INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
21–1039/2022/28–6–366–374
DOI: 10.17957/IJAB/15.1990
http://www.fspublishers.org

Full Length Article

Genetic Diversity among and within Genome Groups of Banana Cultivars Based on ISSR Markers

Didik Wahyudi[1*], Dwi Candra Nursita[1] and Lia Hapsari[2]
[1]*Biology Department, Science and Technology Faculty, Universitas Islam Negeri Maulana Malik Ibrahim Malang, Jl. Gajayana No. 50 Malang, East Java, 65144, Indonesia*
[2]*Research Center for Plant Conservation, Botanic Gardens and Forestry, National Research and Innovation Agency, Kusnoto Building Jl. Ir. H. Juanda No. 18, Bogor, West Java, 16122, Indonesia*
[*]For correspondence: didik_wahyudi@bio.uin-malang.ac.id; didik.s211@gmail.com
Received 14 December 2021; Accepted 14 November 2022; Published 12 December 2022

Abstract

East Java was considered one of Indonesia's banana production centres. More than 90 cultivars have been described, with diverse morphological characteristics and a variety of local languages, making them an intriguing object to research. This research aimed to examine both the genetic diversity and the clustering among and within genome groups of banana cultivars in East Java using ISSR markers. A total of 12 banana cultivars expressing different genome groups were analyzed using five primers *i.e.*, UBC834, UBC835, UBC843, UBC848 and UBC855. UBC-835 primer was considered the best ISSR primer for amplification in terms of PIC, EMR, MI and RP. Clustering analysis resulted in 4 groups of banana cultivars based on their genome. As a result, the ISSR is a powerful marker for identifying banana cultivars at the intraspecific level. Genetic diversity analysis among genome groups showed high genetic richness with Shannon index 0.587 and polymorphic loci 96.97%. Meanwhile, the AA genome group has the highest genetic variation within genome groups, with Shannon index and polymorphic loci of 0.289 and 42.42%, respectively, followed by ABB, AAB, and AAA genome groups. In addition, 50.43% of the molecular variation was present within genome groups and 49.57% of variation lies among genome groups. The genetic variation was considered slightly more conserved within the genome groups of bananas. The findings of this study provide the foundation for sustainable management of East Java's local banana, both *in situ* and *ex situ*.

Keywords: Genetic diversity; Molecular marker; *Musa*; Polymorphism, PCR

Introduction

Banana (genus *Musa*; family Musaceae) is a popular horticultural crop that has grown since agriculture began (Langhe *et al.* 2009). Bananas are commonly cultivated because they are easy to grow and adapt to various environmental situations. Hundreds of millions of people in tropical and subtropical countries rely on bananas as a daily staple meal (Varma and Bebber 2019). In addition, bananas are also considered one of popular export commodity products in the world. In 2017, the global production of bananas was up to 144 million tons, and Indonesia reached 7.1 million tons (Ministry of Agriculture RI 2016).

The Indo-Malesia region is the primary origin of wild and cultivar bananas, which eventually spread throughout Asia, America, and Africa's tropics and subtropics (Perrier *et al.* 2011). At least 325 banana cultivars are recorded in Indonesia (Valmayor *et al.* 2000), distributed across the Indonesian archipelago. In particular, the diversity of bananas in East Java Province is considered high, with more than 90 cultivars reported. It has a wide range of morphological features and is recognized in many local languages (Hapsari *et al.* 2015), which has become an interesting object to study. East Java Province is also listed as a center for banana production in Indonesia, with the highest production volume of up to 2.6 tonnes in 2020 (Central Bureau of Statistics and Directorate General of Horticulture 2021).

The majority of today's banana cultivars are the result of intraspecific and interspecific hybridization between two diploid wild bananas, *Musa acuminata* Colla (donate A genome) and *Musa balbisiana* Colla (donate B genome) (Bakry *et al.* 2009; Perrier *et al.* 2011). As a result, the hybridization generated diploid, triploid, and tetraploid bananas with varied A and B genomic configurations, including AA, AAA, AB, AAB, ABB, AABB, AAAB and ABBB (Davey *et al.* 2013; Jesus *et al.* 2013). The conventional classification of genome groups in bananas was developed by Simmonds and Shepherd in 1955, using a scoring method based on 15 distinguishing morphological

features of the two ancestral parents. The final score will determine the proportional contribution of this ancestral parent to the genomic formation of banana cultivars today. However, research using more advanced molecular markers is needed to confirm the genome classification's validity to overcome the subjectivity weakness of the morphological approach in bananas.

Random amplified polymorphic DNA (RAPD) is the most commonly used molecular marker for assessing genomic formation and genetic variability of banana cultivars (Probojati *et al.* 2019; Wahyudi *et al.* 2020a). In addition, inter simple sequence repeats (ISSR) (Silva *et al.* 2017; Babu *et al.* 2018; Wahyudi *et al.* 2020b), sequence-related amplified polymorphism (SRAP) (Zozimo *et al.* 2018; Boonsrangsom *et al.* 2020) and amplified fragment length polymorphism (AFLP) (Youssef *et al.* 2011; Vroh-Bi *et al.* 2011) were also used for genomic determination of banana cultivars. However, ISSR is a frequently used marker in banana cultivars since it is one of the semi-arbitrary markers that use repeating primers orientated in opposing directions to create segments between two identical microsatellite repeat regions (Reddy *et al.* 2002). ISSR markers have several advantages, including not requiring initial genome sequence information, being effective in differentiating individuals at intraspecies level and individuals with high morphological similarities, relatively low costs with simple techniques, and producing high polymorphisms (Sarwat 2012; Tesfaye *et al.* 2014; Gajera *et al.* 2014). It has been proven powerful to classify the different genome constitutions and evolutionary patterns at intraspecific and interspecific levels of banana cultivars (Poerba and Ahmad 2010; Jesus *et al.* 2013; Babu *et al.* 2018; Poerba *et al.* 2018; Wahyudi *et al.* 2020b).

Understanding genetic diversity and population dynamics are required as a foundation for sustainable conservation management of genetic resources (Jesus *et al.* 2013; Rachmat *et al.* 2016; Resmi *et al.* 2016; Babu *et al.* 2018; Hapsari *et al.* 2018). However, the use of ISSR markers to gather insight into the molecular diversity, clustering, and organization of banana cultivars, particularly in East Java, is currently limited. Therefore, this study attempts to analyze the genetic diversity and the clustering among and within genome groups of twelve selected banana cultivars from East Java using ISSR marker. The information on the genetic diversity and clustering analysis of local banana cultivars would assist in further conservation and breeding strategies.

Materials and Methods

Plant materials

This study used twelve (12) banana cultivars, a collection of Purwodadi Botanic Garden/PBG, located in Pasuruan, East Java, as an ingroup (un-rooted). The banana cultivars were previously collected from populations of several regions in East Java. The used banana cultivars represent four genome groups *i.e*., AA, AAA, AAB and ABB (Table 1 and Fig. 1).

Collection data

DNA extraction: The fresh young banana leaf of each sample was crushed into powder by using liquid nitrogen. DNA extraction was performed using the Promega Wizard® DNA Isolation Kit. The DNA isolation steps follow the guidelines for plants. The isolated DNA was then confirmed quantitatively using the NanoDrop® Spectrophotometer ND-1000 at 260 and 280 nm wavelengths.

PCR ISSR: The PCR-ISSR assays were conducted using five selected primers from the previous study (Wahyudi *et al.* 2020b). The list of primers as well as the information on their sequence are shown in Table 2. The total PCR reaction mixture was 10 μL consisting of 1 μL DNA sample, 3 μL nuclease-free water, 1 μL 10 pmol primer, and 5 μL DreamTaq PCR Master Mix (2X). DNA amplification was performed in Bio-RAD thermal cycler with 40 amplification cycles consisting of denaturation at 94°C for 1 min, annealing with temperature according to the used primer (Table 2) for 45 sec and extension at 72°C for 2 min. The amplification products were separated by electrophoresis in 1,5% agarose gel and TBE (1/2)x buffer at a voltage of 80 V for 30 min. The bands were observed under UV-transilluminator and visualized by Biorad Gel-Documentation system.

Data Analysis

The presence of a band of individual cultivars was scored as 1 and 0 for absent band. The fragment size of the DNA band was estimated using a 1 Kb DNA ladder. The scoring of binary data was then analyzed to determine the most suitable primer for amplification, using four parameters *i.e.*, polymorphic information content (PIC), effective multiplex ratio (EMR), markers index (MI), and resolving power (RP). The PIC value for each ISSR marker was calculated as proposed by Roldan-Ruiz *et al.* (2000), as $\mathrm{PICi} = 2\mathrm{f}(1 - \mathrm{fi})$, where PICi is the polymorphic information content of marker i, fi is the frequency of the marker bands present, and 1-fi is the frequency of absent marker bands. PIC was averaged over all the bands for each primer. The EMR was estimated based on Medhi *et al.* (2014) as $\mathrm{EMR} = \mathrm{np}(\frac{\mathrm{np}}{\mathrm{n}})$; which n is defined as the product of the total number of the present band of each primer and the number of polymorphic bands (np). The MI was calculated by using the formulae described by Varshney *et al.* (2007), as MI = PIC × EMR. The RP value was calculated according to Prevost and Wilkinson (1999) as RP = 1-[2×(0.5-P)], where P is the proportion of genotypes containing bands.

Subsequently, multivariate distance indices and clustering analyses were performed using Palaeontological Statistics (PAST) 3.0 software based on Ward's method of

Table 1: List of banana cultivars examined in this study

No.	Cultivar name (Pisang)	Genome group	Sub-group	Population source
1	Mas Kripik	AA	Sucrier	Senduro, Lumajang
2	Mas Jambe	AA	Sucrier	Tulungagung, Tulungagung
3	Lilin	AA	Pisang Lilin	Krucil, Probolinggo
4	Cebol	AAA	Dwarf Cavendish	Pasrujambe, Lumajang
5	Kongkong	AAA	Gros Michel	Lawang, Malang
6	Kidang	AAA	Red	Kalisat, Jember
7	Candi	AAB	Plantain	Ambulu, Jember
8	Raja Ketan	AAB	Pisang Raja	Siman, Ponorogo
9	Raja Temen	AAB	Pisang Raja	Lawang, Malang
10	Tlekung	ABB	Saba	Tlekung, Batu
11	Tajinan	ABB	Saba	Glagah, Banyuwangi
12	Sabeh Biru	ABB	Bluggoe	Camplong, Sampang, Madura Island

Table 2: List of ISSR primers used in this study (Wahyudi *et al.* 2020b)

No.	Primer name	Sequences (5'-3')	MT (°C)	AT (°C)
1	UBC834	AGA GAG AGA GAG AGA GYT	51.6	46.6
2	UBC835	AGA GAG AGA GAG AGA GYC	53.9	48.9
3	UBC843	CTC TCT CTC TCT CTC TRA	51.6	46.6
4	UBC848	CAC ACA CAC ACA CAC ARG	53.9	48.9
5	UBC855	ACA CAC ACA CAC ACA CYT	51.6	46.6

Remarks: R= A/G; Y= T/C; MT= Melting Temperature; AT= Annealing Temperature

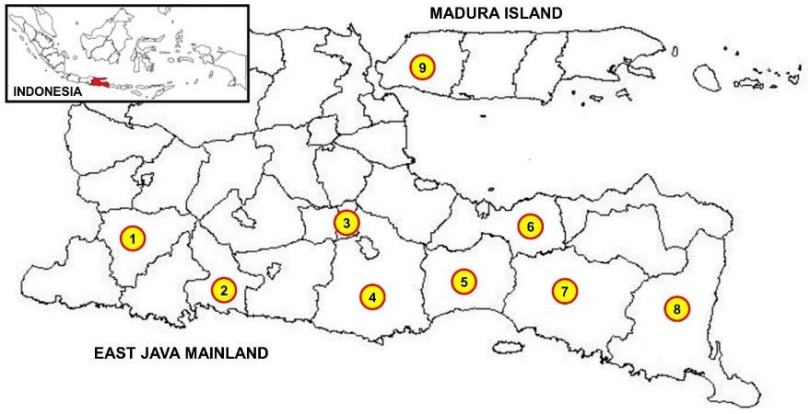

Fig. 1: Map population source of 12 banana cultivars examined: 1. Ponorogo, 2. Tulungagung, 3. Batu, 4. Malang, 5. Lumajang, 6. Probolinggo, 7. Jember, 8. Banyuwangi and 9. Sampang

hierarchical clustering (minimum variance method) with Euclidean dissimilarity index, bootstraps 1000 (Hammer *et al.* 2001). The basic parameters of genetic diversity was also analyzed using GenAlEx 6.5 software (Peakall and Smouse 2012). The parameters include the number of alleles observed (na), the number of effective alleles (ne), the average expected heterozygosity (He) and the Shannon information index (I) as a measure of gene diversity and the percentage of polymorphic loci (P%). This genetic diversity analysis was carried out both among and within genome groups of bananas. Furthermore, the data of distance matrix was also subjected to analysis of molecular variance (AMOVA) using GenAlEx6.5 (Peakall and Smouse 2012). The AMOVA estimated and partitioned the total molecular variance among and within genome groups of the populations and then tested the significance of partitioned variance components using non-parametric testing procedures with 999 permutations (Excoffier *et al.* 1992).

Results

ISSR polymorphisms and marker informativeness

All twelve banana cultivars were successfully amplified using the five ISSR primers employed in this investigation. Thirty-three bands ranging from 250-2000 bp were produced (Fig. 2 and Table 3). In this study, UBC-834 primer produced seven bands ranging from 600-2000 bp, UBC-835 primer produced nine bands ranging from 250-1500 bp, UBC-843 primer produced five bands ranging from 300-1000 bp, and UBC-848 primer produced five bands ranging from 400-1000 bp. Furthermore, each primer had an average band of 6.6 and an average number of polymorphic bands of 6.4.

Primer UBC-835 produced the most polymorphic bands (9), while UBC-843 and UBC-848 produced the least polymorphic bands (5). The percentage of polymorphic

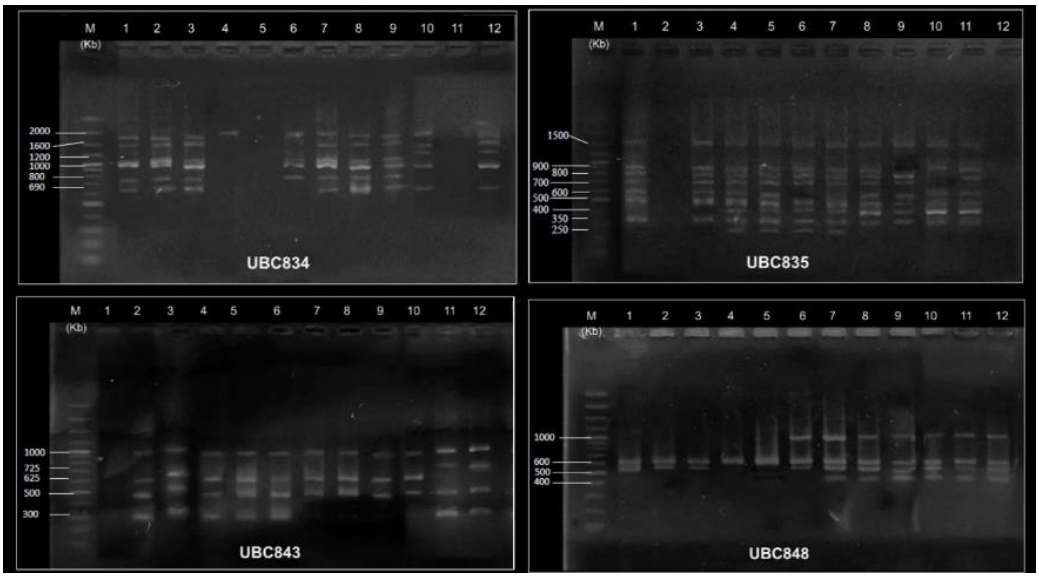

Fig. 2: ISSR amplification profiles of 12 banana cultivars (4 primers = UBC-834, UBC-835, UBC-843, and UBC-848)

Table 3: Polymophisms analysis result of 12 banana cultivars based on ISSR markers

No.	Primer	TNB	NPB	PB (%)	PIC	EMR	MI	RP
1	UBC-834	7	7	100	0.48	49	23.54	8.67
2	UBC-835	9	9	100	0.48	81	38.52	22.50
3	UBC-843	5	5	100	0.40	25	10.12	7.83
4	UBC-848	5	4	80	0.38	25	9.49	5.83
5	UBC-855	7	7	100	0.45	49	21.93	8.83
Total		33	32	480	2.19	229	103.60	53.67
Average		6.60	6.40	96	0.44	45.80	20.72	10.73

Remarks: TNB = Total Number of Bands, NPB = Number of Polymorphic Bands, PB = Polymorphic Band Percentage, PIC = Polymorphic Information Content, EMR = Effective Multiplex Ratio, MI = Marker Index, and RP = Resolving Power

bands varied from 80% for UBC-848 primers to 100% for UBC-834, UBC-835, UBC-843 and UBC-855 primers with an average polymorphism of 96% per primer (Fig. 2 and Table 3). Analysis of polymorphisms showed that ISSR primers which produced the highest PIC value of 0.48 were UBC-834 and UBC-835 primers, while the lowest PIC value of 0.38 was produced by UBC-848 primer, with an average PIC value of 0.44 per primer.

Clustering of banana cultivars in East Java

Twelve banana cultivars from East Java were clustered into four groups following their genome group, with distance values ranging from 1.00 to 4.90 (Fig. 3 and Table 4). Bananas with AA genome (Mas Jambe, Mas Kripik and Lilin) were served as an outgroup at a genetic distance 3.16, and supported by strong bootstrap values (100). Bananas with AAA genome (Kidang, Cebol and Kongkong) were separated at a genetic distance of 1.73 and supported by moderate bootstrap value (70). Furthermore, bananas with AAB genomes (Candi, Raja Ketan and Raja Temen) were clustered with a genetic distance value of 2.65, whilst, bananas with ABB genomes (Tlekung, Tajinan and Sabeh Biru) were grouped at a genetic distance of 2.83. Both genome groups separation was supported by low bootstrap values *i.e.*, 32 and 46, respectively. Nevertheless, from this study, the ISSR markers are proven powerful in classifying banana cultivars at the intraspecific level of bananas, particularly among and within genome groups of bananas.

Genetic variation and structure of banana cultivars

Estimating genetic diversity, like the gene diversity index and percentage of polymorphic loci, provides a measure of the taxa's genetic richness. This study detected high genetic richness across populations of 12 banana cultivars from East Java, with an average Shannon index value of 0.587 and a percentage of polymorphic loci of 96.97 percen (Table 5).

Molecular variance analysis of banana cultivar populations

Analysis of molecular variance (AMOVA) of 12 banana cultivar populations from East Java revealed that 50.43% of the variation is present within the genome groups and 49.57% of variation lies among the genome groups (Table 6). The results of AMOVA were also found comparable to percentage of gene differentiation in this study, depicting that

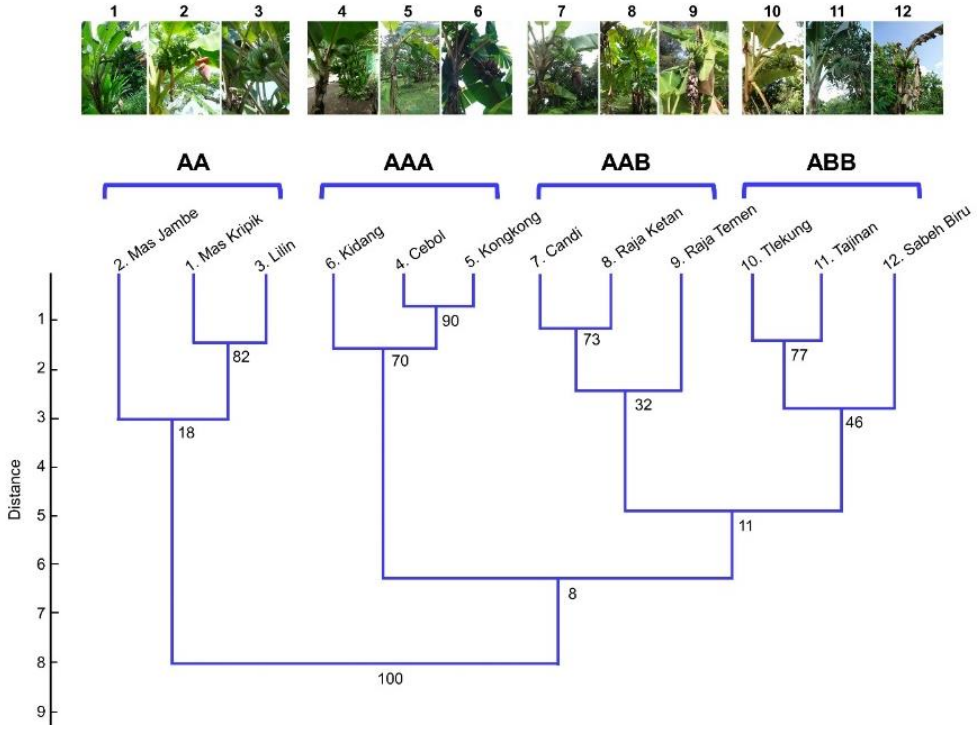

Fig. 3: Dendogram clustering based on ISSR markers and morphological appearance of 12 banana cultivars

Table 4: Euclidean genetic distance of 12 banana cultivars

No.	Cultivar name	Genome group	1	2	3	4	5	6	7	8	9	10	11
2	Mas Jambe	AA	3.74										
3	Lilin	AA	2.00	3.16									
4	Cebol	AAA	3.87	4.58	3.32								
5	Kongkong	AAA	4.00	4.69	3.46	1.00							
6	Kidang	AAA	3.61	4.36	3.00	2.00	1.73						
7	Candi	AAB	3.61	4.12	3.00	3.16	3.00	2.45					
8	Raja Ketan	AAB	3.46	4.24	2.83	3.32	3.16	2.65	1.73				
9	Raja Temen	AAB	3.32	3.32	2.65	3.74	3.87	3.46	2.83	2.65			
10	Tlekung	ABB	3.74	4.47	3.16	3.32	3.16	3.00	2.65	2.45	3.00		
11	Tajinan	ABB	4.24	4.90	3.74	3.00	2.83	3.00	3.32	3.16	3.61	2.00	
12	Sabeh Biru	ABB	4.47	3.46	4.00	4.36	4.24	4.12	3.61	3.74	4.12	2.83	3.46

Table 5: Genetic diversity parameters of 12 banana cultivars among and within genome groups

Parameter	Across populations	AA group	AAA group	AAB group	ABB group
N	12	3	3	3	3
Na (mean ± SE)	1.970 ± 0.030	1.121 ± 0.149	0.848 ± 0.108	1.182 ± 0.102	1.182 ± 0.127
Ne (mean ± SE)	1.741 ± 0.030	1.404 ± 0.083	1.068 ± 0.035	1.228 ± 0.069	1.347 ± 0.081
I (mean ± SE)	0.587 ± 0.048	0.289 ± 0.060	0.064 ± 0.031	0.173 ± 0.051	0.248 ± 0.058
He (mean ± SE)	0.407 ± 0.021	0.207 ± 0.043	0.042 ± 0.021	0.122 ± 0.036	0.177 ± 0.041
uHe (mean ± SE)	0.425 ± 0.022	0.248 ± 0.051	0.050 ± 0.025	0.146 ± 0.043	0.213 ± 0.050
P (%)	96.97	42.42	12.12	27.27	36.36

Remarks: N = number of samples; na = Observed number of alleles; ne = Effective number of alleles; I = Shannon's Information index; He = Expected heterozygosity; uHe = Unbiased Expected Heterozygosity; P = Percentage of polymorphic loci.

Table 6: Analysis of molecular variance of 12 banana cultivars populations

Variation source	df	SS	MS	Est. Var.	%	PhiPT	Prob.
Among populations/genome groups	3	38.50	12.833	3.194	49.57	0.496	0.001
Within populations/genome groups	8	26.00	3.25	3.250	50.43		
Total	11	64.50			100		

Remarks: df = degrees of freedom, SS = sum of squares, MS = mean squares, Est. Var. = estimated of variation, % = percentage of variation, PhiPT = genetic differentiation among populations, Prob. = probability

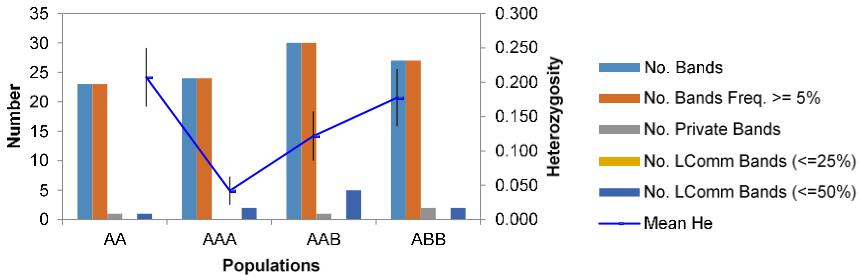

Fig. 4: ISSR band patterns across populations of 12 banana cultivars from East Java

that the variation was slightly more conserved within the genome groups of bananas from East Java. The PhiPT value has inferred the significant level of genetic differentiation of populations. The PhiPT > 0.2 means populations are significantly different. This present study resulted PhiPT value of 0.496 ($P < 0.001$), which indicated a significant level of genetic differentiation among 12 banana cultivar populations (Table 6).

Discussion

ISSR is the most common marker, capable of amplifying DNA segments ranging in size from 100 to 3000 bp between the two microsatellites (Ng and Tan 2015). In order to evaluate which primer is valuable to used, some evaluation including total polymorphic band, polymorphic information criteria (PIC), effective multiplex ratio (EMR), markers index (MI), and resolving power (RP) were performed. The results of this study are in accordance with previous research by Lamare and Rao (2015) on the genetic diversity and population structure of the wild banana *Musa acuminata* Colla where the UBC-835 primer was the primer which produced the highest number of polymorphic bands and the highest PIC value.

The maximum value of PIC is 0.5; the closer PIC value to 0.5, the more informative the primer is to be used in the analysis of genetic diversity (Chesnokov and Artemyeva 2015). The highest EMR value was produced by UBC-835 primer (49) and the lowest value was produced by UBC-843 and UBC-848 primers, with an average of 45.80 per primer. The higher EMR value of a primer, the more effective the primer is in producing polymorphic bands (Laurentin and Karlovsky 2006). Meanwhile, the highest MI and RP values were produced by UBC-835 primer and the lowest value was produced by UBC-848 primer (Table 3). Marker Index (MI) is used to determine which marker is most efficient in analyzing a number of bands simultaneously (Laurentin and Karlovsky 2006), while resolving power is used to measure the strength of a primer in producing polymorphic bands (Chesnokov and Artem'eva 2015). Thus, based on all the polymorphism parameters that have been analyzed, the ISSR primer selected and recommended for amplifying the DNA of banana cultivars is UBC-835.

The level of genetic diversity of a population can be estimated from the value of the genetic distance among the individual members of the population (Babu *et al.* 2018). If the value of the genetic distance between individuals in a population is getting smaller, the more uniform the population is and the higher the percentage of similarity of the observed accessions. This statement is in accordance with the result of this study where the highest genetic distance (4.90) was found between Pisang Mas Jambe and Pisang Tajinan which have different genomes, *i.e.,* AA and ABB, respectively. Genetic distance may indicate variations between individuals in the population due to genetic hybridization from ancestral parents (Poerba *et al.* 2018). Meanwhile, the lowest genetic distance value (1.00) was found between Pisang Kongkong (AAA) and Pisang Cebol (AAA); they are considered very closely related and come from the same genome group but differ in sub-group. A previous study using RAPD markers also showed that intraspecific levels within AAA genome group, particularly Pisang Ambon cultivars were low in the genetic distance (Wahyudi *et al.* 2020a). Likewise, Pisang Tajinan and Pisang Tlekung were close related under the same ABB group and Bluggoe sub-group with a genetic distance of 2.00 (Fig. 3 and Table 4).

Compared to another study to survey the genomes of 30 banana cultivars from Hainan in South China, only 85.10% of loci generated using ISSR were polymorphic (Lu *et al.* 2011). Whilst, Babu *et al.* (2018) reported the ISSR polymorphic loci of 8 banana cultivars from Karnataka in Southwest India was 61.30%.

Furthermore, the diploid AA is considered the most diverse genome group within the genome groups of bananas. It can be seen by the high values of genetic diversity parameters and band patterns accross populations (Table 5 and Fig. 4). Whilst. the triploid AAA genome group was considered the least diverse, then followed by AAB and ABB genome groups. Resmi *et al.* (2016) also reported similar results on banana cultivars in South India, where the diploid AA genome group was the most diverse. On the contrary, another study by Hariyanto *et al.* (2021)

using *mat*K sequences showed that the genetic diversity within the genomic group AAA is slightly higher than AA. However, due to continued genetic diversity and mutations, both genomic groupings (AA and AAA) are unable to be reliably distinguished.

The high degree of genetic diversity in AA diploid cultivars in this study may be due to the isolation and speciation that drives the evolution of particular traits. In addition, differences in genetic composition, geographical conditions or diverse environments will lead to various adaptation patterns and other genetic traits that support a plant's survival. Selection and vegetative propagation are also important factors that may cause high variation in banana cultivars. Species with a lot of genetic variation can withstand environmental strain for a long time (Langhe *et al.* 2009; Hapsari *et al.* 2018).

The AMOVA is a molecular marker-based approach for detecting population divergence. The percentage of gene divergence is a reliable indicator of the proportion of diversity among populations and is proportional to the amount of variation between them (Govindaraj *et al.* 2015). The variation within the genome group in this study is higher than among group. This result in this study is inversely proportional to what was found by Resmi *et al.* (2016) where among 38 South Indian bananas, a higher proportion of genetic variation was found among the genome groups (68%) than within the genome groups (31%).

The low gene flow may cause a high degree of variation among populations. Gene flow between genetically distant populations can decrease genetic variations between populations, whereas gene flow within a population can enhance genetic variation. Further, natural population fragmentation or extinction can result in reduced gene flow across populations, increasing genetic differentiation and structure (Slatkin 1987; Hatmaker *et al.* 2018).

This study's analysis of genetic diversity parameters using ISSR markers showed that the genetic variation of 12 banana cultivars in East Java was considered high among and within genome groups. The detail information from this study can provide the basic data for further conservation efforts and breeding of local banana genetic resources from East Java. Populations with high genetic diversity have a higher chance of survival because they have better environmental adaptability (Varma and Bebber 2019). Therefore, for conservation, banana cultivars with high genetic variability and far genetic distance are prioritized.

Farmers are encouraged to plant varied banana native varieties to minimize climatic risks, enhance the resilience of pest and disease outbreaks, and secure food sources for in-situ/on-farm conservation (Sthapit *et al.* 2009). Thus, all those 12 banana cultivars should be cultivated to keep them conserved on farm. Since, genetic erosion of local bananas may occur through the genetic uniformity of commercially cultivated cultivars in general which replaces and reduces the cultivation of potential local cultivars (Hapsari *et al.* 2017). Furthermore, genetically homogeneous populations are more susceptible to diseases and viruses, and are more likely to become extinct as a result of the spread of a single fatal disease on the farm (Resmi *et al*. 2016).

Ex situ conservation is required as a final line of defence to protect germplasm in the event of catastrophic events threatening their limited natural environment. Further, *ex situ* conservation also preserves the results of certain genes and genotypes sampled at a specific moment (Jesus *et al.* 2013; Rachmat *et al.* 2016; Hapsari *et al.* 2017). Upon this study, the diploid AA group bananas (Mas Kripik, Mas Jambe, Lilin) as the most genetically diverse group were prioritized for conservation. Further, they have viable pollens, potentially as male parents for further breeding (Damaiyani and Hapsari 2018). Meanwhile, more *ex situ* conservation efforts are needed for the least diverse group, *i.e.,* the AAA group followed by AAB and ABB groups, through exploration activities and collecting missions to several areas in East Java. However, for long-term strategy, if *ex situ* conservation resources are limited, any banana cultivars which are very closely related (low genetic diversity, low genetic distance) should be chosen with one of them as representative, such as Pisang Cebol x Pisang Kongkong and Pisang Raja Ketan x Pisang Candi.

Conclusion

Genetic variation of 12 banana cultivars in East Java were considered high both among and within genome groups. Diploid AA group bananas (Mas Kripik, Mas Jambe, Lilin) as the most genetically diverse group were prioritized for conservation. *Ex situ* conservation is also needed for the least diverse group *i.e.*, the AAA group followed by AAB and ABB groups, through exploration activities and collecting missions to several areas in East Java.

Acknowledgements

None to declare

Author Contributions

All authors (DCN, DW, LH) contributed equally in this manuscript from conceptualization, labworks, data analysis, writing the manuscript, review and editing of the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest

Data Availability

Data is available with the corresponding author

Ethics Approvals

No applicable to this study

References

Babu AG, G Prabhuling, RS Karani, D Satish, RK Patil, SR Mulla, G Raghavendra, RC Jagadeesha (2018). Genetic diversity analysis among banana cultivars through ISSR markers. *J Pharmacogn Phytochem* 7:1576–1580

Bakry F, F Carreel, C Jenny, JP Horry (2009). *Genetic Improvement of Banana, Breeding Plantation Tree Crops: Tropical Species*, pp:3–51. Jain SM, PM Priyadarshan (Eds). Springer, New York, USA

Boonsrangsom T, B Phetnin, K Ratanasut, K Sujipuli (2020). Assessment of Genetic Diversity among Musa Cultivars based on Sequence-Related Amplified Polymorphism Technique. *Nares Univ J Sci Technol* 28:52–61

Central Bureau of Statistics and Directorate General of Horticulture (2021). *Produksi Tanaman Buah-buahan* 2020. https://www.bps.go.id/indicator/55/62/1/produksi-tanaman-buah-buahan.html

Chesnokov YV, A Artem'eva (2015). Evaluation of the measure of polymorphism. *Sel'skokhozyaistv Biol* 50:571–578

Damaiyani J, L Hapsari (2018). Pollen viability of 19 Indonesian bananas (*Musa* L.) collection of Purwodadi Botanic Gardens: Preliminary study for breeding. *In: Proceeding of International Conference on Tropical Plant Conservation and Utilization*, pp:42–51

Davey MW, R Gudimella, JA Harikrishna, LW Sin, N Khalid, J Keulmens (2013). A draft *Musa balbisiana* genome sequence for molecular genetics in polyploid, inter- and intra-specific *Musa* hybrids. *BMC Genom* 14:1–20

Excoffier L, PE Smouse, JM Quattro (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131:479–491

Gajera HP, RK Domadiya, SV Patel, BA Golakiya (2014). Appraisal of RAPD and ISSR Markers for genetic diversity analysis among cowpea (*Vigna unguiculata* L.) genotypes. *J Crop Sci Biotechnol* 17:79–88

Govindaraj M, M Vetriventhan, M Srinivasan (2015). Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. *Genet Res Intl* 2015:1–15

Hammer Ø, DAT Harper, PD Ryan (2001). PAST: Paleontological statistics software for education and data analysis. *Palaeontologia Electronica*, 4:1–9 Retrieved from http://palaeo-electronica.org/2001_1/past/past.pdf

Hapsari L, R Azrianingsih, EL Arumingtyas (2018). Genetic variability and relationship of banana cultivars (*Musa* L.) from East Java, Indonesia based on the Internal Transcribed Spacer region nrDNA sequences. *J Trop Biol Conserv* 15:101–120

Hapsari L, J Kennedy, DA Lestari, A Masrum, W Lestarini (2017). Ethnobotanical survey of bananas (Musaceae) in six districts of East Java, Indonesia. *Biodiversitas* 18:160–174

Hapsari L, D Wahyudi, R Azrianingsih, EL Arumingtyas (2015). Genomic Identification of bananas (*Musa* spp.) from East Java assessed by PCR-RFLP of the Internal Transcribed Spacer ribosomal DNA. *Intl J Biosci* 7:42–52

Hariyanto S, RK Ainiyah, ESW Utami, L Hapsari (2021). Genetic diversity and network within dessert bananas (*Musa acuminata* cv. AA and AAA) inferred by newly designed matK marker. *Intl J Conserv Sci* 12:585–598

Hatmaker EA, ME Staton, AJ Dattilo, Đ Hadziabdic, TA Rinehart, EE Schilling, RN Trigiano, PA Wadl (2018). Population Structure and Genetic Diversity Within the Endangered Species Pityopsis ruthii (Asteraceae). *Front Plant Sci* 9:943-957

Jesus OND, EDOE Silva, EP Amorim, CF Ferreira, JMSD Campos, GDG Silva, A Figueira (2013). Genetic diversity and population structure of Musa accessions in *ex-situ* conservation. *BMC Plant Biol* 13:1–22

Lamare A, SR Rao (2015). Efficacy of RAPD, ISSR and DAMD markers in assessment of genetic variability and population structure of wild *Musa acuminata* colla. *Physiol Mol Biol Plants* 21:349–358

Laurentin HE, P Karlovsky (2006). Genetik relationship and diversity in a sesame (*Sesamum indicum* L.) germplasm collection using amplified fragment length polymorphism (AFLP). *BMC Genet* 7:1–10

Langhe ED, L Vrydaghs, PD Maret, X Perrier, T Denham (2009). Why bananas matter: An introduction to the history of banana domestication. *Ethnobot Res Appl* 7:165–177

Lu Y, X Zhang, J Pu, Y Qi, Y Xie (2011). Molecular assessment of genetic identity and genetic stability in banana cultivars (*Musa* spp.) fromChina using ISSR markers. *Aust J Crop Sci* 5:25–31

Medhi K, DK Sarmah, M Deka, BS Bhau (2014). High gene flow and genetic diversity in three economically important *Zanthoxylum* spp. of upper Brahmaputra valley zone of NE India using molecular markers. *Meta Gen* 2:706–721

Ministry of Agriculture RI (2016). *Outlook komoditas pertanian sub sector hortikultura*. http://www.epublikasi.setjen.pertanian.go.id/arsip-outlook/76-outlook-hortikultura/424-outlook-pisang-2016

Ng WL, SG Tan (2015). Inter-simple sequence repeat (ISSR) markers: Are we doing it right. *ASM Sci J* 9:30–39

Peakall R, PE Smouse (2012). GenAlEx 6.5: Genetic analysis in excel. Population genetik software for teaching and research – an update. *Bioinformatics* 28:2537–2539

Perrier X, ED Langhe, M Donohue, C Lentfer, L Vrydaghs, F Bakry, F Carreel, I Hippolyte, J Horry, C Jenny, V Lebot, A Risterucci, K Tomekpe, H Doutrelepont, T Ball, J Manwaring, PD Maret, T Denham (2011). Multidisciplinary perspectives on banana (*Musa* spp.) domestication. *Proc Natl Acad Sci* 108:11311–11318

Poerba YS, F Ahmad (2010). Keragaman genetik kultivar pisang diploid (AA) koleksi Cibinong Science Center berdasarkan barka RAPD dan ISSR. *Biotechnology* 15:308–315

Poerba YS, D Martanti, F Ahmad (2018). Genetic variation of wild *Musa acuminata* Colla from Indonesia based on RAPD and ISSR markers. *Biotropia* 26:1–18

Probojati RT, D Wahyudi, L Hapsari (2019). Clustering analysis and genome inference of pisang raja local cultivars (*Musa* spp.) from Java Island by Random Amplified Polymorphic DNA (RAPD) marker. *J Trop Biodivers Biotechnol* 4:42–53

Prevost A, MJ Wilkinson (1999). A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theor App Genet* 98:107–112

Rachmat HH, A Subiakto, K Kamiya (2016). Genetic diversity and conservation strategy considerations for highly valuable medicinal tree of Taxus sumatrana in Indonesia. *Biodiversitas* 17:487–491

Reddy MP, N Sarla, E Siddiq (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica* 128:9–17

Resmi L, AR Nair, AS Nair (2016). Population genetic structure and diversity analysisof South Indian banana cultivars. *J Plant Breed Crop Sci* 8:1–12

Roldan-Ruiz I, J Dendauw, EV Bockstaele, A Depicker, MD Loose (2000). AFLP marker reveal high polymorphic rates in ryegrasses (*Lollium* spp.). *Mol Breed* 6:125–134

Sarwat M (2012). ISSR: A reliable and cost-effective technique for detection of DNA polymorphism. *In: Plant DNA Fingerprinting and Barcoding. Methods in Molecular Biology (Methods and Protocols)*, Vol. 862, pp:103–121. Sucher N, J Hennell, M Carles (Eds). Humana Press, Totowa, New Jersey, USA

Silva AVC, ALS Nascimento, MF Vitória, ARC Rabbani, ANR Soares, AS Lédo (2017). Diversity and genetic stability in banana genotypes in a breeding program using inter simple sequence repeats (ISSR) markers. *Gen Mol Res* 16:1–9

Simmonds NW, K Shepherd (1955). The taxonomy and origins of the cultivated bananas. *J Linn Soc Bot* 55:302–312

Slatkin M (1987). Gene flow and the geographic structure of populations. *Science* 236:787–792

Sthapit B, S Padulosi, B Mal (2009). Role of on-farm/*in situ* conservation and underutilized crops in the wake of climate change. *In: The National Symposium on Recent Global Developments in the Management of Plant Genetic Resources*, pp:17–18. NBPGR, New Delhi, India

Tesfaye K, K Govers, E Bekele, T Borsch (2014). ISSR fingerprinting of *Coffea arabica* throughout Ethiopia reveals high variability in wild populations and distinguishes them from landraces. *Plant Syst Evol* 300:881–897

Valmayor RV, SH Jamaluddin, B Silayoi, S Kusumo, LD Danh, OC Pascua, RRC Espino (2000). *Banana cultivar names and synonyms in Southeast Asia.* International Network for the Improvement of Banana and Plantain Asia and the Pacific Office. Philippines

Varma V, DP Bebber (2019). Climate change impacts on banana yields around the world. *Nat Clim Chang* 9:752–757

Varshney RK, K Chabane, PS Hendre, RK Aggarwal, A Graner (2007). Comparative assessment of EST-SSR, EST-SNP and AFLP markers for evaluation of genetik diversity and conservation of genetik resources using wild cultivated and elite barleys. *Plant Sci* 173:638–649

Vroh-Bi I, C Anagbogu, S Nnadi, A Tenkouano (2011). Genome characterization of natural and somaclonal variations in bananas (*Musa* spp.). *Plant Mol Biol Rep* 29:440–448

Wahyudi D, NI Adnin, L Hapsari (2020a). Intraspecific variation of thirteen Pisang Ambon cultivars (*Musa acuminata* cv. AAA) from Eat Java and Central Java (Indonesia) based on Random Amplified Polymorphic DNA (RAPD) Marker. *Intl J Conserv Sci* 11:1059–1070

Wahyudi D, K Rifliyah, Uslan (2020b). Genome evaluation of banana cultivars based on morphological character and Inter-Simple Sequence Repeat (ISSR) molecular marker. *Biodiversitas* 21:2982–2990

Youssef M, A James, R Rivera-Madrid, R Ortiz, RM Escobedo-Gracia (2011). *Musa* genetic diversity revealed by SRAP and AFLP. *Mol Biotechnol* 47:189–199

Zozimo ROB, K Ratanasut, T Boonsrangsom, K Sujipuli (2018). Assesment of genetic diversity among Thai banana cultivars (*Musa* spp.) based on RAPD and SRAP markers. *Intl J Biosci* 12:172–180