banana for the banana breeding program. Utilization of more advance molecular marker is essential for future studies on the conservation and preservation of the wild *M. acuminata*. The variability of *M. acuminata* in Indonesia is so important and even higher than that of *M. acuminata* reported in neighboring countries (Pollefeys *et al.* 2004). The intra-specific variation of *Musa acuminata* is far more complex than what is known from its subspecies classification and can provide more promising parent forms/cultivars for breeding than had been exploited until now (MusaNet 2016). Studies on a more advanced molecular marker are essential for determining multilocus markers that can correlate alleles between domesticated bananas and their wild relatives (Volkaert 2011). This study is a cornerstone for a better understanding of Indonesian banana genetics, which is useful for banana breeding and conservation programs.

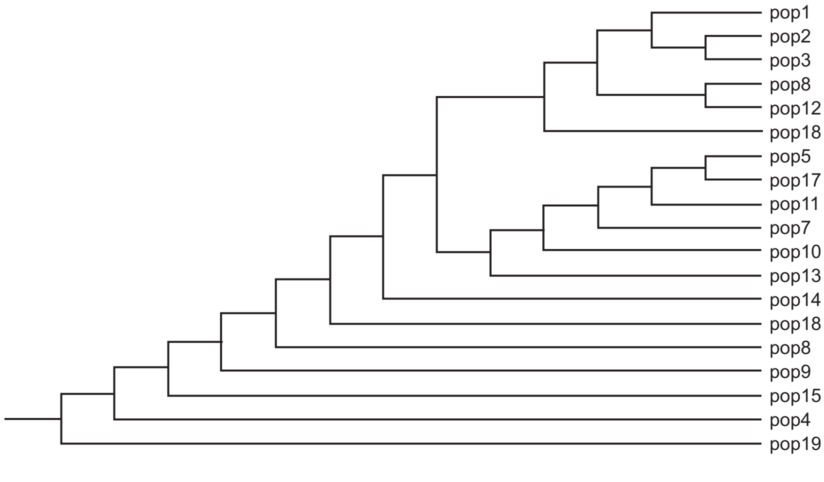

Figure 1 Dendrogram of the 19 populations of *Musa* spp. from Indonesia based on Nei's genetic distance

Notes: 1 = *Musa acuminata* Colla subsp. *banksii* (F. Muell.) NW Simmonds (Sulawesi Utara), 2 = *Musa acuminata* Colla subsp. *banksii* (F. Muell.) NW Simmonds (Papua), 3 = *M. acuminata* Colla var. *acuminata*, 4 = *M. acuminata* Colla var. *alasensis* Nasution, 5 = *M. acuminata* Colla var. *bantamensis* Nasution, 6 = *M. acuminata* Colla var. *breviformis* Nasution, 7 = *M. acuminata* Colla var. *cerifera* (Backer) Nasution, 8 = *M. acuminata* Colla var. *flava* (Ridl.) Nasution, 9 = *M. acuminata* Colla var. *halabanensis* (Meijer) Nasution, 10 = *M. acuminata* Colla var. *longipetiolata* Nasution, 11 = *M. acuminata* Colla var. *malaccensis* (Ridl.) Nasution, 12 = *M. acuminata* Colla var. *microcarpa* (Becc.) Nasution, 13 = *M. acuminata* Colla var. *nakaii* Nasution, 14 = *M. acuminata* Colla var. *rutilifes* Nasution, 15 = *M. acuminata* Colla var. *sumatrana* (Becc.) Nasution, 16 = *M. acuminata* Colla var. *tomentosa* (K.Sch.) Nasution, 17 = *M. acuminata* Colla var. *zebrina* (v. Houtte) Nasution, 18 = *M. schizocarpa* NW Simmonds, and 19 = *M. balbisiana* Colla.

CONCLUSION

The twenty RAPD and ISSR markers used in this study generated a high percentage of polymorphic bands (95.95%). Seventeen populations of wild *M. acuminata* from Indonesia possessed different genetic properties; with genetic distances (Nei 1973) ranging from 0.3676 to 0.1634 and genetic diversity of 20.42%. The highest genetic distance (0.1634) was observed between *M. acuminata* var *rutilifes* (from East Java) and *M. acuminata* var *sumatrana* (from West Sumatra). *Musa acuminata* var *malaccensis* had the highest percentage (39.73%) of polymorphic loci and Nei's (1973) gene diversity (0.2106) among the 17 populations of wild *M. acuminata*, while *M. acuminata* var. *acuminata* had the lowest (9.93% and 0.0596).

Cluster analysis of combined data of RAPD and ISSR produced a dendrogram which separated the population of *M. acuminata* (A genome) from *M. balbisiana* (B genome), but not from *M. schizocarpa* (S genome). The genetic variation of wild *M. acuminata* from Indonesia confirmed that the species is genetically diverse. The samples used in this study are a highly valuable economic material that needs further studies on its population genetic diversity, trait variations and subsequently, on its combination with the cultivated banana for the enhancement of the banana breeding program.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the funding support from the Indonesian Institute of Sciences through its Competitive Programs, and the technical support and assistance of Ms. Herlina from Plant Genetic Laboratory, Research Center for Biology, Indonesian Institute of Sciences.

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