

1 **ACCEPTED MANUSCRIPT**

2

3 GENETIC VARIATION OF WILD *Musa acuminata* COLLA FROM INDONESIA BASED ON
4 RAPD AND ISSR MARKERS

5

6 Poerba YS, Martanti D, Ahmad F

7

8 DOI: 10.11598/btb.2019.26.2.896

9

10 To appear in : BIOTROPIA Vol. 26 No. 2 August 2019 Issue

11

12 Received date : 25 August 2017

13 Accepted date : 11 May 2018

14

15 **This manuscript has been accepted for publication in BIOTROPIA journal. It is unedited,**
16 **thus, it will undergo the final copyediting and proofreading process before being published in**
17 **its final form.**

18

ACCEPTED MANUSCRIPT

19 GENETIC VARIATION OF WILD *Musa acuminata* COLLA FROM INDONESIA
20 BASED ON RAPD AND ISSR MARKERS

21
22 **Yuyu Suryasari Poerba^{*}, Diyah Martanti and Fajarudin Ahmad**

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

24 ^{*}Corresponding author, email: yyspoerba@yahoo.com
25

26 Running title: Genetic of wild *Musa acuminata* Colla from Indonesia
27

28 **ABSTRACT**

29 *Musa acuminata* Colla is one of the progenitors of cultivated bananas today, and Indonesia
30 is the center of origin and diversity of *M. acuminata*. Genetic variation of wild *M. acuminata* from
31 Indonesia has not been studied extensively. This study was conducted to assess genetic variation of
32 wild *M. acuminata* from Indonesia based on 10 RAPD (Random Amplified Polymorphic DNA) and
33 10 ISSR (Inter Simple Sequence Repeats) markers. The genetic properties of 17 populations of wild
34 *M. acuminata* were analyzed by Popgene 1.32 based on Nei's unbiased measures of genetic identity
35 and genetic distance. A total of 443 DNA bands were produced, 425 (95.94%) of which were
36 polymorphic. Cluster analysis of combined data of RAPD and ISSR produced a dendrogram which
37 separated the population of *M. acuminata* (A genome) from *M. balbisiana* (B genome), but not
38 from *M. schizocarpa* (S genome). Genetic distance (Nei, 1978) of the 17 populations of wild *M.*
39 *acuminata* ranged from 0.3676 to 0.1634. The highest genetic distance was observed between *M.*
40 *acuminata* var *rutilifes* (from East Java) and *M. acuminata* var *sumatrana* (from West Sumatra).
41 The percentages of polymorphic loci among the 17 populations of *M. acuminata* ranged from
42 9.93% to 39.73%. Nei's (1973) gene diversity (h) ranged from 0.041 to 0.1418. *M. acuminata* var
43 *malaccensis* population was the most diverse population among the 17 *M. acuminata* populations
44 studied. The high level of genetic diversity of the wild *M. acuminata* from Indonesia emphasizes
45 the need for conservation and preservation of the natural population and its use in banana breeding
46 program.
47

48 **Keywords:** Indonesia, genetic variation, ISSR, RAPD, wild *Musa acuminata*
49

50 **INTRODUCTION**

51 Bananas (*Musa* spp., family *Musaceae*, order Zingiberales) are important crops in Indonesia
52 and the world. They are essential components of the diet and important sources of income for about
53 400 million people in over 120 countries in the tropical and sub-tropical zones (Jones, 2000). The
54 *Musa* genus, together with other genera (*Ensete* and *Musella*), is an herbaceous monocot plant.
55 There are two sections within the genus, *Callimusa* and *Musa*. Section *Callimusa* is a combination
56 of three former sections i.e. *Australimusa* (x=10), *Ingentimusa* (x=7) and *Callimusa* (x=10), while
57 section *Musa* is formerly *Eumusa* (x=11) and *Rhodochlamys* (x=11) (Häkkinen, 2013). Genus
58 *Musa* carries A genome (*M. acuminata* Colla), B genome (*M. balbisiana* Colla), S genome (*M.*
59 *schizocarpa* Simmonds), and T genome (*M. textilis* Née). There is no observed natural
60 hybridization between B, T, or S genome, but *M. acuminata* hybridizes with *M. balbisiana*, *M.*

61 *schizocarpa* and *M. textilis*. Although there are few cultivated bananas with S and T genomes, only
62 two species are regarded as the ancestors of cultivated bananas, i.e. *M. acuminata* and *M.*
63 *balbisiana* (Simmond and Shepherd, 1955). The fruits of these plants contain many seeds with a
64 small amount of edible pulp. The wild-seeded bananas in the genus *Musa* represent some of the best
65 sources of genetic diversity that can be used in the breeding of new edible bananas (Häkkinen and
66 Wallace, 2011).

67 *Musa acuminata* is a complex species that is divided into at least 7 sub-species with
68 different geographic distributions (Simmonds and Shepherd, 1955; Perrier et al., 2011). The 7 sub-
69 species are: *M. acuminata* subsp. *acuminata*, *M. acuminata* subsp. *errans* (Blanco) RV Valmayor,
70 *M. acuminata* subsp. *halabanensis* (Meijer) M Hotta, *M. acuminata* subsp. *malaccensis* (Ridl.) NW
71 Simmonds, *M. acuminata* subsp. *microcarpa* (Becc.) NW Simmonds, *M. acuminata* subsp. *siamea*
72 NW Simmonds, and *M. acuminata* subsp. *truncata* (Ridl.). Nasution (1991) described 15 varieties
73 of *M. acuminata* from Indonesia based on their morphology, and three of them (*M. acuminata* var.
74 *alasensis*, *M. acuminata* var *nakaii* and *M. acuminata* var. *rutilifes*) are classified as rare plants of
75 Indonesia (Mogea et al. 2001). Wild *M. acuminata* serves an important role in banana breeding,
76 specifically in providing genetic resources for disease resistance (Javed et al., 2004; Uma et al.,
77 2006; Sutanto et al., 2014; Fraser-Smith et al., 2016).

78 Assessment of genetic variation is a representative tool for the management of genetic
79 resources and plant breeding program. Although genetic diversity of wild *M. acuminata* has been
80 studied extensively (Wong et al., 2001; Wong et al., 2002, Bartos et al., 2005; Raboin et al., 2005;
81 Li et al., 2010; Liu et al., 2010; Christelová et al., 2011; Perrier et al., 2011; D'Hont et al., 2012;
82 Mukunthakumar et al., 2013; Čížková et al., 2015; Martanti et al., 2015; Sardos et al., 2016), only a
83 small amount of the material used for these studies originated from Indonesia. Herein, we study and
84 analyze the genetic variation of wild bananas within *Musa acuminata* species from Indonesia using
85 Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR)
86 markers.

87 RAPD has been used as a cost-effective method for analyzing genetic variation (Williams et
88 al., 1990; Welsh and McClelland, 1990). Although it has been reported for limited reproducible
89 results for DNA amplification, RAPD has been used for analyzing banana genetic diversity
90 (Kaemmer et al., 1992; Howell et al., 1994; Bhat and Jarret, 1995; Uma et al., 2006; Jain et al.,
91 2007; Poerba and Ahmad, 2010a, 2010b, 2012; Poerba and Ahmad, 2013; Faure et al., 1993; Pillay
92 et al., 2000, 2006; Lamare and Rao, 2015). Likewise, Inter Simple Sequence Repeats (ISSR) is a
93 cost-effective method for studying genetic variation, with no need of previous genome sequence,
94 fast, and a dominant marker (Zietkiewicz et al., 1994). The use of dominant marker for assessing
95 the genetic variation within individual genotype and population is promising because many

96 polymorphic loci could be generated with ease at a short time without previous genome sequence
97 information (Nybom and Bartish, 2000; Nybom, 2004). Polymorphisms within individual genotype
98 are mainly caused by different sequences in one or two primer binding sites and could indicate the
99 presence or absence of the amplified products (Sperisen and Bucher, 1998).

101 MATERIALS AND METHODS

102 Materials and Methods

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

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

114 PCR reactions for RAPD analyses were conducted at a total volume of 15 μ l, containing 0.2
115 nM dNTPs; 1X reaction buffer; 2 mM MgCl₂; 25 ng DNA sample; 1 pmole single primer; and
116 1 unit Taq DNA polymerase (Promega Go Taq Flexy DNA Polymerase) using Thermocycler
117 (Takara) for 45 cycles. The first heating was at a temperature of 94°C for 2 minutes, then followed
118 by 45 cycles which consisted of 1 minute of denaturation at 94°C, 1 minute of annealing at 36°C,
119 and 2 minutes of extension at 72°C. After the 45 cycles ended, 5 minutes of DNA extension at 72°C
120 and cooling at 25°C followed. PCR reactions for ISSR analyses were conducted as follows: 5
121 minutes of denaturation at 94°C, followed by 30 cycles of 1 minute of denaturation at 94°C, 45
122 seconds of annealing at 50°C, and 2 minutes of DNA extensions at 72°C. After the 30 cycles were
123 finished, the reaction was terminated by 5 minutes of extension at 72°C.

125 Visualization of RAPD and ISSR Bands

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

130

131 **Data Analysis**

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

139

140

RESULTS AND DISCUSSION

141 **RAPD and ISSR bands**

142 A total of 443 RAPD and ISSR bands with sizes ranging from 100-2600 bp were produced,
143 and 425 bands (95.95%) were polymorphic. The highest number of bands (27 bands) was produced
144 by OPN-12, while the least number of bands (15 bands) was made by UBC-843. Primers of OPU-
145 06, UBC-826, UBC-835, UBC-843, and UBC-844 generated 100% polymorphic bands. Each
146 primer produced 22.15 bands, with an average of 21.15 polymorphic bands (Table 2). Primer OPN-
147 14 produced the lowest number of polymorphic bands (90.91%). The results suggested that each
148 wild banana genotype observed had DNA variations.

149 In this study, more polymorphism bands were produced than those of Mukunthakumar et al.
150 (2013), which produced 87.5% RAPD polymorphism bands - Das et al. (2018) which produced
151 53.83% ISSR polymorphism bands and Lamere & Rao (2015) which produced 85.09% and 90.06%
152 for RAPD and ISSR polymorphic bands respectively. RAPD is easy, fast and affordable and widely
153 used for banana diversity studies (Kaemmer et al., 1992; Howell et al., 1994; Bhat and Jarret, 1995;
154 Uma et al., 2006; Jain et al., 2007; Poerba and Ahmad, 2010a; Poerba et al., 2010), 2012; Poerba
155 and Ahmad, 2013; Faure et al., 1993; Pillay et al., 2000, 2006; Lamare and Rao, 2015), meanwhile
156 ISSR provide higher reproducibility (Bornet and Branchard, 2012).

157 The polymorphism level of arbitrary markers, RAPD and ISSR, is according to primer
158 sequences and either the position or number of annealing region in the template sequences (William
159 et al., 1990). Therefore, studies with different genetic property and marker will results in diversity
160 of polymorphic content. The combined markers of RAPD and ISSR and the more genotypes used in
161 this study were probably the most influenced factors that contributed to the high polymorphism.
162 This result showed that the 20 primers were effective in bringing out differences among the wild
163 *Musa* genotypes.

164

165

166

167

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

No	Primer code	Nucleotide sequence (5' - 3')	Σ DNA bands	Σ 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	ACCTTTGCCG	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		

170

171 Population genetic diversity of *M. acuminata*

172 Nei's genetic distances (1973) of the 17 populations of *M. acuminata* ranged from 0.3676 to
 173 0.1634, with 20.42% genetic diversity. The lowest genetic distance was observed between *M.*
 174 *acuminata* subsp. *banksii* (from Papua) and *M. acuminata* var *acuminata* (from Papua), *M.*
 175 *acuminata* var. *flava* (from Kalimantan) and *M. acuminata* var. *microcarpa* (from Kalimantan),
 176 while the highest genetic distance observed between *M. acuminata* var *rutilifes* (from East Java)
 177 and *M. acuminata* var *sumatrana* (from West Sumatra) (Table 3). *Musa acuminata* ssp. *banksii* and
 178 *M. acuminata* var. *acuminata* originated in Papua, geographically isolated from the other subspecies
 179 or varieties, and *M. acuminata* subsp. *banksii* is a preferential autogamous (Carreel et al., 2002).
 180 Likewise *M. acuminata* var. *flava* and *M. acuminata* var. *microcarpa* originated in Kalimantan,
 181 isolated from other subspecies or varieties, they constitute the same species according to De Langhe
 182 et al. (2009). The lower genetic distance of populations in the same geographic region is might be
 183 because isolation drives the evolution for a particular trait with similar genetic properties, in
 184 contrast distinct environment or geographical condition will lead in to other adaptation pattern and
 185 genetic properties.

186

187

188

189

190

191 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	****

192

193 Notes:

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

195 1 = *Musa acuminata* Colla subsp. *banksii* (F. Muell.) NW Simmonds (Sulawesi Utara); 2 = *Musa acuminata* Colla
 196 subsp. *banksii* (F. Muell.) NW Simmonds (Papua); 3 = *M. acuminata* Colla var. *acuminata*, 4 = *M. acuminata* Colla
 197 var. *alasensis* Nasution, 5 = *M. acuminata* Colla var. *bantamensis* Nasution, 6 = *M. acuminata* Colla var. *breviformis*
 198 Nasution, 7 = *M. acuminata* Colla var. *cerifera* (Backer) Nasution, 8 = *M. acuminata* Colla var. *flava* (Ridl.) Nasution,
 199 9 = *M. acuminata* Colla var. *halabanensis* (Meijer) Nasution, 10 = *M. acuminata* Colla var. *longipetiolata* Nasution, 11
 200 = *M. acuminata* Colla var. *malaccensis* (Ridl.) Nasution, 12 = *M. acuminata* Colla var. *microcarpa* (Becc.) Nasution,
 201 13 = *M. acuminata* Colla var. *nakaii* Nasution, 14 = *M. acuminata* Colla var. *rutilifera* Nasution, 15 = *M. acuminata*
 202 Colla var. *sumatrana* (Becc.) Nasution, 16 = *M. acuminata* Colla var. *tomentosa* (K.Sch.) Nasution 17 = *M. acuminata*
 203 Colla var. *zebrina* (v. Houtte) Nasution, 18 = *M. schizocarpa* NW Simmonds, dan 19 = *M. balbisiana* Colla.

204

205 Genetic properties of 17 populations of *M. acuminata* varied. The percentages of
 206 polymorphic loci for the 17 populations of *M. acuminata* ranged from 9.93% to 39.73%. It means
 207 that genetic variation within each population of *M. acuminata* varies from the lowest 9.93% (within
 208 *M. acuminata* var. *acuminata*) to the highest 39.73% (within *M. acuminata* var. *malaccensis*).
 209 Nei's (1973) gene diversity (h) ranged from 0.041 to 0.1418. *Musa acuminata* var *malaccensis* had
 210 the highest percentages of polymorphic loci and Nei's (1973) gene diversity among the 17
 211 population of wild *M. acuminata*; while *M. acuminata* var. *acuminata* had the lowest (Table 4). *M.*
 212 *balbisiana* had the lowest polymorphic loci among 19 populations. The fact that *M. balbisiana* was
 213 not originated from Indonesia, and it was introduced and naturalized (De Langhe, 2009), could be
 214 the possible explanation why its genetic variation in Indonesia was the lowest. All the 209 samples
 215 had na, ne, Nei's gene diversity, and Shannon Information Index values of 1.9594, 1.5277, 0.3126,
 216 and 0.4727, respectively (Table 4).

217

218

219

220

221

222 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	<i>M. acuminata</i> subsp. <i>banksii</i> (Sulawesi)	6	1.2099	1.1557	0.0855	0.1239	93	20.99%
2	<i>M. acuminata</i> subsp. <i>banksii</i> (Papua)	21	1.2777	1.0511	0.0387	0.0714	123	27.77%
3	<i>M. acuminata</i> var. <i>acuminata</i> (Papua)	10	1.0993	1.0725	0.041	0.0596	44	9.93%
4	<i>M. acuminata</i> var. <i>alasanensis</i>	10	1.1174	1.0749	0.043	0.0637	52	11.74%
5	<i>M. acuminata</i> var. <i>bantamensis</i>	8	1.2054	1.1463	0.082	0.1196	91	20.54%
6	<i>M. acuminata</i> var. <i>breviformis</i>	7	1.14	1.1127	0.0603	0.0863	62	14.00%
7	<i>M. acuminata</i> var. <i>cerifera</i>	6	1.1693	1.1147	0.0645	0.0948	75	16.93%
8	<i>M. acuminata</i> var. <i>flava</i>	13	1.2754	1.1773	0.1024	0.1516	122	27.54%
9	<i>M. acuminata</i> var. <i>halabanensis</i>	10	1.1332	1.084	0.0484	0.0719	59	13.32%
10	<i>M. acuminata</i> var. <i>longipetiolata</i>	14	1.1558	1.1061	0.0608	0.0894	69	15.58%
11	<i>M. acuminata</i> var. <i>malaccensis</i>	19	1.3973	1.2458	0.1418	0.2106	176	39.73%
12	<i>M. acuminata</i> var. <i>microcarpa</i>	9	1.1287	1.0884	0.0502	0.0736	57	12.87%
13	<i>M. acuminata</i> var. <i>nakaii</i>	7	1.1986	1.1476	0.0816	0.1183	88	19.86%
14	<i>M. acuminata</i> var. <i>rutilifera</i>	10	1.1174	1.0984	0.0533	0.0758	52	11.74%
15	<i>M. acuminata</i> var. <i>sumatrana</i>	14	1.1219	1.0813	0.0471	0.0693	54	12.19%
16	<i>M. acuminata</i> var. <i>tomentosa</i>	11	1.1309	1.0725	0.0432	0.0657	58	13.09%
17	<i>M. acuminata</i> var. <i>zebrina</i>	11	1.2483	1.1139	0.0719	0.1129	110	24.83%
18	<i>M. schizocarpa</i>	11	1.1174	1.05	0.0321	0.051	52	11.74%
19	<i>M. balbisiana</i>	12	1.0767	1.0436	0.0257	0.0389	34	7.67%
		209	1.9594	1.5277	0.3126	0.4727	425	95.94%

223 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)]

224

225 Population diversity indexes such as allele frequencies (na and ne), Nei's gene diversity (h),
 226 and Shannon's information index (I) define the genome composition of a population. In this study,
 227 observed number of alleles (na) and effective number of allele (ne) from combined RAPD and ISSR
 228 data were lower than those of other research (Lamare and Rao, 2015; Mukunthakumar et al., 2013).
 229 Combination of a marker and genotypes will produce observed and diversity of alleles in a certain
 230 level, this is make a sense that our result are different to the previous studies. This could be due to
 231 different markers and genotypes used. Lamare and Rao (2015) used more markers (58) on 25
 232 different genotypes. In this study, na and ne values ranged from 1.0767 to 1.3973 and from 1.0436
 233 to 1.072, respectively. Nei's (1973) gene diversity within population ranged from 0.057 to 0.1418.
 234 Total Nei's (1973) gene diversity was 0.3126. *Musa acuminata* var *malaccensis* had the highest
 235 percentage of polymorphic loci and Nei's (1973) gene diversity among the 17 populations of wild

236 *M. acuminata*, with the values of na, ne, h, I and percentage of polymorphism of 1.3973, 1.2458,
237 0.1418, 0.2106, and 39.73%, respectively. *M. acuminata* var. *acuminata* had the lowest percentage
238 of polymorphic loci and Nei's (1973) gene diversity, with the values of na, ne, h, I, and percentage
239 of polymorphism of 1.0993, 1.0725, 0.0596, and 9.95%, respectively.

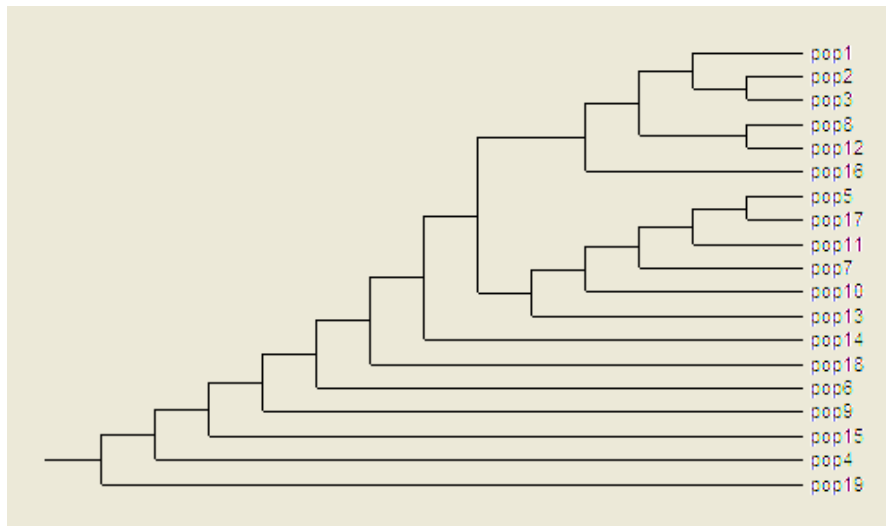
240

241 **Cluster Analysis**

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

250 Cluster 1 consisted of 6 populations, namely: 1 *M. acuminata* subsp *banksii* (North
251 Sulawesi), 2 (*M. acuminata* subsp *banksii* (Papua), 3 *M. acuminata* var *acuminata* (Papua), 8 *M.*
252 *acuminata* var *flava* (Central Kalimantan), 12 *M. acuminata* var *microcarpa* (Central Kalimantan)
253 and 16 *M. acuminata* var *tomentosa* (North Sulawesi). Cluster 2 consisted of 6 populations of 5 *M.*
254 *acuminata* var *bantamensis*, 17 *M. acuminata* var *zebrina*, 11 *M. acuminata* var *malaccensis*, 7 *M.*
255 *acuminata* var *cerifera*, 10 *M. acuminata* var *longipetiolata*, and *M. acuminata* var *nakaii*. The rest
256 6 populations (14 *M. acuminata* var *rutilifes*, 18 *M. schizocarpa*, 6 *M. acuminata* var *breviformis*, 9
257 *M. acuminata* var *halabanensis*, 15 *M. acuminata* var *sumatrana*, 4 *M. acuminata* var *alasisensis*)
258 were separated within cluster of *M. acuminata*–*M. schizocarpa* (Figure 1). The results were different
259 from those of Nasution's study (1991) using morphological characters. Based on morphological
260 characters, *M. acuminata* was divided into two groups. Group 1 were varieties of *alasisensis*,
261 *halabanensis*, *acuminata*, *tomentosa*, *flava* and *sumatrana*. The other group were varieties of
262 *nakaii*, *zebrina*, *cerifera*, *longipetiolata*, *bantamensis*, *rutilifes*, *breviformis*, *malaccensis* and
263 *microcarpa*. Some of the varieties such as *flava*, and *microcarpa* which was in different
264 morphological group were in the same cluster (1). Likewise, varieties of *halabanensis*, and *rutilifes*
265 which was in different morphological group were in the same cluster (3).

266



267

268

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

271 Notes: 1 = *Musa acuminata* Colla subsp. *banksii* (F. Muell.) NW Simmonds (Sulawesi Utara); 2 = *Musa acuminata*
272 Colla subsp. *banksii* (F. Muell.) NW Simmonds (Papua); 3 = *M. acuminata* Colla var. *acuminata*, 4 = *M. acuminata*
273 Colla var. *alasensis* Nasution, 5 = *M. acuminata* Colla var. *bantamensis* Nasution, 6 = *M. acuminata* Colla var.
274 *breviformis* Nasution, 7 = *M. acuminata* Colla var. *cerifera* (Backer) Nasution, 8 = *M. acuminata* Colla var. *flava*
275 (Ridl.) Nasution, 9 = *M. acuminata* Colla var. *halabanensis* (Meijer) Nasution, 10 = *M. acuminata* Colla var.
276 *longipetiolata* Nasution, 11 = *M. acuminata* Colla var. *malaccensis* (Ridl.) Nasution, 12 = *M. acuminata* Colla var.
277 *microcarpa* (Becc.) Nasution, 13 = *M. acuminata* Colla var. *nakaii* Nasution, 14 = *M. acuminata* Colla var. *rutilifera*
278 Nasution, 15 = *M. acuminata* Colla var. *sumatrana* (Becc.) Nasution, 16 = *M. acuminata* Colla var. *tomentosa* (K.Sch.)
279 Nasution 17 = *M. acuminata* Colla var. *zebrina* (v. Houtte) Nasution, 18 = *M. schizocarpa* NW Simmonds, and 19 =
280 *M. balbisiana* Colla.
281

282 The different properties of genetic variation of wild *M. acuminata* from Indonesia confirm
283 that genetically the wild *M. acuminata* is diverse. The wild *M. acuminata* collection in this study is
284 high valuable material that need further population genetic diversity as as well as trait variation
285 studies, subsequently combined with cultivated banana study to contribute in banana breeding
286 program. Utilization of more advance molecular marker is essential for the future studies, for
287 example, the need for conservation and preservation of the wild *M. acuminata*. Further studies of
288 population genetic as well as genetic diversity of wild *M. acuminata* using more advanced
289 molecular techniques would be needed for better understanding of genetics for use in conservation
290 and banana breeding programs. The variability of *M. acuminata* is important and far above the
291 number of *M. acuminata* reported in neighbouring countries (Pollefeys et al., 2004). The infra-
292 specific variation of *Musa acuminata* is far more complex than is understood from its subspecies
293 classification, and can provide more promising parent forms/cultivars for breeding than have been
294 exploited until now (MusaNet, 2016). Utilization of more advance molecular marker is essential for
295 the future studies, for example, multi locus markers can correlate alleles between domesticated
296 banana from the wild relatives (Volkaert, 2011) or SNPs which can provide a high resolution of
297 durum wheat genetic diversity (Ren et al., 2013). This study is a corner stone for better

298 understanding of Indonesian banana genetics which useful for banana breeding and conservation
299 programs.

300

301

CONCLUSIONS

302 Twenty RAPD and ISSR markers used in this study could generate a high percentage of
303 polymorphic bands (95.95%). A total of 17 populations of wild *M. acuminata* from Indonesia have
304 different genetic properties with genetic distances (Nei 1973) ranging from 0.3676 to 0.1634, with
305 20.42% genetic diversity. The highest genetic distance was observed between *M. acuminata* var
306 *rutilifes* (from East Java) and *M. acuminata* var *sumatrana* (from West Sumatra). *Musa acuminata*
307 var *malaccensis* had the highest percentage of polymorphic loci and Nei's (1973) gene diversity
308 among the 17 populations of wild *M. acuminata*, while *M. acuminata* var. *acuminata* had the
309 lowest.

310 Cluster analysis of combined data of RAPD and ISSR produced a dendrogram which
311 separated the population of *M. acuminata* (A genome) from *M. balbisiana* (B genome), but not
312 from *M. schizocarpa* (S genome). The different properties of genetic variation of wild *M.*
313 *acuminata* from Indonesia confirm that genetically the wild *M. acuminata* is diverse. The wild *M.*
314 *acuminata* collection in this study is high valuable material that need further population genetic,
315 genetic diversity as well as trait variation studies, subsequently combined with cultivated banana
316 study to contribute in banana breeding program.

317

318

ACKNOWLEDGEMENTS

319 The authors would like to acknowledge the funding support of the Indonesian Institute of
320 Sciences through its Competitive Programs, and the technical support and assistance of Ms. Herlina.

321

322

REFERENCES

- 323 Bartos J, Alkhimova O, Dolezelova M, Langhe E, Dolezel J. 2005. Nuclear genome size and
324 genomic distribution of ribosomal DNA in *Musa* and *Ensete* (*Musaceae*): Taxonomic
325 implications. *Cytogenetics and Genome Research* 109:50–57.
- 326 Bhat KV, Jarret RL. 1995. Random amplified polymorphic DNA and genetic diversity in Indian
327 *Musa* germplasm. *Genet Resour Crop Evol* 42(2):107–118.
- 328 Boret B and Branchard M. 2001. Nonanchored Inter Simple Sequence Repeat (ISSR) Markers:
329 Reproducible and Specific Tools for Genome Fingerprinting. *Plant Molecular Biology*
330 Reporter International Society for Plant Molecular Biology 19: 209-215
- 331 Carreel F, Gonzalez de Leon D, Lagoda P, Lanaud C, Jenny C, Horry JP, Tezenas du Montcel H.
332 2002. Ascertaining maternal and paternal lineage within *Musa* by chloroplast and
333 mitochondrial DNA RFLP analyses. *Genome* 45(4):679-92.
- 334 Christelová P, Valárik M, Hřibová E, van den Houwe I, Channeliere S, Roux N *et al.* 2011. A

- 335 platform for efficient genotyping in *Musa* using microsatellite markers. *AoB PLANTS*.
336 plr024, doi: 10.1093/aobpla/plr024. pmid: 22476494.
- 337 Christelová P, De Langhe E, Hřibová E, Čížková J, Sardos J, Hušáková Ines M, Van den Houwe I,
338 Sutanto A, Kepler AK, Swennen S, Roux N and Doležel J. 2017. Molecular and cytological
339 characterization of the global *Musa* germplasm collection provides insights into the treasure
340 of banana diversity. *Biodiversity and Conservation* 26(4):801–824
- 341 Čížková J, Hřibová E, Christelová, P, van den Houwe I, Häkkinen M, Roux N, *et al.*
342 2015. Molecular and Cytogenetic Characterization of Wild *Musa* Species. *PLoS ONE* 10(8):
343 e0134096. doi:10.1371/journal.pone. 0134096.
- 344 De Langhe ED, EL Vrydaghs, P de Maret, X Perrier and T Denham. 2009. Why bananas matter: An
345 introduction to the history of banana domestication. *Ethnobotany Research and Applications*
346 7:165-177.
- 347 D’Hont A, Denoeud F, Aury J, Baurens F, Carreel F, Garsmeur O, et al. 2012. The banana (*Musa*
348 *acuminata*) genome and the evolution of monocotyledonous plants. *Nature* 213–317.
- 349 Faure S, Noyer JL, Horry JP, Bakry F, Lanaud C, Gozalez de Lean D. 1993. A molecular marker-
350 based linkage map of diploid bananas (*Musa acuminata*). *Theor Appl Genet* 87(4) 517-526.
- 351 Fraser-Smith S, Czislowski E, Daly A, Meldrum R, Hamill S, Smith M, and Aitken E. 2016.
352 Single gene resistance to *Fusarium oxysporum* f.sp. *cubense* race 4 in the wild banana *Musa*
353 *acuminata* subsp. *malaccensis*. *Acta Horticulturae* 1114, ISHS Proceeding of the
354 International ISHS-ProMusa Symposium on Unravelling the Banana’s Genomic Potential.
355 Cited 16 June 2017. Available from www.musalit.org.
- 356 Häkkinen M. 2013. Reappraisal of sectional taxonomy in *Musa* (Musaceae). *Taxon* 62:809-813.
- 357 Häkkinen M and Wallace R. 2011. Genetic resources for banana improvement. In: Pillay M and
358 Tenkouano A, editors. *Banana Breeding: Progress and Challenges*. Boca Raton London New
359 York: CRC Press. p. 41–52
- 360 Howell EC., HJ. Newbury, RL. Swennen RL, LA. Withers LA, BV. Ford-Lloyd. 1994. The use
361 of RAPD for identifying and classifying *Musa* germplasm. *Genome* 37(2):328–332.
- 362 International Plant Genetic Resources Institute-International Network for the Improvement of
363 Banana and Plantain/Centre de Coopération internationale en recherche agronomique pour
364 le développement [IPGRI-INIBAP/CIRAD]. 1996. Description for Banana (*Musa* spp.). Int.
365 Network for the Improvement of Banana and Plantain, Montpellier, France; Centre de
366 coopération int. en recherche agronomique pour le développement, Montpellier, France;
367 International Plant Genetic Resources Institute Press, Rome.
- 368 Jain PK, Saini MK, Pathak H, Gupta VK. 2007. Analysis of genetic variation in different banana
369 (*Musa* species) variety using random amplified polymorphic DNAs (RAPDs). *Afri J*
370 *Biotechnol* 6(17) 1987–1989.
- 371 Javed MA, Chai M, Othman RY. 2004. Study of resistance of *Musa acuminata* to *Fusarium*
372 *oxysporum* using RAPD markers. *Biol Plant* 48:93–99.
- 373 Jones DR. 2000. Introduction to banana, abaca and enst. In: Jones Dr, editor. *Disease of banana,*
374 *abaca and enst*. Wallllingford, UK CABI Publishing, CAB International p 1-36.
- 375 Kaemmer D. 1992. Oligonucleotide and amplification fingerprinting of wild species and cultivars
376 of banana (*Musa* spp.). *Bio/Technology* 10(9):1030-1035.
- 377 Lamare A, Rao SR. 2015. Efficacy of RAPD, ISSR and DAMD markers in assessment of

378 genetic variability and population structure of wild *Musa acuminata* Colla. *Physiology and*
379 *Molecular Biology of Plants* 21(3):349-358.

380 Li L-F, Häkkinen M, Yuan Y-M, Hao G, Ge X-J. 2010. Molecular phylogeny and systematics of
381 the banana family (*Musaceae*) inferred from multiple nuclear and chloroplast DNA
382 fragments, with a special reference to the genus *Musa*. *Molecular Phylogenetics and*
383 *Evolution* 57:1-10.

384 Li L-F, Wang H-Y, Zhang C, Wang X-F, Shi F-X, Chen W-N, *et al.* 2013. Origins and
385 Domestication of Cultivated Banana Inferred from Chloroplast and Nuclear Genes. *PLoS*
386 *ONE* 8(11): e80502. doi:10.1371/journal.pone.0080502

387 Liu AZ, Kress WJ, Li DZ. 2010. Phylogenetic analyses of the banana family (*Musaceae*) based on
388 nuclear ribosomal (ITS) and chloroplast (trnL-F) evidence. *Taxon* 59:20–28.

389 Martanti D, Widyastuti U, Poerba YS and Megia R. 2015. Identification of Gene Candidate of
390 Nucleotide Binding Site (NBS) from Banana *Musa acuminata* Colla var *malaccensis*
391 (Riddl.) Nasution and *Musa*, AAA, Cavendish Sub-group. *Pakistan Journal of Biological*
392 *Sciences* 18(3): 99-106.

393 Mogeia JP, Gandawidjaja D, Wiriadinata H, Nasution RE and Irawati. 2001. Rare Plants of
394 Indonesia. Bogor: Herbarium Bogoriense. Research and Development Center of Biology,
395 the Indonesian Institute of Sciences (In Bahasa Indonesia).

396 Mukunthakumar S, Padmesh P, Vineesh PS, Skaria R, Kumar KH, Krishnan PN. 2013. Genetic
397 diversity and differentiation analysis among wild antecedents of banana (*Musa*
398 *acuminata* Colla) using RAPD markers. *Indian J Biotechnol* 12:493–498.

399 MusaNet 2016. Global Strategy for the Conservation and Use of *Musa* Genetic Resources (B. Laliberté,
400 compiler). Bioversity International, Montpellier, France.

401 Nasution RE. 1991. A taxonomic study of the species *Musa acuminata* Colla with its
402 intraspecific taxa in Indonesia. 1991. *Memoirs of the Tokyo University of Agriculture*
403 32:1-122.

404 Nei M. 1972. Genetic distance between populations. *The American Naturalist* 106(949):283-292.

405 Nei M. 1973. Analysis of gene diversity in subdivided populations. *Proc Natl Acad Sci USA*
406 70:3321–3323.

407 Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of
408 individuals. *Genetics* 89:583-590.

409 Nybom H. 2004. Comparison of different nuclear DNA markers for estimating intraspecific
410 genetic diversity in plants. *Mol Ecol* 13(5):1143-55.

411 Nybom H and Bartish IV. 2000. Effects of life history traits and sampling strategies on genetic
412 diversity estimates obtained with RAPD markers in plants. *Perspect. Plant Ecol. Evol. Syst.*
413 3:93-114.

414 Opara UL, Jacobson D and Al-Saady NA. 2010. Analysis of genetic diversity in banana cultivars
415 (*Musa* cvs.) from the South of Oman using AFLP markers and classification by
416 phylogenetic, hierarchical clustering and principal component analyses. *J Zhejiang Univ Sci*
417 *B* (5):332-41. doi: 10.1631/jzus.B0900310.

418 Page RDM. 1998. TreeView (Win 32). [cited 2017 March 31] Available at
419 <http://www.taxonomy.zoology.gla.ac.uk/rod/rod.html>.

- 420 Perrier X, De Langhe E, Donohue M, Lentfer C, Vrydaghs L, Bakry F, Carreel F, Hippolyte I,
421 Horry JP, Jenny C, Lebot V, Risterucci AM, Tomekpe K, Doutrelepont H, Ball T,
422 Manwaring J, de Maret P, Denham T. 2011. Multidisciplinary perspectives on banana
423 (*Musa* spp.) domestication. *Proceedings of the National Academy of Sciences* 108(28),
424 11311-11318.
- 425 Pillay M, Nwakanma DC, Tenkouano A. 2000. Identification of RAPD markers linked to A
426 and B genome sequences in *Musa* L. *Genome* 43(3):763–767.
- 427 Pillay M, Ogundiwin E, Tenkouano A, Dolezel J. 2006. Ploidy and genome composition
428 of *Musa* germplasm at the International Institute of Tropical Agriculture (IITA). *Afr J*
429 *Biotechnol* 5(13):1224–1232.
- 430 Poerba YS and Ahmad F. 2010a. Genetic variation of diploid banana cultivars (AA) Cibinong
431 Science Center Collection based on RAPD and ISSR markers. *Biota* 15(3):308-315 (In
432 Bahasa Indonesia).
- 433 Poerba YS and Ahmad F. 2010b. Genetic variability among 18 cultivars of cooking bananas
434 and plantain by RAPD and ISSR markers. *Biodiversitas* 11(3):118-123.
- 435 Poerba YS and Ahmad F. 2013. Genetic variation analyses of *Musa balbisiana* Colla based on
436 RAPD and ISSR marker. *Berita Biologi* 12(2):259-267 (In Bahasa Indonesia).
- 437 Poerba YS, Ahmad F and Witjaksono. 2012. Hybridization of wild diploid *Musa acuminata nata*
438 *Colla var malaccensis* (Ridl.) Nasution as pollen source with tetraploid Pisang Madu. *Jurnal*
439 *Biologi Indonesia* 8(1):181-196 (in Bahasa Indonesia).
- 440 Pollefeys P, Sharrock S and Arnaud E. 2004. Preliminary analysis of the literature on the
441 distribution of wild *Musa* species using MGIS and DIVA-GIS. IPGRI.
- 442 Raboin L, Carreel F, Noyer J, Baurens F, Horry J, Bakry F, *et al.* 2005. Diploid ancestors of triploid
443 export banana cultivars: molecular identification of 2n restitution gamete donors and n
444 gamete donors. *Molecular Breeding* 16(4):333–341.
- 445 Sardos J, Perrier X, Doležel J, Hřibová E, Christelová P, van den Houwe I, Kilian A and Roux N.
446 2016. DArT whole genome profiling provides insights on the evolution and taxonomy of
447 edible banana (*Musa* spp.). *Ann Bot* 7:1269-1278.
- 448 Simmonds NW and Shepherd K. 1955. Taxonomy and origin of cultivated bananas. *Botanical*
449 *Journal of Linnean Society* 55: 302–312.
- 450 Sperisen C and Bucher U. 1998. Cloning of random amplified polymorphic DNA (RAPD) to
451 generate codominant genetic marker. In: Karp A, Issac PG and Ingram D, editors. *Molecular*
452 *Tools for Screening Biodiversity*. Chapman & Hall, London, p. 217-222.
- 453 Syamkumar S, Lowarence B, Sasikumar B. 2003. Isolation and amplification of DNA from
454 rhizomes of tumiric and ginger. *Plant Mol Biol Rep* 212:171a-171e.
455 <http://dx.doi.org/10.1007/BF02774243>
- 456 Sutanto A, Sukma D, Hermanto C and Sudarsono S. 2014. Isolation and characterization of
457 resistance gene ana-logue (RGA) from *Fusarium* resistant banana cultivars. *Emirates*
458 *Journal of Food and Agriculture* 26(6): 508-518.
- 459 Uma S, Siva SA, Saraswathi MS, Manickavasagam M, Durai P, Relvarajan S, Sathiamoorthy S.
460 2006. Variation and intraspecific relationships in Indian wild *Musa balbisiana* (BB)

- 461 population as evidenced by random amplified polymorphic DNA. *Genetic Resources in*
462 *Crop Evolution* 53(2):349-355.
- 463 Volkaert H. 2011. Molecular Analysis Reveals Multiple Domestications of Edible Bananas.
464 Proceeding of the International ISHS-ProMusa Symposium on Global Perspectives on Asian
465 Challenges. *Acta Horticultura* 897.
- 466 Welsh J and McClelland M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucl*
467 *Acids Res* 18,7213-7218.
- 468 Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. 1990. DNA polymorphisms
469 amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*
470 18(22):6531–6535.
- 471 Wong C, Kiewj R, Loh JP, Gan LH, Set O, Lee SK, Lum S and Gan YY. 2001. Genetic Diversity
472 of the Wild Banana *Musa acuminata* Colla in Malaysia as Evidenced by AFLP. *Annals of*
473 *Botany* 88: 1017-1025.
- 474 Wong C, Kiew R, Argent GCG, Set O, Lee SK, Gan YY. 2002 Assessment of the validity of
475 the sections in *Musa (Musaceae)* using AFLP. *Annals of Botany* 90: 231–238.
- 476 Yeh FC, Yang RC, Boyle T, Ye YZ, Mao JZ. 1997. POPGENE: the user-friendly shareware
477 for population genetic analysis. Molecular Biology and Biotechnology Centre, University of
478 Alberta, Canada. [cited 2017 April 17]. Available at <http://www.ualberta.ca/~fyeh/>
- 479 Zietkiewicz E, Rafalski JA and Labuda D. 1994. Genome fingerprinting by simple sequence re-
480 peat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20(2):176–183.

481

ACCEPTED MANUSCRIPT

Table 1. List of samples used in the study

No	Coll. number	Scientific name	Local name	Origin
1	PAN01	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Yaki, Pisang Wolai	Sulawesi Utara
2	PAN08	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Yaki, Pisang Wolai	Sulawesi Utara
3	PAN09	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Yaki, Pisang Wolai	Sulawesi Utara
4	A12X	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Yaki, Pisang Wolai	Sulawesi Utara
5	A42X	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Yaki, Pisang Wolai	Sulawesi Utara
6	A62X	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Yaki, Pisang Wolai	Sulawesi Utara
7	PA 01	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
8	PA 02	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
9	PA 03	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
10	PA 04	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
11	PA 07	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
12	PA 08	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
13	PA 09	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
14	PA 10	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
15	PA 11	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
16	PA 13	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
17	PA 14	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
18	PA 15	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
19	PA 16	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
20	PA 17	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
21	PA 18	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
22	PA 19	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
23	PA 21	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
24	PA 23	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
25	PA 24	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
26	PA 25	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
27	PA 26	<i>Musa acuminata</i> Colla subsp. <i>banksii</i> (F.Muell.) Simmonds	Pisang Hutan	Timika, Papua
28	PA61	<i>Musa acuminata</i> Colla var. <i>acuminata</i>	Pisang Hutan	Sentani, Papua
29	PA79a	<i>Musa acuminata</i> Colla var. <i>acuminata</i>	Pisang Hutan	Manokwari, Papua Barat
30	PA79b	<i>Musa acuminata</i> Colla var. <i>acuminata</i>	Pisang Hutan	Manokwari, Papua Barat
31	PA84	<i>Musa acuminata</i> Colla var. <i>acuminata</i>	Pisang Hutan	Manokwari, Papua Barat
32	PA85	<i>Musa acuminata</i> Colla var. <i>acuminata</i>	Pisang Hutan	Manokwari, Papua Barat
33	PA86	<i>Musa acuminata</i> Colla var. <i>acuminata</i>	Pisang Hutan	Manokwari, Papua Barat
34	PA89	<i>Musa acuminata</i> Colla var. <i>acuminata</i>	Pisang Hutan	Manokwari, Papua Barat
35	PA90	<i>Musa acuminata</i> Colla var. <i>acuminata</i>	Pisang Hutan	Manokwari, Papua Barat
36	PA91	<i>Musa acuminata</i> Colla var. <i>acuminata</i>	Pisang Hutan	Manokwari, Papua Barat
37	PA96	<i>Musa acuminata</i> Colla var. <i>acuminata</i>	Pisang Hutan	Manokwari, Papua Barat
38	PN 07	<i>Musa acuminata</i> Colla var. <i>alasensis</i> Nasution	Pisang Runcim	Aceh Tenggara
39	PN 11	<i>Musa acuminata</i> Colla var. <i>alasensis</i> Nasution	Pisang Keken	Aceh Tenggara
40	PN 18	<i>Musa acuminata</i> Colla var. <i>alasensis</i> Nasution	Pisang Keken	Aceh Tenggara
41	PN 20	<i>Musa acuminata</i> Colla var. <i>alasensis</i> Nasution	Pisang Keken	Aceh Tenggara
42	PN 22	<i>Musa acuminata</i> Colla var. <i>alasensis</i> Nasution	Pisang Keken	Aceh Tenggara
43	PN 23	<i>Musa acuminata</i> Colla var. <i>alasensis</i> Nasution	Pisang Keken	Aceh Tenggara
44	PN 24	<i>Musa acuminata</i> Colla var. <i>alasensis</i> Nasution	Pisang Keken	Aceh Tenggara
45	PN 25	<i>Musa acuminata</i> Colla var. <i>alasensis</i> Nasution	Galuh Rincim	Aceh Tenggara
46	PN 27	<i>Musa acuminata</i> Colla var. <i>alasensis</i> Nasution	Galuh Rincim	Aceh Tenggara
47	PN 28	<i>Musa acuminata</i> Colla var. <i>alasensis</i> Nasution	Galuh Rincim	Aceh Tenggara
48	II 23B#3	<i>Musa acuminata</i> Colla var. <i>bantamensis</i> Nasution	Cau Kole	Jawa Barat
49	II23B#4	<i>Musa acuminata</i> Colla var. <i>bantamensis</i> Nasution	Cau Kole	Jawa Barat
50	II 23B#5	<i>Musa acuminata</i> Colla var. <i>bantamensis</i> Nasution	Cau Kole	Jawa Barat
51	APH 192	<i>Musa acuminata</i> Colla var. <i>bantamensis</i> Nasution	Cau Kole	Jawa Barat
52	APH 193	<i>Musa acuminata</i> Colla var. <i>bantamensis</i> Nasution	Cau Kole	Jawa Barat
53	APH 194	<i>Musa acuminata</i> Colla var. <i>bantamensis</i> Nasution	Cau Kole	Jawa Barat
54	APH 195	<i>Musa acuminata</i> Colla var. <i>bantamensis</i> Nasution	Cau Kole	Jawa Barat
55	APH 196	<i>Musa acuminata</i> Colla var. <i>bantamensis</i> Nasution	Cau Kole	Jawa Barat
56	IV2B#1	<i>Musa acuminata</i> Colla var. <i>breviformis</i> Nasution	Cau Kole	Jawa Barat
57	IV2B#2	<i>Musa acuminata</i> Colla var. <i>breviformis</i> Nasution	Cau Kole	Jawa Barat
58	IV2B#3	<i>Musa acuminata</i> Colla var. <i>breviformis</i> Nasution	Cau Kole	Jawa Barat
59	IV2B#4	<i>Musa acuminata</i> Colla var. <i>breviformis</i> Nasution	Cau Kole	Jawa Barat
60	IV2B#5	<i>Musa acuminata</i> Colla var. <i>breviformis</i> Nasution	Cau Kole	Jawa Barat
61	PHD25	<i>Musa acuminata</i> Colla var. <i>breviformis</i> Nasution	Cau Kole	Jawa Barat
62	PHD26	<i>Musa acuminata</i> Colla var. <i>breviformis</i> Nasution	Cau Kole	Jawa Barat
63	PSNA02	<i>Musa acuminata</i> Colla var. <i>cerifera</i> Nasution	Cau Kole	Jawa Barat
64	PSNA03	<i>Musa acuminata</i> Colla var. <i>cerifera</i> Nasution	Cau Kole	Jawa Barat
65	PSNA04	<i>Musa acuminata</i> Colla var. <i>cerifera</i> Nasution	Cau Kole	Jawa Barat
66	PSNA05	<i>Musa acuminata</i> Colla var. <i>cerifera</i> Nasution	Cau Kole	Jawa Barat
67	PSNA07	<i>Musa acuminata</i> Colla var. <i>cerifera</i> Nasution	Cau Kole	Jawa Barat
68	PSNA09	<i>Musa acuminata</i> Colla var. <i>cerifera</i> Nasution	Cau Kole	Jawa Barat
69	PNK18b	<i>Musa acuminata</i> Colla var. <i>flava</i> (Ridl.) Nasution	Pisang Hutan/Pisang Monyet	Kalimantan Tengah
70	PNK18c	<i>Musa acuminata</i> Colla var. <i>flava</i> (Ridl.) Nasution	Pisang Hutan/Pisang Monyet	Kalimantan Tengah
71	PNK19b	<i>Musa acuminata</i> Colla var. <i>flava</i> (Ridl.) Nasution	Pisang Hutan/Pisang Monyet	Kalimantan Tengah
72	PNK26	<i>Musa acuminata</i> Colla var. <i>flava</i> (Ridl.) Nasution	Pisang Hutan/Pisang Monyet	Kalimantan Tengah
73	PNK27b	<i>Musa acuminata</i> Colla var. <i>flava</i> (Ridl.) Nasution	Pisang Hutan/Pisang Monyet	Kalimantan Tengah
74	PNK28a	<i>Musa acuminata</i> Colla var. <i>flava</i> (Ridl.) Nasution	Pisang Hutan/Pisang Monyet	Kalimantan Tengah

150	PAA107	<i>Musa acuminata</i> Colla var. <i>sumatrana</i> (Becc.) Nasution	Pisang Palapah	Sumatera Barat
151	PAA108	<i>Musa acuminata</i> Colla var. <i>sumatrana</i> (Becc.) Nasution	Pisang Palapah	Sumatera Barat
152	PAA110	<i>Musa acuminata</i> Colla var. <i>sumatrana</i> (Becc.) Nasution	Pisang Palapah	Sumatera Barat
153	PAA190	<i>Musa acuminata</i> Colla var. <i>sumatrana</i> (Becc.) Nasution	Pisang Palapah	Sumatera Barat
154	PAA200	<i>Musa acuminata</i> Colla var. <i>sumatrana</i> (Becc.) Nasution	Pisang Palapah	Sumatera Barat
155	PH 04	<i>Musa acuminata</i> Colla var. <i>rutilifis</i> Nasution	Pisang Cici	Jawa Timur
156	PH 05	<i>Musa acuminata</i> Colla var. <i>rutilifis</i> Nasution	Pisang Cici	Jawa Timur
157	PH 06	<i>Musa acuminata</i> Colla var. <i>rutilifis</i> Nasution	Pisang Cici	Jawa Timur
158	PH 07	<i>Musa acuminata</i> Colla var. <i>rutilifis</i> Nasution	Pisang Cici	Jawa Timur
159	PH 08	<i>Musa acuminata</i> Colla var. <i>rutilifis</i> Nasution	Pisang Cici	Jawa Timur
160	PH 09	<i>Musa acuminata</i> Colla var. <i>rutilifis</i> Nasution	Pisang Cici	Jawa Timur
161	PH 12	<i>Musa acuminata</i> Colla var. <i>rutilifis</i> Nasution	Pisang Cici	Jawa Timur
162	PH 15	<i>Musa acuminata</i> Colla var. <i>rutilifis</i> Nasution	Pisang Cici	Jawa Timur
163	PH 16	<i>Musa acuminata</i> Colla var. <i>rutilifis</i> Nasution	Pisang Cici	Jawa Timur
164	PH 19	<i>Musa acuminata</i> Colla var. <i>rutilifis</i> Nasution	Pisang Cici	Jawa Timur
165	PAR 72	<i>Musa acuminata</i> Colla var. <i>tomentosa</i> (K.Sch.) Nasution	Unti Darek	Sulawesi Selatan
166	PAR 73	<i>Musa acuminata</i> Colla var. <i>tomentosa</i> (K.Sch.) Nasution	Unti Darek	Sulawesi Selatan
167	PAR 74	<i>Musa acuminata</i> Colla var. <i>tomentosa</i> (K.Sch.) Nasution	Unti Darek	Sulawesi Selatan
168	PAR 76	<i>Musa acuminata</i> Colla var. <i>tomentosa</i> (K.Sch.) Nasution	Unti Darek	Sulawesi Selatan
169	PAR 78	<i>Musa acuminata</i> Colla var. <i>tomentosa</i> (K.Sch.) Nasution	Unti Darek	Sulawesi Selatan
170	PAR 93	<i>Musa acuminata</i> Colla var. <i>tomentosa</i> (K.Sch.) Nasution	Unti Darek	Sulawesi Selatan
171	PAR 94	<i>Musa acuminata</i> Colla var. <i>tomentosa</i> (K.Sch.) Nasution	Unti Darek	Sulawesi Selatan
172	PAR 100	<i>Musa acuminata</i> Colla var. <i>tomentosa</i> (K.Sch.) Nasution	Unti Darek	Sulawesi Selatan
173	PAR 101	<i>Musa acuminata</i> Colla var. <i>tomentosa</i> (K.Sch.) Nasution	Unti Darek	Sulawesi Selatan
174	PAR 103	<i>Musa acuminata</i> Colla var. <i>tomentosa</i> (K.Sch.) Nasution	Unti Darek	Sulawesi Selatan
175	PAR 106	<i>Musa acuminata</i> Colla var. <i>tomentosa</i> (K.Sch.) Nasution	Unti Darek	Sulawesi Selatan
176	III 21A#1	<i>Musa acuminata</i> Colla var. <i>zebrina</i> (v. Houtte) Nasution	Cau Kole Beureum	Jawa Barat
177	III 21A#3	<i>Musa acuminata</i> Colla var. <i>zebrina</i> (v. Houtte) Nasution	Cau Kole Beureum	Jawa Barat
178	III 21A#4	<i>Musa acuminata</i> Colla var. <i>zebrina</i> (v. Houtte) Nasution	Cau Kole Beureum	Jawa Barat
179	III 21A#5	<i>Musa acuminata</i> Colla var. <i>zebrina</i> (v. Houtte) Nasution	Cau Kole Beureum	Jawa Barat
180	III20G#1	<i>Musa acuminata</i> Colla var. <i>zebrina</i> (v. Houtte) Nasution	Cau Kole Beureum	Jawa Barat
181	III20G#2	<i>Musa acuminata</i> Colla var. <i>zebrina</i> (v. Houtte) Nasution	Cau Kole Beureum	Jawa Barat
182	III20G#3	<i>Musa acuminata</i> Colla var. <i>zebrina</i> (v. Houtte) Nasution	Cau Kole Beureum	Jawa Barat
183	III20G#5	<i>Musa acuminata</i> Colla var. <i>zebrina</i> (v. Houtte) Nasution	Cau Kole Beureum	Jawa Barat
184	II23A#3	<i>Musa acuminata</i> Colla var. <i>zebrina</i> (v. Houtte) Nasution	Cau Kole Beureum	Jawa Barat
185	II23B#1	<i>Musa acuminata</i> Colla var. <i>zebrina</i> (v. Houtte) Nasution	Cau Kole Beureum	Jawa Barat
186	II23B#2	<i>Musa acuminata</i> Colla var. <i>zebrina</i> (v. Houtte) Nasution	Cau Kole Beureum	Jawa Barat
187	PA 64	<i>Musa schizocarpa</i> NW Simmonds	Pisang Hutan	Papua
188	PA 66	<i>Musa schizocarpa</i> NW Simmonds	Pisang Hutan	Papua
189	PA 67	<i>Musa schizocarpa</i> NW Simmonds	Pisang Hutan	Papua
190	PA 68	<i>Musa schizocarpa</i> NW Simmonds	Pisang Hutan	Papua
191	PA 69	<i>Musa schizocarpa</i> NW Simmonds	Pisang Hutan	Papua
192	PA 76	<i>Musa schizocarpa</i> NW Simmonds	Pisang Hutan	Papua
193	PA 87	<i>Musa schizocarpa</i> NW Simmonds	Pisang Hutan	Papua
194	PA 92	<i>Musa schizocarpa</i> NW Simmonds	Pisang Hutan	Papua
195	PA 93	<i>Musa schizocarpa</i> NW Simmonds	Pisang Hutan	Papua
196	PA 95	<i>Musa schizocarpa</i> NW Simmonds	Pisang Hutan	Papua
197	PA 103	<i>Musa schizocarpa</i> NW Simmonds	Pisang Hutan	Papua
198	II20C#1	<i>Musa balbisaniana</i> Colla	Pisang Klutuk	Jawa Barat
199	II20C#2	<i>Musa balbisaniana</i> Colla	Pisang Klutuk	Jawa Barat
200	PHD22	<i>Musa balbisaniana</i> Colla	Pisang Klutuk	Jawa Barat
201	PHD97	<i>Musa balbisaniana</i> Colla	Pisang Klutuk	Jawa Barat
202	PHD101	<i>Musa balbisaniana</i> Colla	Pisang Klutuk	Jawa Barat
203	PHD106	<i>Musa balbisaniana</i> Colla	Pisang Klutuk	Jawa Barat
204	P001	<i>Musa balbisaniana</i> Colla	Pisang Klutuk	Bali
205	PAR62	<i>Musa balbisaniana</i> Colla	Pisang Klutuk	Sulawesi Selatan
206	PAR63	<i>Musa balbisaniana</i> Colla	Pisang Klutuk	Sulawesi Selatan
207	II18C#1	<i>Musa balbisaniana</i> Colla	Pisang Klutuk Sukun	Yogyakarta
208	II18C#3	<i>Musa balbisaniana</i> Colla	Pisang Klutuk Sukun	Yogyakarta
209	I3C#1	<i>Musa balbisaniana</i> Colla	Pisang Klutuk Wulung	Yogyakarta