Asia-Pacific Journal of Science and Technology: Volume: 29. Issue: 05. Article ID.: APST-29-05-16. Research Article

APST

Asia-Pacific Journal of Science and Technology

https://www.tci-thaijo.org/index.php/APST/index

Published by the Research Department,
Khon Kaen University, Thailand

Study on kinship among kepok banana cultivars using resistance gene analogue (RGA) based single nucleotide polymorphism (SNP) markers

Eti Ernawiati[1,2], Wasinton Simanjuntak[3], Rochmah Agustrina[2,*], Bambang Irawan[2] and Sutopo Hadi[3]

[1] Doctoral Student at Postgraduate School, Faculty of Mathematics and Natural Sciences, University of Lampung, Indonesia
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, Bandar Lampung, Indonesia
[3]Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Lampung, Bandar Lampung, Indonesia
[*]Corresponding author: rochmah.agustrina@fmipa.unila.ac.id

Received 6 July 2023
Revised 7 February 2024
Accepted 15 March 2024

Abstract

The primary goal of the research is to implement Resistance Gene Analog (RGA) markers to determine the kinship of three kepok banana cultivars from Bandarlampung: kepok abu (AAB genome), kepok batu (ABB genome), and kepok kuning (BBB genome). The banana RGA genome sequence from GenBank (ID: KF034945 to KF034953) was used to examine the sequencing data for parallels. Phylogenetic trees were constructed with 1000 bootstrap replicates of neighbor-joining using MEGA X. It was determined that the primer SNP2-MNBS-Ref. may identify Single Nucleotide Polymorphism (SNP) sites at the 327th nucleotide base pair, which is the cytosine nucleotide base, in the RGA of the three banana cultivars that have been validated in GenBank. The phylogenetic tree showed that the three banana cultivars have a common ancestor, which is *Musa balbisiana* cv. Klutuk Wulung. With a genetic distance of 29%, the progenitor created a branch where Kepok Batu and Kepok Kuning are in the same clade as *Musa acuminata* subsp. burmannica cv. Calcuta4. Kepok Abu is in the same clade as *Musa acuminata* cv. Rejang and Musa acuminata subsp. Malaccensis, with a genetic distance of 28%. This indicates that Kepok Batu and Kepok Kuning are one cultivar but distinct variations. Consequently, it may be said that each of the three banana cultivars studied has an SNP location in the R gene. Kepok Abu's reduced genetic distance indicates that it may be a good source of R gene material for the Musa improvement program, which aims to produce a cultivar with increased pathogen resistance.

Keywords: Kepok banana, single nucleotide polymorphism, resistance gene analog, phylo-genetic three

1. Introduction

Bananas (*Musa* spp.) are native to the tropics of Southeast Asia and play an important role in food security in tropical and subtropical countries [1]. *Musa* spp. are derived from two wild species of the diploid genome, *Musa acuminata* Colla (genome A) and *Musa balbisiana* Colla (genome B). Interspecific and intraspecific hybridization of the two wild species resulted in hybrids with increased resistance to pathogens and pests, short growth cycles, high fruit yields, and parthenocarpy. Other desirable plant traits of hybrid cultivars are still a challenge and many studies are needed to genetically improve the quality of Musa plants [2]. According to Sardos *et al.* [3], the complex genome structure and phylogenetic relationships between hybrid cultivars and wild type require further investigation.

One of the biggest threats to global banana production is pests and diseases. There are more than 50 species of fungi, as well as a number of bacterial and viral species that are banana microbial pathogens. Pathogens in banana plants can also include nematodes and insects [2,4]. According to Sardos *et al.* [3], Unexplored knowledge of banana genetics makes banana cultivation vulnerable to pests and diseases. Information on DNA markers, DNA marker-based genetic maps, and banana genome sequences are ways to gain more information on Musa

genetics and identify genes that can accelerate the improvement of Musa quality. Innovations in molecular markers have a high potential to genetically improve Musa quality to cope with environmental stress due to climate change [5]. A previous study by Yang *et al.* [6] successfully obtained a chitinase gene, which is believed to play an important role in the resistance of banana plants to FocR4 disease, and the accession 'Rastali Mutiara' (AAB) is known as a potential source to obtain disease resistance genes in banana molecular breeding. A similar gene, the ChiI1 gene, which has a motif of the GH19 protein from the chitinase family, was found in the leaves of the *ambon lumut* banana (group M. *acuminata* AAA), which also has potential as a candidate gene for resistance to pathogens [7].

Naturally, many types of plants are capable of developing effective mechanisms for recognizing and responding to pathogen infection. Such abilities are possible because plants have molecules called resistance genes (R genes) that recognize molecules to respond to infection caused by pathogens. In response to incoming infections, plants multiply the R gene and position the genes in the plant genome. Gene analogs (RGAs) have nucleotide sequences rich in leucine repeats and can act as receptors, such as for kinase. Thousands of RGAs have been identified from plant genomes, randomly distributed in plant chromosomes and genomes, and many in the form of clusters [2,3],[8,9].

Many varieties of commercial banana do not have a resistance system against pests and diseases [4]. In Indonesia, one of the banana cultivars of high economic value and cultivated mostly by the farmer community is locally known as the *kepok* banana (*Pisang Kepok*). However, the productivity of this banana cultivar is still very low due to several factors, including inappropriate cultivation techniques and high pest and disease disturbances. In addition, it is acknowledged that the cultivated bananas are characterized by narrow genetic diversity and sterility. On the contrary, their wild relatives exhibit greater variability and show a greater pool of disease resistance genes (R genes) [4]. Many workers have reported several studies on resistance gene analog (RGA) in bananas [6,10,11].

According to Sardos *et al.* [3], variation in the banana genome is formed through a process of evolution, mutation, and demographic changes in the banana habitat. Therefore, tracing the evolutionary track of the formation of variations in the banana genome is important to better understand the evolution of bananas in a specific region. The evolution path can be traced through the construction of phylogenetic trees. As Baum suggested [12], a phylogenetic tree (phylogeny) is very useful to describe the evolutionary lineage of various species, organisms, or genes of a common ancestor. Furthermore, phylogenetic trees are also useful for understanding biodiversity, constructing classifications, and events that occur during evolution.

Molecular markers have been widely used in (phylogenetic) studies of banana germplasm, such as nuclear ribosomal (ITS) and chloroplast (trnL-F) [13], multigene sequences [14], nucleotide binding site (NBS) gene [15], Diversity array technology marker (DArT) [3], S16 protein gene (Rps16) chloroplast intron [16], internal transcription spacer 2 (ITS2) region [17], matK genes [11], plastomics, 45S rDNA, and mitochondria [18]. This molecular approach is considered capable of overcoming the limitations of the application of morphological characters in the construction of banana phylogenetic trees.

Sutanto *et al.* [19] developed a method for analyzing Single Nucleotide Polymorphism (SNP) in the genome sequence of the RGA based on Polymerase Chain Reaction (PCR) of banana plants, however the application of SNP molecular markers to study *kepok* bananas grown in the region of Bandar Lampung City, Lampung province has never been reported [20]. SNPs are a new generation of molecular markers that are abundant, robust, and widely feasible due to their distribution throughout the genome. In plants, SNPs occur approximately once every 100 to 300 base pairs, or about one in every 1,000 nucleotides in genomic DNA. They play a crucial role in inducing phenotypic variation. SNPs are variations that arise at a single nucleotide position in the DNA sequence of a chromosome between two accessions. Specifically, SNPs refer to defined positions on a chromosome where the DNA sequences of two genotypes differ by a single base. These polymorphisms result from transitions (purine-to-purine or pyrimidine-to-pyrimidine changes), transversions (purine-to-pyrimidine changes), or small deletions or insertions (indels) [21-23]. Therefore, the use of RGA-based markers to study the kinship of *kepok* banana cultivars is important to gain the scientific information required for the selection and assembly of disease resistant kepok banana cultivars.

2. Materials and methods

2.1 Banan sample

The kepok banana kinship study was carried out in 3 different cultivars of kepok banana, locally known as *kepok abu* (AAB), *kepok batu* (ABB), and *kepok kuning* (BBB) from a collection of previous studies in the city of Bandar Lampung [20]. This research was carried out at the Integrated Laboratory and Center for Technology Innovation (LTSIT), University of Lampung.

2.2. DNA Extraction

The plant DNA was extracted from the young leaves using the manufacturer's protocol (Dneasy Plan Mini Kit (50) from Qiagen, Cat#69104). A mass of 50 mg of fresh leaves was mixed with 400 µL AP1 buffer and 4 µL Rnase A Stock Solution 100 mg / ml, then crushed into a fine slurry using a sterile mortar and then transferred into a 1.5 ml microtube for further incubation in a 65°C heating block for 10 minutes. The microtube was inverted 2-3 times during incubation and 130 µL Buffer P3 was added to the microtube and then incubated again for 5 minutes on ice.

The supernatant was centrifuged for 5 minutes at 20,000 x g, and the obtained lysate was transferred to a 2 ml collection tube contained in the QIAshredder column and then centrifuged for 2 minutes at 20,000 x g. The lysate was taken and transferred to a new tube, then 1.5 volumes of Buffer AW1 was added and the mixture was homogenized by pipetting. A total of 650 µL of the mixture was transferred to a 2 ml Eppendorf tube contained in the DNeasy Mini spin column and then centrifuged for 1 minute at 6000 x g.

The supernatant portion of the was discarded while the pellets in the centrifugation column were transferred to a new 2 ml collection tube and 500 µL Buffer AW2 buffer was added. The mixture was centrifuged again for 1 minute at 6000 x g, and the supernatant was discarded. An aliquot of 500 µL Buffer AW2 was added to the tube and then centrifuged for 2 minutes at 20,000 x g. The pellet i was then transferred to a new 1.5 mL or 2 mL Eppendorf tube and 40 µL Buffer AE was added for elution. The sample was incubated for 15 minutes and centrifuged for 1 minute at 6000 xg to obtain DNA extracts.

2.3 DNA Amplification

DNA amplification was performed using the Sensoquest Sensodirect Gradient Labcycler. DNA analysis was based on the SNP markers of the RGA of the genome sequence of banana plants to identify the genetics of 3 kepok banana cultivars with different genomes [19]. The primer pairs used were SNP1_MNBS_Ref, SNP1_MNBS_Alt, SNP2_MNBS_Ref, SNP2_MNBS_Alt, SNP4_MNBS_Ref, SNP4_MNBS_Alt, SNP5_MNBS_Ref, SNP5_MNBS_Alt, SNP6_MNBS_Ref, and SNP6_MNBS_Alt [19]. First, a mixture of PCR reagents with a total volume of 25 µL was prepared using the KAPA2G TM PCR kit. The PCR reagent consists of: 5.0 µL 5 X PCR buffer; 0.5 µL 25 mM MgCl2; 0.5 µL 10 mM dNTPs; 1.0 µL 10µM each primer (forward and reverse primers); 2.5 µL genomic DNA (± 30 ng/µL); 0.1 µL Taq DNA polymerase (5 U/µL), and 14.4 µL ddH_2O. Table 1 shows that the nucleotide primer sequences designed by Sutanto *et al.* [19] use DNA from the banana cultivars klutuk wulung and barangan which have BB and AAA genomes respectively

Table 1 The nucleoide primer sequences of PCR designed by Sutanto *et al.* [19]

No.	Primer name	Forward sequence	Reverse sequence
1	SNP1_MNBS_Ref	5'-CCGCGATTACCATGTGGTGCTCA-3'	5'-GCAGGGCATCTCCATGGGCTC-3'
2	SNP1_MNBS_Alt	5'-CCGCGATTACCATGTGGTGCTCC-3'	5'-GCAGGGCATCTCCATGGGCTC-3'
3	SNP2_MNBS_Ref	5'-GGCGAGGATCAGCTTCCACCCTTT-3'	5'-GGTGATCATGATCGAGGTTGCCAACT-3'
4	SNP2_MNBS_Alt	5'-GGCGAGGATCAGCTTCCACCCTTA-3'	5'-GGTGATCATGATCGAGGTTGCCAACT-3'
5	SNP4_MNBS_Ref	5'-TGGTTCGACCGCGAGGCCAG-3'	5'-GGAATGGGGAAAACGACGCTCCT-3'
6	SNP4_MNBS_Alt	5'-TGGTTCGACCGCGAGGCCAC-3'	5'-GGAATGGGGAAAACGACGCTCCT-3'
7	SNP5_MNBS_Ref	5'-AACCTCAGCGATTCATTGGCGC-3'	5'-ATGCAGAAGATCATCGCCAATCGG-3'
8	SNP5_MNBS_Alt	5'-ACAACCTCAGCGATTCATTGGCGT-3'	5'-ATGCAGAAGATCATCGCCAATCGG-3'
9	SNP6_MNBS_Ref	5'-CGGAACAACCTCAGCGATTCCTTG-3'	5'-GGTGATCATGATCGAGGTTGCCAACT-3'
10	SNP6_MNBS_Alt	5'-CCGGAACAACCTCAGCGATTCACTC-3'	5'-GGTGATCATGATCGAGGTTGCCAACT-3'

The PCR instrument was established following the procedure reported by Sutanto *et al.* [19]. The initial denaturation was performed at 95°C for 3 minutes, followed by 35 cycles of 95°C for 10 seconds, 60°C to 62°C for 10 seconds, and 72°C for 3 seconds. After the denaturation cycles were completed, the sample was subjected to the final 1 cycle at 72°C for 10 minutes. All PCR samples were electrophoresed with the QIAxcel Advanced digital electrophoresis inhibitor from Qiagen using the DNA High Resolution Kit. Electrophoresis was performed following the procedure described in the instrument operation manual. The PCR results that indicate the presence of an amplicon were followed by a sequencing process using the Sanger method with the assistance of PT. Indonesian bioner.

2.4 Data Analysis

Sequencing data was analyzed for alignment with the banana RGA genome sequence in Genebank (access codes KF034945 to KF034953) using Bioedit software. The differences in nitrogen bases at each SNP were analyzed. Phylogenetic trees were constructed using the MEGA X software developed by Kumar *et al.* used the neighbor joining method and the stability of the branch was tested by bootstrap 1000 replicates [24].

3. Results and discussion

3.1 Electrophoresis Data

The results of the purity test and DNA quantification on the three cultivars of kepok banana investigated, *kepok kuning*, *kepok Abu* and *kepok batu*) showed good results so that it can be continued to the next stage, DNA amplification. The purity value of the DNA samples from the three tested kepok banana cultivars has a purity level ranging from 1.769 to 1.800 and a quantity ranging from 10.5 to 12.5 ng/µL.

The results of electrophoresis derived from the PCR analysis of the DNA of the three banana cultivars investigated are presented in Table 2.

Table 2 Electrophoresis data of the samples investigated

No.	Primer Name	*Kepok kuning*	*Kepok batu*	*Kepok abu*
1	SNP1_MNBS_Ref			
2	SNP1_MNBS_Alt			
3	SNP2_MNBS_Ref			
4	SNP2_MNBS_Alt			
5	SNP4_MNBS_Ref			
6	SNP4_MNBS_Alt			
7	SNP5_MNBS_Ref			
8	SNP5_MNBS_Alt			
9	SNP6_MNBS_Ref			
10	SNP6_MNBS_Alt			

Note: The red color indicates that the electrophoresis results are not in accordance with the reference, and the blue color indicates that the electrophoresis results are in accordance with the reference [19].

The electrophoresis results in Table 2 indicate that the samples of the three banana cultivars could be amplified by the five primers used, which showed the effectiveness of the primers as SNAP markers. Some PCR results did not produce DNA bands, which indicated that the primers used allegedly could not recognize the PCR product and therefore were not included in the sequencing process. Another possibility is that there is an opportunity for the ends of a primer to form double strands with itself (high-end self-complementarity), thus allowing the formation of hairpins or dimer primers. If a hairpin or primary dimer is formed, it has the potential to disrupt the fragment amplification process so that the target and desired PCR product are not achieved [19].

The results of this study show that the PCR results were less effective in recognizing the SNP markers used in this study. The SNP marker used is predicted to be variant specific and can only recognize PCR results from *klutuk wulung* (BB) and *barangan* (AAA) bananas. In this study, we used the same primers designed by Sutanto et al. [19], but for PCR from bananas from *kepok abu* (AAB), *batu* (ABB) and *kuning* (BBB) whose genomes are different. This condition is predicted to have caused the PCR results in this study to be less effective.

3.2 SNP analysis

Electrophoresis results that show a good band were then purified and sequenced using the services of PT. Indonesian bioner. The resulting DNA sequences were compared with banana RGA (Resistance Gene Analogue) sequences in the Gene bank (access codes KF034945 to KF034953) using Bioedit software. The SNP analyses of the sequenced results are shown in Figures 1-5.

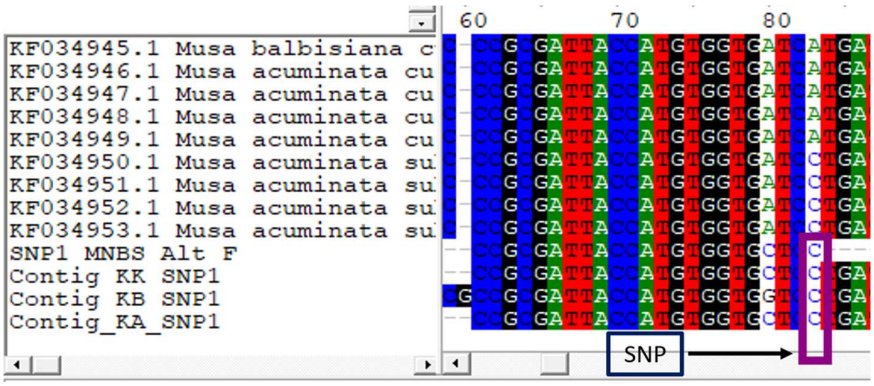

Figure 1 The SNP sites of the RGA gene for the three cultivars investigated using the SNP1 MNBS Alt primer.

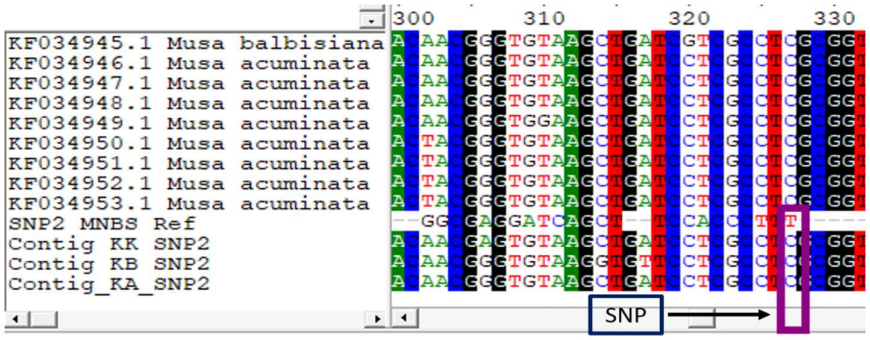

Figure 2 The SNP sites of the RGA gene for the three cultivars investigated using the SNP2 MNBS Ref primer.

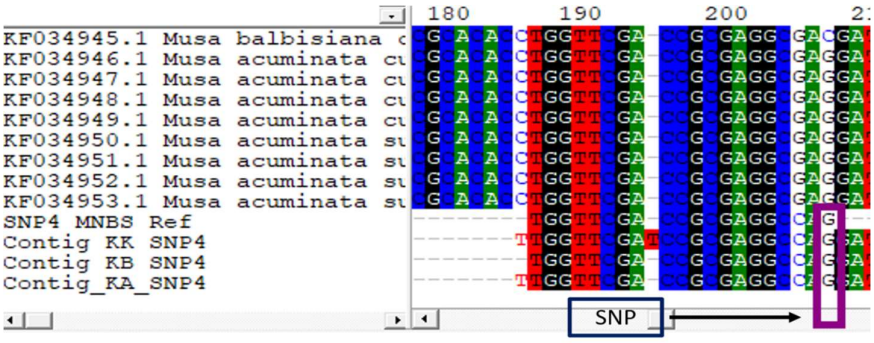

Figure 3 The SNP sites of the RGA gene for the three cultivars investigated using the SNP4 MNBS Ref Primer.

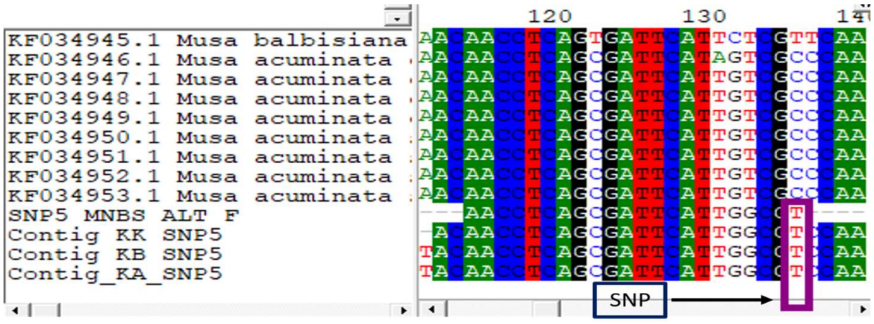

Figure 4 The RGA gene SNP sites for the three cultivars investigated using the SNP5 MNBS Alt. Primer.

Figure 5 The SNP sites of the RGA gene for the three cultivars investigated using the SNP6 MNBS Ref primer.

From Figures 1 through 5 it can be seen that the SNP sites of the RGA gene on the three cultivars were found at the positions of the 82 to 327 base pairs. The SNP sites of the three cultivars were not different from the SNPs 1, 4, 5 and 6. Meanwhile, the SNP site of the RGA gene on the 327th base pair shows differences, in which the three cultivars showed a cytosine base, while the primer (SNP 2 MNBS Ref.) showed a thymine base. This result is in agreement with Gupta *et al.* who explain that the frequency is about 1 SNP per 1000 nucleotides in genomic DNA or 1 SNP every 100-300 bp in plants [21].

3.3 Phylogenetic analysis

The results of the identification of the SNP sites of the RGA gene of the three cultivars were then used to construct phylo-genetic trees using the MEGA X software developed by Kumar *et al.* using the neighbor joining method, and the stability of the branch was evaluated using bootstrap with 1000 replications [24]. The results are shown in Figure 6.

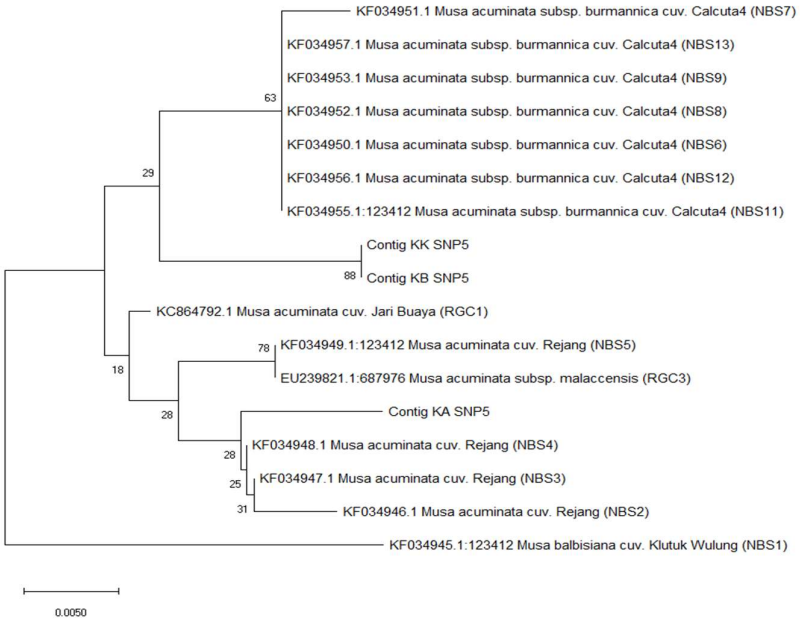

Figure 6 Phylogenetic tree of the banana cultivars investigated based on SNP markers in RGA.

The phylogenetic tree presented in Figure 6 shows that the *kepok abu*, *kepok batu* and *kepok kuning* bananas have the same ancestor, namely *Musa balbisiana* cuv. Klutuk Wulung. The three then separated to form different branches. *Kepok batu* and *kepok kuning* are in the same group as *Musa acuminata* subsp. Calcuta4 with a genetic distance of 29%. Furthermore, *kepok batu* and *kepok kuning* are closely related with a genetic distance of 88%. *Kepok abu* belongs to the same group as *Musa acuminata* cuv. Rejang and Musa acuminata subsp. Malaccensis with a genetic distance of 28%. Pei *et al.* found a high level of diversity and considerable sequence variation among RGAs in the genomes of the 33 Musa RGAs studied [25].

The *kepok kuning* and *kepok batu* are in clade 2 while the *kepok abu* is in clade 4, which means that *kepok kuning* and *kepok batu* are more closely related to their common ancestor, *M. balbisiana* cuv. Klutuk Wulung. These results are in agreement with the genomes of the three *kepok* banana cultivars according to their morphological characteristics, in which *kepok kuning* has a BBB genome, *kepok batu* has an ABB genome, and *kepok abu* has an AAB genome [20]. Thus the kinship of the *kepok batu* is closer to the *kepok kuning* because it has two "B" genomes and is farther related to the *kepok abu,* which has only one "B" genome.

These results are in agreement with the results of Meitha *et al.* who used ITS2 sequences in kepok bananas from various regions of Indonesia, which showed that there was no genetic variation in ITS2 kepok bananas and that they were located in the same clade in the phylogenetic tree, namely clustered in the B genome of banana cultivars (*M. balbisiana*) [17]. Sukartini stated that accessions with large genetic distances from one another or accessions with distant kinship are good accessions used as a source of breeding activities, and vice versa [26]. The recommendation is based on the fact that closely related cultivars have high similarity, so that their genetic diversity tends to be low and less profitable for breeding activities.

4. Conclusions

Previous research showed that a frequency of 1 SNP per 80 bp was detected in the LYB 7 gene and I SNP per 32 bp in the PSY 11 gene, each with a size of 160 bp. These genes play a role in the synthesis of high levels of beta-carotene in banana (plantain genome AAB). Another research stated that an MNBS fragment of 524 bp from banana plants, where 30 SNP sites were found in this segment with an SNP density of 1 SNP per 17 bp. Meanwhile, our research found that SNP2 MNBS primers Ref. can mark the presence of an SNP site in the RGA of the three kepok banana cultivars at the 327th base pair, the base for cytosine. Based on the results of this research, it is known that the three kepok banana cultivars used in this study have SNP sites in RGA, which can add important information about the R gene in GenBank and have the potential to be used as molecular markers to screen for resistance genes in kepok banana. Future studies could aim to clone the R gene in banana and characterize the banana defense response. This study is important for the kepok banana quality improvement program, especially for increasing its genetic resistance to pathogens. These results show similarities with those of others, although the molecular markers used are different. Based on the results of genetic analysis using ITS2 markers, the flores kepok banana, kepok jawa, kepok bali and kepok papua are located in the same group as the sobo banana, kepok tanjung, poh sabe tiger, bali, siam (ABB genome), klutuk, klutuk wulung (BB genome) and empedu (AB genome). This indicates that kepok bananas originating from various regions of Indonesia are identical or show the same cultivar and do not show any genetic variation.

5. Acknowledgments

The authors gratefully acknowledged financial support by Faculty of Mathematics and Natural Sciences, through research grant BLU DIPA 2018, contract number of 918/UN26.17/DT.02.00.01/2018. The authors also appreciate the help of Mr. Wawan Abdullah Setiawan from LTSIT, University of Lampung.

6. References

[1] D'Hont A, Denoeud F, Aury JM, Baurens FC, Carreel F, Garsmeur O, et al .The banana (*Musa acuminata*) genome and the evolution of monocotyledonous plants. Nature. 2012;488:213-217.

[2] Ortiz R, Swennen R. From crossbreeding to biotechnology-facilitated improvement of banana and plantain. Research review paper. Biotechnol Adv. 2014;32:158-169.

[3] Sardos J, Rouard M, Hueber Y, Cenci A, Hyma KE, van den Houwe I. A genomewide association study on the seedless phenotype in banana (*Musa* spp.) reveals the potential of a selected panel to detect candidate genes in a vegetatively propagated crop. PLoS ONE. 2016;11(5):e0154448.

[4] Miller RNG, Bertiole DJ, Baurens FC, Santos CMR, Alves PC, Martins NF, et al. Analysis of non-TIR NBS-LRR resistence gene analogs in *Musa acuminata* Colla: Isolation, RLFP marker development and physical mapping. BMC Plant Biol. 2008;8(1):1-15.

[5] Hinge VR, Shaikh IM, Chavhan RL, Deshmukh AS, Shelake RM, Ghuge SA, et al. Assessment of genetic diversity and volatile content of commercially grown banana (Musa spp.) cultivars. Sci Rep. 2022;12:7979.

[6] Yang C-Y, Sathyapriya H, Wong M-Y. Characterisation of pathogenesis-related genes and resistance gene candidates in banana (*Musa acuminata*) and their expression during host-pathogen interaction. Pertanika J Trop Agric Sci. 2016;39(1):55-72.

[7] Dwivany FM, Stefani G, Sutanto A, Nugrahapraja H, Wikantika K, Hiariej A, et al. Genetic relationship between Tongka Langit Bananas (*Musa troglodytarum L.*) from Galunggung and Maluku, Indonesia, Based on ITS2. Hayati J Biosci. 2020;27(3):258-265.

[8] Fluhr R. Sentinels of disease. Plant resistance genes. Plant Physiol. 2001;127:1367-1374.

[9] Sekhwal MK, Li P, Lam I, Wang X, Cloutier S, You FM. Disease resistance gene analogs (RGAs) in plants. Int J Mol Sci. 2015;16:19248-19290.
[10] Wang X, Yu R, Li J. Using genetic engineering techniques to develop banana cultivars with Fusarium wilt resistance and ideal plant architecture. Front Plant Sci. 2021;11:617528.
[11] Probojati RT, Listyorini D, Sulisetijono S, Wahyudi D. Phylogeny and estimated genetic divergence times of banana cultivars (*Musa* spp.) from Java Island by maturase K (matK) genes. Bull Natl Res Cent. 2021; 45(1):33.
[12] Baum D. Reading a phylogenetic tree: The meaning of monophyletic groups. Nature Edu. 2008;(1):190.
[13] Liu A-Z, Kress JW, Li D-Z. Phylogenic analyses of the banana family (Musaceae) based on nuclear ribosomal (ITS) and chloroplast (*trnL*-F) evidence. Taxon. 2010;59(1):20-28.
[14] Christelová P, Valárik M, Hÿibová E, de Langhe E, Doležel J. A multi gene sequence-based phylogeny of the Musaceae (banana) family. BMC Evol Biol. 2011;11:1-13.
[15] Martanti D, Widyastuti U, Poerba YS, Megia R. Identification of gene candidate of nucleotide binding site (NBS) from banana *Musa acuminate* Colla var. Malaccensis (Ridll.) Nasution and Musa, AAA, Cavendish Sub-group. Pak J Biol Sci. 2015;18(3):99-106.
[16] Noor FN. Phylogenetic analysis and time divergence of genus *Musa* spp. using S16 protein genes (Rps16) intron chloroplast. Bioinform Biomed Res J. 2019;2(1):1-7.
[17] Meitha K, Nugrahapraja H, Wikantika K. Phylogenetic analysis of 23 accessions of Indonesian banana cultivars based on Internal Transcribed Spacer 2 (ITS2) region. Indo J Biotechnol. 2020;25(1):1-11.
[18] Wu C-S, Sudianto E, Chiu H-L, Chao C-P, Chaw S-M. Reassessing banana phylogeny and organelle inheritance modes using genome skimming data. Front Plant Sci. 2021;12:713216.
[19] Sutanto A, Hermanto C, Sukma D, Sudarsono S. Development of SNAP marker based on resistance gene analogue genomic sequences in banana (*Musa* spp.). J Hort. 2013;23(4):300-309. (in Indonesian)
[20] Ernawiati E, Agustrina R, Irawan B, Nurhasanah E, Kanedi M. Germplasm diversity of banana (*Musa* Spp) in the city of Bandar Lampung, Indonesia by type of genome and number of chromosome. Sch J Agric Vet Sci. 2018;5(4):251-254.
[21] Gupta PK, Roy JK, Prasad M. Single Nucleotide Polymorphisms: A new paradigm for molecular marker technology and DNA polymorphism detection with emphasis on their use in plants. Curr Sci. 2001;80(4):524-535
[22] Akter S, Huq MA, Jung Y-J, Cho Y-G, Kang K-K. Application of single nucleotide polymorphism markers for selection of male sterility in crop plants. Plant Breed Biotech. 2016;4(4):379-386.
[23] Shehzad M, Ditta A, Iqbal MS, Jarwar AH. Role of molecular markers and importance of SNP for the development of cotton programs. J Biol Agric Healthcare. 2017;7(21):61-73.
[24] Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol. 2018;35:1547-1549.
[25] Pei X, Li S, Jiang Y, Zhang Y, Wang Z, Jia S, Isolation, characterization and phylogenetic analysis of the resistance gene analogues (RGAs) in Banana (Musa spp). Plant Sci. 2007;172(6):1166-1174.
[26] Sukartini, S. The grouping of banana accessions using the morphological character of IPGRI. J Hort. 2007; 17(1):26-33.