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Research Article

Genetic Diversity of Black Pepper in Bangka Island Based on SSR Markers

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ABSTRACT

The Bangka Belitung Islands Province is a major centre of black pepper production in Indonesia. Black pepper production in Indonesia has dropped, so it needs to be increased using superior varieties. Identifying the genetic diversity of black pepper plants is important for breeding superior varieties. The study's objective was to determine the genetic diversity and relationships of Bangka landrace black pepper using SSR markers. This research was conducted from June to August 2024 at the Biology Laboratory, Universitas Bangka Belitung. The black pepper genotypes used in this study were as follows: Lampung Daun Kecil (LDK), Nyelungkup, Petaling 1, Chunuk, Peremis, Balunijuk 1, Balunijuk 2, and Jerambah Gantung. The SSR markers employed in this study included Psol3, Psol6, Psol9, Psol10, Psol11, Psol17, and Psol19. The genetic relationship of Bangka black pepper was analysed using the UPGMA method. Genetic relationship revealed two main clusters of eight Bangka black pepper genotypes. Cluster I consisted of the Peremis and Balunijuk 2 genotype, while the remaining six genotypes were contained within cluster II. The structure population exhibited two subpopulations, with FST values of 0.30 and 0.61, respectively. The results showed that the seven primers used produced polymorphic (82.1%) and monomorphic (17.9%) bands on eight pepper genotypes. The Polymorphic Information Content (PIC) value of all primers tested was found to be medium, with the exception of Psol9, which exhibited a low PIC value. The average PIC value was 0.32, which is categorized as medium.

1. Introduction

Black pepper (*Piper nigrum* L.) is a plantation crop cultivated in Indonesia and classified as a spice plant. It is evident that black pepper constitutes a pivotal export commodity in Indonesia, playing a substantial role in the nation's economy, particularly in terms of its contribution to foreign exchange earnings (Shaliha *et al.* 2022). A significant decrease has been observed in black pepper production in Indonesia. A recent study of data reported by the Indonesian Directorate General of Plantations (2024) reveals that the production of black pepper in Indonesia has decreased significantly from 86,083 tons in 2020 to 73,536 tons in 2024. The decline in black pepper production in Indonesia can be caused by pest and disease attacks on black pepper plants, such as stem base rot, stem and flower borers, and dwarfism (Suryanti *et al.* 2017). As asserted by Bande *et al.* (2015), the emergence of diseases within black pepper agroecosystems is likely to lead to a decline in productivity.

The Bangka Belitung Islands are a province that serves as the centre for the development of black pepper cultivation in Indonesia, particularly in the West Bangka, South Bangka, and Belitung regencies, as stated

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in Kepmentan No. 830/KPTS/RC.040/12/2016. The black pepper cultivated in the Bangka Belitung Islands is known as Muntok White Pepper (Pranoto 2016).

The production of black pepper in the Bangka Belitung Islands, as reported by the Indonesian Directorate General of Plantations (2024), was recorded at 25,458 tons. This suggests a potential for augmentation to support the national-scale advancement of black pepper production. Shaliha *et al.* (2022) state that the Bangka Belitung Islands have the perfect climate and geography for black pepper plantations in Indonesia. Using superior varieties that meet high production criteria and are resistant to plant pests and diseases helps increase black pepper production. This can be achieved through black pepper plant breeding programmes.

Genetic diversity is a critical factor in the development of superior varieties. The enhancement of genetic diversity can be achieved through the utilisation of existing germplasm or by cross-breeding between germplasm (Widyaningtyas *et al.* 2023). Genetic diversity in the assembly of high-yielding black pepper varieties can be sourced from local, native, and wild relatives (Prayoga *et al.* 2020). Between 1998 and 2018, a total of ten superior black pepper varieties were released in Indonesia, including Petaling 1, Petaling 2, Natar 1, Natar 2, Chunuk, Bengkayang, Lampung Daun Kecil (LDK), Malonan 1, Cinten, and Nyelungkup (Rostianana and Ruhnayat 2020). Bangka black pepper has distinctive characteristics for each variety. For instance, the Nyelungkup variety can be distinguished by its downward-curved leaf pattern. Therefore, it is essential to analyze its genetic diversity as a resource for developing superior varieties in black pepper plant breeding programs. I am running a few minutes late; my previous meeting is running over.

The analysis of genetic diversity in black pepper can be performed using molecular markers. Molecular markers are defined as DNA fragments that are present within a genome and are associated with specific characteristics (Hafizah *et al.* 2018). Genetic diversity analysis can utilise Simple Sequence Repeat (SSR) markers. Analysis using SSR markers has been shown to have several advantages, including its codominant, abundant presence in the plant genome, based on PCR techniques, high levels of reproducibility, high polymorphism, and its ease of scoring (Andarini and Nugroho 2023). The utilisation of SSR markers has been previously employed by Christ *et al.* (2018) for the identification of multiple species of piper within the family Piperaceae. Meilawati *et al.* (2020), analysed the genetic relationships in nine superior black pepper varieties grown in the Indonesian Spice and Medicinal Research Institute. The analysis, based on SSR and RAPD markers, divided the varieties into three main clusters, namely cluster 1 (Natar 1, Natar 2, Petaling 1, Petaling 2), cluster 2 (Chunuk, LDK, Malonan), and cluster 3 (Ciinten, Bengkayang).

An analysis of the morphological diversity of Bangka landrace black pepper has been conducted; however, a genetic level analysis has not been carried out (Prayoga *et al.* 2020). The analysis revealed the distribution of nine black pepper plant accessions, which were divided into five clusters at the 50% similarity level based on morphological characters. The objective of this study is to obtain genetic information regarding the genetic relationship of black pepper in Bangka and to ascertain the reliability of SSR markers in its identification. This information can subsequently be utilised as a foundation for the cultivation of superior varieties of black pepper plants.

2. Materials and Methods

2.1. Plant Materials

The materials employed in this study were eight Bangka black pepper genotypes (Lampung Daun Kecil (LDK), Nyelungkup, Petaling 1, Chunuk, Peremis, Balunijuk 1, Balunijuk 2, Jerambah Gantung (JG)). The plant samples were obtained from the Indonesian Agency for the Assembly and Modernisation of Agriculture (BRMP), Bangka Belitung Island, and farmers' fields in Merawang District, Bangka.

2.2. DNA Extraction

Leaves from each black pepper genotype were extracted for DNA analysis, with three leaves selected per genotype as the biological sample. The optimal sample criteria should be fresh, young leaves that are free from pest and disease infestations. During the fieldwork, the leaves were stored in an icebox and subsequently stored at -20°C in the laboratory for DNA extraction.

The extraction of black pepper DNA was conducted in accordance with the protocol outlined by the Vivantis GF-1 Plant Extraction Kit. Extracted DNA samples were stored at -20°C until the next use. The quality of the extracted DNA was evaluated through the implementation of agarose gel electrophoresis, utilising a 1% (w/v) agarose solution. This procedure was conducted for a duration of 30 minutes, employing an electric current of 100 volts.

2.3. PCR Amplification and Visualization

The seven SSR primers used in this study are listed in Table 1. The SSR primers utilised in this study align with those reported in the research conducted by Meilawati *et al.* (2020) and Yoshida *et al.* (2014). The PCR reaction utilized was 25 µL of reaction solution, composed of 12 µL Taq polymerase, 1 µL of each forward and reverse SSR primer (0.5 µl each), 8 µl Nuclease-free water, and 4 µl DNA. The process of DNA amplification was conducted in accordance with the following steps: initial denaturation at 94°C for 4 minutes; denaturation at 94°C for 4 minutes; annealing at 37°C for 1 minute; and extension at 72°C for 1 minute. The process was repeated for a total of 35 cycles, after which the final extension stage was initiated at a temperature of 72°C for a duration of 5 minutes (Meilawati *et al.* 2020) PCR products were electrophoresed on a 2.5% agarose gel, run at 100 V for 30 minutes. The gel was then visualized under UV light at 70% intensity. The 50 bp DNA ladder from Tiangen Biotech was used as a standard marker.

2.4. Data Analysis

The data were analyzed by interpreting the DNA bands formed during visualization. The analysis was conducted by examining the variations in the size of DNA bands (polymorphism) formed, utilizing binary data visualization through the implementation of scoring, categorized as band (1) and non-band (0). The DNA band size reading was conducted using GenAnalyzer software. Clustering analysis for genetic relationships was performed using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) and Gower distance by PBSTAT-CL 2.2.1 (Suwarno *et al.* 2025). The population structure was analyzed using the STRUCTURE v2.3.4 software, with K values ranging from 2 to 4. The determination of the optimal K was achieved through the implementation of Evanno's ΔK method, which was executed by the Structure Selector (Li and Liu 2018).

The reliability of each primer used to measure diversity in Bangka black pepper was evaluated using Polymorphic Information Content (PIC) value analysis. The PIC value was calculated using the following formula:

$$PIC_j = 1 - \sum_i^n Pi^2$$

Where:
i : the i-th allele of the j-th marker
n : number of j-th marker allele
P : allele frequency

The PIC value is divided into three categories: PIC > 0.5 = very informative, then 0.25 > PIC > 0.5 = medium, and PIC < 0.25 = low (Botstein *et al.* 1980).

3. Results

3.1. PCR Visualization Result

The PCR visualisation revealed that amplification with seven SSR primers resulted in the identification of 19 alleles across eight black pepper genotypes (Table 2). The Psol6 and Psol19 primers exhibited the highest number of alleles, with four alleles each, while the Psol9 primer exhibited the fewest alleles, with only one allele. The results of the DNA band visualisation process demonstrated that several DNA bands were not present in black pepper genotypes, namely Balunijuk 2 (Psol10, Psol17, and Psol19), Peremis (Psol3 and Psol19), and Chunuk (Psol19). The size of the bands formed varies for each primer, with a band size range of 50-271 bp. For instance, the PCR visualisation on eight

Table 1. List of seven SSR primers for genetic diversity analysis of Bangka black pepper

Primer	Genebank accession number	Primer sequence (5'-3')	Source
Psol3	JQ924478	F: CACGACGTTGTAAAACGACGCGGATCTTACCAGAATCAG R: GAGTAGCCTTTGGTTGTTGC	Meilawati *et al.* (2020)
Psol6	JQ924481	F: CACGACGTTGTAAAACGACCTCTTGGCAAAAGTCACCTG R: ATCCCATACCGATCTCCTTC	Meilawati *et al.* (2020)
Psol9	JQ924484	F: CACGACGTTGTAAAACGACGGAACCCACGAGTTTCTTG R: GGGGTCCTTTTTACGTTGAG	Meilawati *et al.* (2020)
Psol10	JQ924485	F: CACGACGTTGTAAAACGACCAGACGGATTCCCACTGAT R: GGACTTGTAACCCATCGAGA	Meilawati *et al.* (2020)
Psol11	JQ924486	F: CACGACGTTGTAAAACGACTTATTTGGTTGGAGCTGTGTG R: CCACGGTGGGTTATCACAC	Meilawati *et al.* (2020)
Psol17	JQ924492	F: CACGACGTTGTAAAACGACTATTCCCATGCGAGATGC R: CGGCATAACCACTAAACCAC	Yoshida *et al.* (2014)
Psol19	JQ924494	F: CACGACGTTGTAAAACGACCGCGTGATGCATGCTTAT R: GCTCAACTCCGGAATCTACA	Yoshida *et al.* (2014)

black pepper genotypes with Psol11 primers revealed the presence of DNA bands for all genotypes tested. The total number of alleles identified was two, with sizes of 50 bp and 58 bp, respectively. Six genotypes were observed to exhibit the same DNA band size (50 bp), namely Nyelungkup, Balunijuk 2, Lampung Daun Kecil, Peremis, Petaling 1, and Chunuk. Meanwhile, Balunijuk 1 and Jerambah Gantung genotypes exhibited a distinct pattern of band size variation (58 bp) (Figure 1).

The total number of alleles formed was 17, with 88.2% of these (15 alleles) being polymorphic and the remaining 11.8% (2 alleles) being monomorphic. The presence of polymorphic alleles was detected using the following primers: Psol3, Psol6, Psol10, Psol11, Psol17, and Psol19. In contrast, monomorphic alleles were only identified on Psol9.

3.2. Genetic Relationships

The genetic relationship among eight Bangka black pepper genotypes was investigated based on seven SSR markers. The analysis revealed two main clusters, as illustrated in the dendrogram (Figure 2). Cluster I consisted of the Peremis and Balunijuk 2 genotypes. In contrast, the remaining six genotypes were contained within cluster II. A close genetic relationship was identified between the Petaling 1 and Nyelungkup genotypes, as well as between the Balunijuk 1 and Lampung Daun Kecil (LDK) genotypes.

The results of the population structure analysis revealed two subgroups among the 8 Bangka black pepper genotypes at the K = 2 value. Peremis and Balunijuk 2 genotypes were in subgroup 1 (P1), while the other six genotypes were in subgroup 2 (P2) (Figure 3). These results are consistent with the findings of the genetic kinship analysis. The FST values in P1 and P2 were 0.30 and 0.61, respectively. Subgroup P2 was the most genetically diverse and less differentiated compared to the other subpopulations.

3.3. Polymorphic Information Content (PIC) Values

The results of PIC analysis on seven primers showed an average value of 0.32, which is categorized as medium (Table 3). All of the primers examined exhibited medium PIC values, except for PSol9, which

Table 2. Amplification results of 7 SSR primers on 8 Bangka black pepper genotypes

Primer	Number of alleles	Band size (bp)	Polymorphic		Monomorphic	
			Total	%	Total	%
Psol3	3	61-88	3	100.0	0	0.0
Psol6	4	129-271	3	75.0	1	25.0
Psol9	1	122-143	0	0.0	1	100.0
Psol10	3	69-264	3	100.0	0	0.0
Psol11	2	50-58	2	100.0	0	0.0
Psol17	2	124-199	2	100.0	0	0.0
Psol19	2	50-100	2	100.0	0	0.0
Total	17		15	88.2	2	11.8

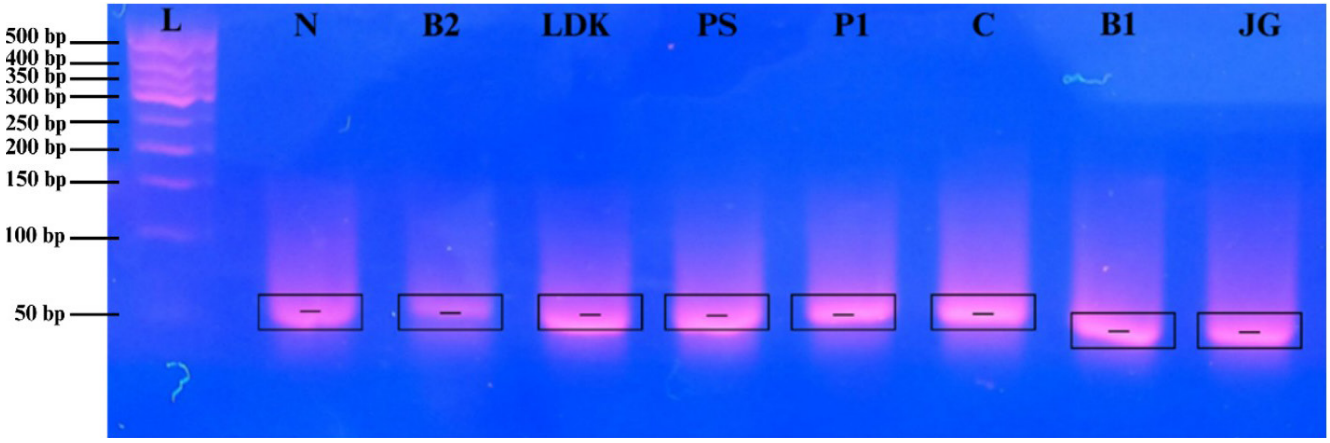

Figure 1. The following example illustrates a representative DNA banding pattern of Bangka black pepper with Psol11 primers. Ladder 50Bp (L), Nyelungkup (N), Balunijuk 2 (B2), Lampung Daun Kecil (LDK), Peremis (PS), Petaling 1 (P1), Chunuk (C), Balunijuk 1 (B) and Jerambah Gantung (JG)

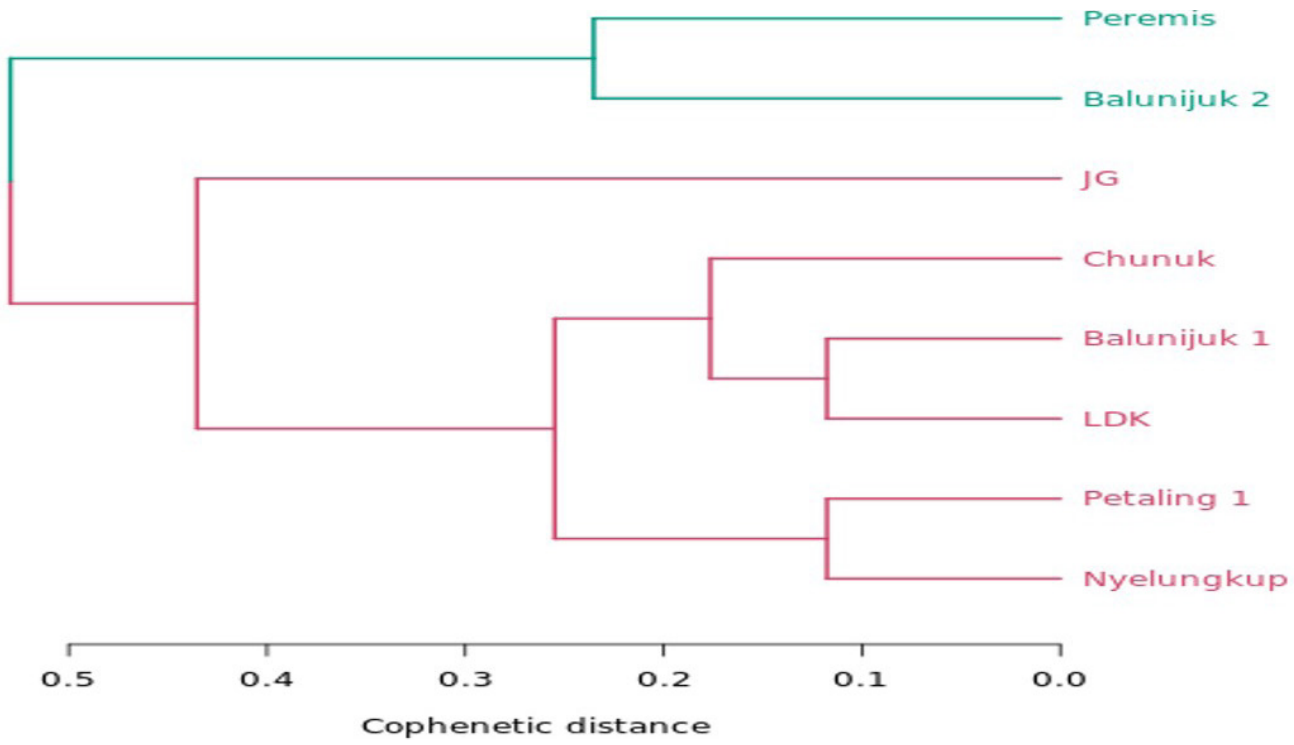

Figure 2. Dendrogram of the genetic relationship between Bangka black pepper genotypes

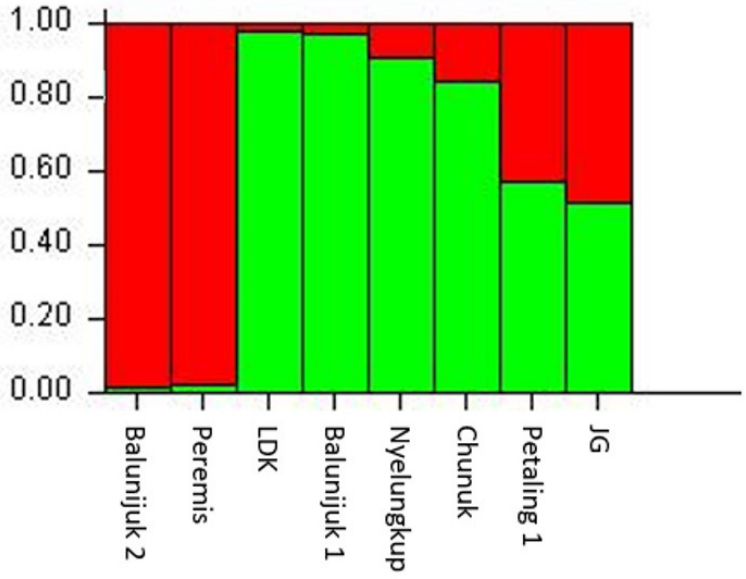

Figure 3. Population structure analysis of eight Bangka black pepper genotypes

resulted in a low PIC value. Psol17 and Psol19 primers exhibited the highest PIC value, while primer Psol19 showed the lowest PIC value.

4. Discussion

The presence of DNA bands indicates the successful execution of the primer amplification process on Bangka local black pepper DNA. The process of amplifying DNA relies on the existence of a sequence that is compatible with the primers utilised (Adeputri *et al.* 2016). The success of primers to amplify a DNA fragment is influenced by many factors, such as primer constituent sequences, primer size, attachment temperature, and the composition of GC content (Indradewi *et al.* 2022). It was hypothesized that the

Table 3. PIC values of seven primers

Primer	PIC	Category
Psol3	0.39	Medium
Psol6	0.30	Medium
Psol9	0.00	Low
Psol10	0.27	Medium
Psol11	0.38	Medium
Psol17	0.47	Medium
Psol19	0.47	Medium
	0.32	Medium

The PIC value is divided into three categories: PIC > 0.5 is considered very informative, PIC between 0.25 and 0.5 is medium, and PIC < 0.25 is low (Botstein *et al.* 1980)

primers used did not match the local Bangka black pepper tested, as DNA bands that did not appear on some genotypes were identified. Despite repeated optimization of the PCR process, DNA bands have not been observed for several genotypes when using specific primers. Dalimunthe *et al.* (2020) explain that the utilisation of primers that are not in accordance with the DNA sequences under study will result in the product not being amplified, due to the absence of a complementary match between the DNA and primer sequences employed.

The primers used showed the presence of alleles formed in 8 genotypes of Bangka black pepper. The results of the present study demonstrated that the number of alleles formed did not exceed four per primer, with a size range of 50-271 bp, across the eight black pepper genotypes utilised. Meilawati *et al.* (2020) conducted a study utilising five SSR primers on nine black pepper genotypes (Petaling 1, Petaling 2, Natar 1, Natar 2, Chunuk, Lampung Daun Kecil, Bengkayang, Malonan, and Cilinten). Their findings revealed the presence of up to 20 distinct alleles, exhibiting a size range of 50-250 base pairs (bp). Furthermore, multiple alleles in Psol17 and Psol19 were identified in Yoshida *et al.* (2014). The presence of different alleles in individuals observed in SSRs is due to variations in the number of repeats of the motif, which are caused by polymerase strand slippage during DNA replication or by recombination errors (Vieira *et al.* 2016). Primers that bind to multiple locations in the genome are also known as non-specific primers. These primers are not optimally designed for the target SSR locus, and as a consequence, they can lead to off-target amplification. Furthermore, the observed variation in the number of alleles may be attributable to differences in the genotypes of the black pepper utilized. The DNA sequence of an organism is the basic unit that determines its genetic diversity (Anchana *et al.* 2020). Salgotra and Chauhan (2023) explained that each individual or variety has genetic diversity due to differences in DNA sequences.

An additional factor that affects the number of alleles is the type of electrophoresis gel used. The present study employed agarose gel, while the research of Meilawati *et al.* (2020) utilised polyacrylamide gel electrophoresis (PAGE). Agarose gel is known to produce suboptimal band resolution and is not well-suited for samples with small DNA fragment sizes (Djankpa *et al.* 2021). Polyacrylamide gels have been demonstrated to yield high-resolution separation results (Harahap 2018). As posited by Green and Sambrook (2020), polyacrylamide gels have been demonstrated to possess high separation power, rendering them most efficacious for the purpose of distinguishing between small DNA fragments (50-500 bp). It has been established that DNA fragments differing in size by only 1 bp can be effectively separated from each other. Therefore, PAGE utilisation is highly recommended for DNA visualisation of Bangka black pepper DNA, which has a size range of 50-271 bp.

The amplification results of seven primers on eight genotypes of local Bangka black pepper produced polymorphic and monomorphic bands. The total percentage value of polymorphic bands was found to be 88.2%, while the percentage of monomorphic bands was determined to be 11.8%. The results of this study are in accordance with the research of Meilawati *et al.* (2020), which showed the presence of polymorphic and monomorphic bands in the results of SSR primer amplification on black pepper DNA.

Polymorphic bands are defined as DNA bands that are present in only some DNA samples, while monomorphic bands are those present in all samples (Ballo and Nge 2020). Sulistyawati and Widyatmoko (2017) further reinforced by explaining that polymorphic bands are DNA bands that appear at a specific size and are not found in other DNA samples. Differences in the arrangement of bases in each DNA sample cause the presence of polymorphic banding patterns. Polymorphic bands have been utilised to demonstrate the diversity or variation between local Bangka black pepper genotypes. The presence of variations in banding patterns serves as an indication of genetic differences between samples. The greater the number of polymorphic bands produced, the more evident the variations between the sample DNA (Sinaga *et al.* 2017).

The genetic relationship of eight Bangka black pepper genotypes, as determined by seven SSR markers,

revealed two main clusters. Cluster I consisted of the Peremis and Balunijuk 2 genotypes, while the other six genotypes were in Cluster II. A cluster will consist of accessions with the highest genetic similarity (Lestari *et al.* 2022). The genetic relationships of black pepper are supported by, or in accordance with, the morphological characteristics of black pepper plants (Meilawati *et al.* (2020). The Petaling 1 variety is frequently referred to as Lampung Lebar Daun (LDL) by farmers. The Petaling 1 pepper variety was selected from local accessions of the Lampung Daun Lebar (LDL) variety, which were cultivated in Bangka (Sudarsono *et al.* 2019). The Petaling 1 (LDL) and Nyelungkup genotypes have been shown to exhibit morphological similarities, including the character of leaf vein, leaf margin, leaf base shape, leaf tips, leaf color, flowering nature, fruit shape, unripe and ripe fruit color, ripe stem color, and growth habits (Prayoga *et al.* 2020).

A population structure analysis was conducted, which revealed the presence of two distinct subpopulations. The FST values for these subpopulations were determined to be 0.30 and 0.61, respectively. The Peremis and Balunijuk 2 genotypes were classified as belonging to subgroup 1 (P1), while the remaining six genotypes were designated as belonging to subgroup 2 (P2). FST values greater than 0.25 indicate significant population differentiation (Eltaher *et al.* 2018). These findings indicated that subgroup P2 exhibited higher genetic diversity and less differentiation compared to the other subpopulations.

The PIC is a value that is used to determine the level of polymorphism of a marker (Terryana *et al.* 2020). A low PIC value was found in the Psol9 primer, while the other primers used showed a medium PIC value. The PIC value is influenced by the type of primer used or the characteristics of the primer (Fatimah *et al.* 2019). The absence of DNA bands can cause low PIC values, or the PCR products may show the same banding pattern on all the DNA used (Dalimunthe *et al.* 2020). The use of non-specific primers during genetic diversity analysis can lead to the production of low PIC values or uninformative primers. The employment of non-specific primers has been demonstrated to result in the amplification of other regions within the genome that were not targeted during the process (Sinaga *et al.* 2017). In addition, the selection of appropriate primers is a critical component in the development of molecular markers for specific plant traits or characteristics (Herison *et al.* (2020)). In this study, all of the primers utilized by Yoshida *et al.* (2014) for *Piper solmsianum*, have also been employed by Meilawati *et al.* (2020) for the purpose of conducting a genetic relationship analysis on black pepper (*Piper nigrum*) plants. A critical consideration in the utilization of SSR markers pertains to the necessity of optimization of the primers prior to their application to specific plant species, given the inherent variations among different plants (Dalimunthe *et al.* 2020). According to the PIC value analysis, the average PIC value of the primers used to analyze black pepper genetic diversity is medium to moderate, with the exception of the Psol9 primer, which showed a low PIC value.

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References

Adeputri, E., Rustikawati, Suryati, D., Herison, C., 2016. Penapisan tiga puluh tujuh genotipe tomat dan seleksi primer RAPD untuk toleransi terhadap layu bakteri (*Ralstonia solanacearum*). *Akta Agrosia.* 19, 28-42. https://doi.org/10.31186/aa.19.1.28-42

Anchana, A., Deepudas, D.T., Jabbar, M.A., Beevy, S., Mathew, P.J. 2020. Assesment of genetic diversity among the three unique and potential genotypes of *Piper nigrum*-"PMM", "PAJ", and CV. "Karimunda" using RAPD markers. *Journal of Cytology and Genetics*. 21, 107-114.

Andarini, Y.N., Nugroho, K., 2023. Review pemanfaatan marka simple sequence repeat (SSR) dalam kegiatan analisis keragaman genetik plasma nutfah padi lokal di Indonesia. *Vegetalika*. 12, 47-63. https://doi.org/10.22146/veg.77050

Ballo, A., Nge, S.T., 2020. Analisis keragaman genetik pada tanaman kelor (*Moringa oleifera*) berdasarkan penanda molekuler random amplified polimorphic DNA (RAPD). *Jurnal Biotropikal Sains.* 17, 35-44.

Bande, L.O.S., Hadisutrisno, B., Somowiyarjo, S., Sunarminto, B. H., 2015. Peran unsur cuaca terhadap peningkatan penyakit busuk pangkal batang lada di Sentra Produksi Lada Daerah Sulawesi Tenggara. *Jurnal Manusia dan Lingkungan*. 22, 187-193. https://doi.org/10.22146/jml.18741

Botstein, D., White, R.L., Skolnick, M., Davis, R.W., 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics.* 32, 314.

Christ, J.A., Hollunder, R.K., Carvalho, M.S., Ferreira, M.F.D.S., Garbin, M.L., Carrijo, T.T., 2018. DNA fingerprinting based on SSR amplification profiles for *Piper* species identification (Piperaceae). *Acta Botanica Brasilica.* 32, 511-520. https://doi.org/10.1590/0102-33062017abb0268

Dalimunthe, S.R., Siregar, L.A.M., Putri, L.A.P., Chairunnisa, T., Hairmansis, A., 2020. Polymorphism levels of some SSR markers (simple sequence repeat) for parental line identification on low temperature tolerance. *IOP Conf. Ser.: Earth Environ. Sci.* 454, 012165. https://doi.org/10.1088/1755-1315/454/1/012165

Direktorat Jenderal Perkebunan. 2024. Buku Statistik Perkebunan 2022-2024 Jilid I. Seketariat Direktorat Jenderal Perkebunan, Direktorat Jenderal Perkebunan, Kementrian Pertanian.

Djankpa, F.T., Wiafe, A.G., Boateng, B.N., Tsegah, K.M., Baidoo, S. E., Ulanja, M.K., Affram, K.O., Ussif, A.M., Agyeman, D.O., Asante, G. 2021. Assessment of corn starch as substitute for agarose in DNA gel electrophoresis. *BMC Research Notes.* 14, 1-6. https://doi.org/10.1186/s13104-021-05483-1

Eltaher, S., Ahmed, S., Vikas, B., Hamdy, A.E., Nower, A.A., Salem, K.F.M., Poland, J., Baenziger, P.S., 2018. Genetic diversity and population structure of F3:6 nebraska winter wheat genotypes using genotyping-by-sequencing. *Front Genet.* 9, 76. https://doi.org/10.3389/fgene.2018.00076

Fatimah, Masumah, Prasetiyono, J., Sustiprijatno, 2019. Evaluasi kemudahan transfer marka SSR padi untuk menganalisis keragaman genetik famili Poaceae toleran kekeringan. *Jurnal Biologi Indonesia*. 15, 41-51. https://doi.org/10.47349/jbi/15012019/41

Green, M.R., Sambrook, J., 2020. Analysis of DNA by agarose gel electrophoresis. *Cold Spring Harb Protoc.* 2019, 1-15. https://doi.org/10.1101/pdb.top100388

Hafizah, R.A., Adawiyah, R., Harahap, R.M., Hannum, S., Santoso, P.J., 2018. Aplikasi marka SSR pada keanekaragaman genetik durian (*Durio zibethinus* Murr.) di Kabupaten Deli Serdang, Sumatera Utara. *Journal of Biology*. 11, 49-56. https://doi.org/10.15408/kauniyah.v11i1.5668

Harahap, M.R., 2018. Elektroforesis: analisis elektronika terhadap biokimia genetika. *CIRCUIT: Jurnal Ilmiah Pendidikan Teknik Elektro.* 2, 21-26. https://doi.org/10.22373/crc.v2i1.3248

Herison, C., Rustikawati, Meita, R., Hasanudin, 2020. Analisis cluster dan seleksi primer SSR (simple sequence repeats) untuk sifat toleran aluminium pada cabai. *Jurnal Hort. Indonesia.* 11, 61-71. https://doi.org/10.29244/jhi.11.1.61-71

Indradewi, R., Saksono, B., Damayanti, S., 2022. Teknik desain primer untuk amplifikasi gen tujuan kloning dari DNA agrobacterium tumefaciens. *Perbal: Jurnal Pertanian Berkelanjutan.* 10, 305-309. https://doi.org/10.30605/perbal.v10i3.2090

Lestari, P., Sulastri, A., Manguntungi, B., Nugroho, K., 2022. Keragaman genetik 50 aksesi plasma nutfah kacang hijau (*Vigna radiata* L.) berdasarkan marka SSR. *Vegetalika.* 11, 220-232. https://doi.org/10.22146/veg.74076

Li, Y.L., Liu, J.X., 2018. Structureselector: a web-based software to select and visualize the optimal number of clusters using multiple methods. *Mol Ecol Resour.* 18, 176-177. https://doi.org/10.1111/1755-0998.12719

Kepmentan No. 830/Kpts/RC.040/12/2016 Tahun 2016 Tentang Lokasi Pengembangan Kawasan Pertanian Nasional.

Meilawati, N.L.W., Susilowati, M., Bermawie, N., 2020. Phyllogenetic of nine superior black pepper (*Piper nigrum* L.) varieties based on morphological and molecular markers. *IOP Conf. Ser.: Earth Environ. Sci.* 418, 012056. https://doi.org/10.1088/1755-1315/418/1/012056

Pranoto, Y.P., 2016. Penyebaran informasi teknologi lada putih ramah lingkungan dalam upaya peningkatan mutu dan produksi yang berkelanjutan di Desa Serdang Kecamatan Toboali Kabupaten Bangka Selatan. *Jurnal Pengabdian Kepada Masyarakat Universitas Bangka Belitung*. 3, 1-7. https://doi.org/10.33019/jpu.v3i2.148

Prayoga, G.I., Ropalia, Aini, S.N., Mustikarini, E.D., Rosalin, Y., 2020. Diversity of black pepper plant (*Piper nigrum*) in Bangka Island (Indonesia) based on agro-morphological characters. *Biodiversitas.* 21, 652-660. https://doi.org/10.13057/biodiv/d210230

Rostianana, O., Ruhnayat, A., 2020. *Booklet Varietas Unggul Lada*. Balai Penelitian Tanaman Rempah dan Obat, Bogor.

Salgotra, R.K., Chauhan, B.S., 2023. Genetic diversity, conservation, and utilization of plant genetic resources. *Genes.* 14, 174. https://doi.org/10.3390/genes14010174

Shaliha, M.B., Jahroh, S., Johar, S., 2022. Strategi pengembangan agribisnis lada putih di Provinsi Kepulauan Bangka Belitung. *Jurnal Pendidikan Tambusai*. 6, 13718-13724. https://doi.org/10.31004/jptam.v6i2.4622

Sinaga, A., Putri, L.A.P., Bangun, M.K., 2017. Analisis pola pita andaliman (*Zanthoxylum Acanthopodium* D.C) berdasarkan primer OPD 03, OPD 20, OPC 07, OPM 20, OPN 09. *Jurnal Agroteknologi FP USU*. 5, 55-64. https://doi.org/10.32734/joa.v5i1.2289

Sudarsono, H., Syarif, A., Erwanto, Barusman, M.Y.S., Redaputri, A.P., Noer, I., Zakaria, W.A., Hasanudin, U., Persada, C., Nurdin, B.V., Berawi, K., Rangga, A., Hikam, S., Evizal, R., Prasmatiwi, F.E., Astuti, H., Anggraini, N., Hendarto, K., 2019. *Revitalisasi Lada Lampung Sebagai Komoditas Warisan.* AURA publishing, Bandar Lampung.

Sulistyawati, P., Widyatmoko, A., 2017. Keragaman genetik populasi kayu merah (*Pterocarpus indicus* Willd) menggunakan penanda random amplified polymorphism DNA. *Jurnal Pemuliaan Tanaman Hutan.* 11, 67-76. https://doi.org/10.20886/jpth.2017.11.1.67-76

Suryanti, Hadisutrisno, B., Mulyadi, Widada, J., 2017. Interaksi *Meloidogyne incognita* dan *Fusarium solani* pada penyakit kuning lada. *Jurnal Perlindungan Tanaman Indonesia.* 21, 127-134. https://doi.org/10.22146/jpti.29760

Suwarno, W.B., Sobir, Sitaresmi, T., 2025. PBSTAT-CL: cluster analysis for plant genetic diversity studies (Version 2.2.1) [Computer software]. IPB University. http://www.pbstat.com

Terryana, R.T., Ningrum, N.D.S.A., Nugroho, K., Saptadi, D., Kurniawan, H., Lestari, P., 2020. Analisis keragaman genetik dan pengembangan profil sidik jari DNA 20 varietas cabai lokal Indonesia berdasarkan marka SSR. *Jurnal AgroBiogen.* 16, 45-58. https://doi.org/10.21082/ip.v26n2.2017.p121-132

Vieira, M.L., Santini, L., Diniz, A.L., Munhoz, C.D., 2016. Microsatellite markers: what they mean and why they are so useful. *Genetics and Molecular Biology.* 4, 312-28. http://doi.org/10.1590/1678-4685-GMB-2016-0027

Widyaningtyas, N.W., Moeljani, I.R., Sulistyono, A., 2023. Keragaman genetik tanaman bawang merah (*Allium ascalonicum* L.) varietas bauji hasil iradiasi sinar gamma 60Co (Generasi 5). *AGROSAINSTEK: Jurnal Ilmu Dan Teknologi Pertanian.* 7, 48-60. https://doi.org/10.33019/agrosainstek.v7i2.447

Yoshida, N.C., Lima, P.F., Priolli, R.H.G., Kato, M.J., Colombo, C.A. 2014. Isolation and characterization of nine polymorphic microsatellite loci in *Piper solmsianum* (piperaceae). *Applications in Plant Sciences*. 2, 3-5. https://doi.org/10.3732/apps.1300092