RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
57 (4) 1377-1388, 2025
http://doi.org/10.54910/sabrao2025.57.4.4
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

MOLECULAR IDENTIFICATION OF PINEAPPLE (*ANANAS COMOSUS*) ACCESSIONS BASED ON RANDOM AMPLIFIED POLYMORPHIC DNA MARKERS

E. YUNIASTUTI[1*], I. ZAKARIA[1], PARJANTO[1], and A.P.D. MASAILLA[2]

[1]Department of Agrotechnology, Faculty of Agriculture, Sebelas Maret University, Surakarta, Indonesia
[2]Department of Agronomy, Faculty of Agriculture, Sebelas Maret University, Surakarta, Indonesia
*Corresponding author's email: yuniastutisibuea@staff.uns.ac.id
Email addresses of co-authors: ibnuzak01@student.uns.ac.id, parjanto@staff.uns.ac.id, sukaya@staff.uns.ac.id, anamasailla@student.uns.ac.id

SUMMARY

Pineapple (*Ananas comosus* L.) is an edible fruit and the most economically important tropical plant of the family Bromeliaceae. Genetic diversity analysis can better support in pineapple advanced breeding that ultimately leads to improved quality of pineapple. The following study aimed to analyze the genetic diversity of five pineapple accessions originating from the Nusantara Pineapple Garden, Kudus, Indonesia, i.e., Blitar, Indralaya, Jambi, Riau Siak, and Madu Subang. The study collected plant samples from each accession, consisting of pineapple fruit crowns. The DNA isolated from each sample underwent amplification by the PCR using eight RAPD primers, viz., OPA-02, OPA-07, OPA-14, OPA-15, OPB-10, OPB-15, OPC-05, and OPD-01. The results showed the lowest genetic diversity among the five pineapple accessions, with variations in the number of amplified bands and DNA fragment size. Primer OPA 15 (900 bp) was the choice used to evaluate genetic variability in *Ananas comosus* between the smooth cayenne and queen types. Two clusters' identification resulted from the UPGMA analysis. Cluster 1, comprising four pineapple accessions, Blitar, Indralaya, Riau Siak, and Jambi, indicated the highest genetic similarity. However, the pineapple accession Madu Subang, placed in cluster 2, showed the lowest genetic similarity with other pineapple accessions.

Keywords: Pineapple (*A. comosus* L.), bromeliaceae, accessions, genetic diversity, RAPD primers, DNA, genetic similarity

Key findings: Genetic diversity assessment among five pineapple (*A. comosus* L.) accessions using RAPD primers revealed significant variability. Specifically, the primers OPA-15, OPB-10, OPB-15, and OPC-05 effectively distinguished the Madu Subang pineapple from other studied cultivars.

Communicating Editor: Dr. Anita Restu Puji Raharjeng

Manuscript received: June 13, 2024; Accepted: February 22, 2024.

Citation: Yuniastuti E, Zakaria I, Parjanto, Masailla APD (2025). Molecular identification of pineapple (A*nanas comosus*) accessions based on random amplified polymorphic DNA markers. *SABRAO J. Breed. Genet.* 57(4): 1377-1388. http://doi.org/10.54910/sabrao2025.57.4.4.

INTRODUCTION

Pineapple (*Ananas comosus* L. Merr.) is an important fruit commodity in Indonesia with steadily increasing production over the past few years. Pineapple production from 2018 to 2022 was 1.80, 2.20, 2.45, 2.89, and 3.20 million tons, respectively (BPS, 2022). Sustainable pineapple production has a positive impact on the Indonesian economy, both in the domestic and international markets. Besides being a considerable export commodity and due to its fresh, sweet taste and beneficial nutritional content, pineapples are also popular in the Indonesian community.

Genetic diversity analysis has become crucial in supporting the development of quality pineapples. The pineapple commodity needs a boost in improving its quality, either by its size, taste, or its nutrients. Traits in Indonesia (size and taste), especially the taste of pineapple, are sour, resulting in most people disliking pineapple due to its original taste. By modifying its genotype, researchers can invent pineapples with desirable characteristics. This can succeed by studying the genetic diversity of pineapples. Genetic diversity decoding can depend on the alleles of genes found in pineapple populations. The said information can help breeders maintain crossbred cultivars, retaining desirable traits, such as quality traits and tolerance to various stress factors (Salgotra and Chauhan, 2023).

In the context of agriculture, genetic diversity is most crucial in breeding programs, enabling the development and maintenance of hybrid cultivars with desired traits and adaptability to changing climatic conditions (Swarup *et al.,* 2021). Analysis of genetic diversity can be effective to assess the genetic composition among the populations and identify the cause of diversity. For example, genetic identification at the chromosome level shows that *P. aquatica* species have a variable number of chromosomes, namely, 2n = 88, 2n = 34, 2n = 92, and 2n = 66, and this variation caused the lack of phylogenetic signals for the number of chromosomes (Baum and Oginuma, 1994; Lima *et al.,* 2012; Costa *et al.,* 2017; Yuniastuti *et al*., 2022). Therefore, the presented study has chosen the DNA-level genetic analysis since the observed pineapples belong to the same species.

Genetic diversity analysis can be helpful for the development of superior cultivars with desirable fruit and quality traits in pineapple. Past research conducted by Amda *et al.* (2020) characterized the morphology and quality of local pineapples in Indonesia, and the pineapple accessions found in each location mostly have a wide range of similarity distances among the observed characters. Additionally, research on the evaluation of relationships among the pineapple populations relied on the isozyme analysis (Prasetyo *et al.*, 2023). In pineapples, the genetic diversity analysis using molecular markers succeeded in evaluating the relationships among the pineapple populations (Harahap *et al.*, 2013).

For the genetic diversity analysis, the random amplified polymorphic DNA (RAPD) technology has become one of the most applied and important approaches. RAPD is a molecular technique that employs different markers to study the genetic diversity at different levels. This technique relies on a PCR machine, which can amplify the DNA sequences in vitro (Khoiriyah *et al.,* 2018). RAPD is an effective and easy-to-use method, requiring no prior information about the target genome and allowing the use of limited samples. RAPD markers have been widely used to analyze the genetic diversity and the relationship of populations in a species (Yin *et al*., 2021).

In matoa identification (*Pometia pinnata*), namely, green matoa (*Emme Anokhong*), yellow matoa (*Emme Khabelaw*), and red matoa (*Emme Bhanggahe*), using five RAPD primers showed 28 total polymorphic bands, with an average of 52.8%, and 25 monomorphic bands, with an average of 47.2% (Yuniastuti *et al.,* 2023). Boomibalagan *et al.* (2021) analyzed the genetic variation of Asclepiadaceae using 16 RAPD primers. Another study by Hayati and Kasiamdari (2024) on the genetic variability of pineapple has also progressed using four ISSR primers, showing the existence of two groups separating 'Spanish' accessions from 'Queen' and 'Cayenne' accessions. However, the RAPD as a method for analyzing the genetic diversity

of pineapple plants in Indonesia is still very rare and confined. Therefore, further research in genetic diversity analysis of pineapples employing the RAPD method could provide better insights to support the development of superior pineapple cultivars in the field of plant breeding. Thus, the potential research aimed to analyze the genetic diversity of five pineapple accessions collected from Kebun Nanas Nusantara, Kudus, Indonesia, including Blitar, Indralaya, Jambi, Siak Riau, and Madu Subang pineapples.

MATERIALS AND METHODS

Breeding material

The pineapple accessions taken from Kebun Nanas Nusantara Kudus, Kudus Regency, Central Java, Indonesia (-6.791736, 110.860178), were a collection of pineapples from various regions. All the accessions, Blitar Pineapple (NBl), Indralaya Pineapple (NI), Jambi Pineapple (NJ), Siak Riau Pineapple (NSR), and Madu Subang Pineapple (NMS), underwent analysis. The pineapple fruit crown samples from each accession served for molecular analysis.

DNA isolation, quantification, and amplification

Performing DNA isolation followed the modified method of Doyle *et al.* (1990). The modification involved the addition of 1.4 M NaCl, 100 mM Tris HCl pH 8, 20 mM EDTA pH 8, 2% PVP, and 2% β-mercaptoethanol.

The use of the spectrophotometry method helped assess the DNA quantification. Spectrophotometric analysis measures the UV light absorption by DNA, allowing estimation of its concentration (Passos and Saraiva, 2019). Then, the quantification of purified DNA using a GeneQuant spectrophotometer determined the DNA concentration. The concentration and purity values of DNA samples depended on light absorption at wavelengths of 260/280 nm. According to Bunu *et al.* (2020), the ideal 260/280 ratio for pure DNA typically ranged from 1.8 to 2.0. This ratio indicates that the DNA was free from protein contamination or other organic compounds.

The DNA amplification results sustained visualization by electrophoresis, which is a technique to separate and analyze the DNA molecules based on their size and charge (Shahabadi *et al*., 2022). During electrophoresis, an electric field treatment of a gel matrix occurs, which contained the earlier inserted DNA into wells in the gel. The negatively charged DNA molecules move through the gel toward the positively charged electrodes. As they pass through the gel matrix, smaller DNA fragments move faster than larger DNA fragments, resulting in a visible banding pattern on the gel. The migration of DNA bands during electrophoresis enables visualization and characterization of DNA samples.

DNA amplification proceeded using the polymerase chain reaction (PCR), which aims to duplicate the DNA sequences based on the primers used (Foo *et al.,* 2020). The primers used were OPA-02 (TGCCGAGCTG), OPA-07 (GAAACGGGTG), OPA-14 (TCTGTGCTGG), OPA-15 (GGAGGGTGTT), OPB-10 (CTGCTGGGAC), OPB-15 (GGAGGGTGTT), OPC-05 (GATGACCGCC), and OPD-01 (ACCGCGAAGG). PCR reactions continued in a total volume of 12.5 µL for each PCR tube. Each PCR reaction consists of 6.25 µL GoTaq Green Master Mix (Promega), 1 µL of 10 µM primer from IDT-Integrated DNA Technologies, 2.5 µL DNA templates, and 2.75 µL nuclease-free water. DNA amplification succeeded using the PCR System BIO-RAD T100. The initial denaturation step transpired at 94 °C for 3 min, followed by 35 cycles consisting of a denaturation step at 94 °C for 1 min, annealing at 37 °C for 1 min, and elongation at 72 °C for 1 min and 30 s. After the repeated cycles, a final elongation step ensued at 72 °C for 8 min, followed by inactivation at 12 °C.

Electrophoresis

The PCR product DNA later underwent electrophoresis using a horizontal electrophoresis tank. A 1.5% (w/v) agarose powder, dissolved in 1X TBE buffer (comprising 0.45 M Tris-HCl pH 8, 0.45 M boric acid, and

20 mM EDTA), acquired Floro Safe DNA stain as a dye. The electrophoresis ran at 100 volts and a current of 400 mA for 70 min. The results' visualization used gel documentation, a system used to detect, record, and analyze electrophoresis gel results by visualizing the separated bands of DNA, RNA, or protein. This system utilizes UV or LED light sources, a camera, and software to produce fluorescent images that can be analyzed and stored.

Data analysis

The visualization results of electrophoresis attained scores to determine the molecular weight of the DNA bands using Gel Analyzer 23.1 to calculate the total bands, polymorphic bands, and percentage of polymorphic bands. It is basically counting the appearance of DNA bands (1 if present and 0 if not). The polymorphic information content (PIC) value, as calculated for each RAPD primer, relied on the formula PIC = 1 - $\sum(P_{ij})^2$, where P_{ij} is the frequency of the i-th pattern produced by the j-th primer summed for all bands produced by that primer (Akçali, 2020). The scoring results are binary data that undergo further analysis using the NTSYSpc version 2.1 software with the Dice coefficient to obtain a genetic similarity matrix. The Dice similarity coefficient's calculation followed the formula 2a/(2a+b+c), where a = both have a value of 1, b = 1-0, and c = 0-1, as developed by Czekanowski (1932), Dice (1945), and Sørensen (1948). Using the genetic similarity matrix helped construct a dendrogram representing the relationship among the pineapple accessions using the Unweighted Pair Group with Arithmetic Averaging (UPGMA) method.

RESULTS AND DISCUSSION

DNA purity value

The most widely used technique was to determine the concentration and purity of DNA with UV absorption measurement and a spectrophotometer (Table 1). The λ260/λ280 ratio provides the information on the type of nucleic acids found in the DNA/RNA solution which indicates purity. The nucleic acids' concentration determination resulted from measuring the absorption of ultraviolet light. The highest purity value appeared in the pineapple accession Nanas Blitar (NBl), with a purity value of 2.000, while the lowest value emerged in the accession Nanas Siak Riau (NSR), with a value of 1.750. The purity values of other pineapple accessions were as follows: Indralaya Pineapple (NI 1.860), Madu Subang Pineapple (NMS 1.882), and Jambi Pineapple (NJ 1.860). Overall, the purity values of the five pineapple accessions were still within a good range for DNA purity (1.8-2), although one accession has a purity value below 1.8.

Generally, protein contamination detection can come from a decrease in this ratio, which implies that the smaller the ratio, the greater the protein contamination. The RNA contamination can also be notable through an increase in this ratio, and the higher the ratio value, the greater the RNA contamination. Pure DNA has an A260/A280 ratio of about 1.8-2, while pure RNA has a ratio of about 2.1.

Table 1. Purity and concentration values of the DNA of five pineapple accessions resulting from extraction with the CTAB method.

No.	Sample code	Purity value (λ260/λ280)	Concentration value (ng/uL)
1	NBl	2.000	400
2	NI	1.860	799
3	NJ	1.941	301
4	NSR	1.750	599
5	NMS	1.882	513

NBl = Blitar Pineapple; NI = Indralaya Pineapple; NJ = Jambi Pineapple; NSR = Siak Riau Pineapple; and NMS = Madu Subang Pineapple.

The pineapple accession Riau Siak has a purity value of below 1.8, and it was evident from the findings of previous studies that a 260/280 ratio falling below 1.8 signifies the presence of protein contamination (Russo *et al*., 2022). Protein contamination can arise from the improper lysis process. Li *et al.* (2021) stated that the lysis process is a crucial initial step, playing a pivotal role in determining the quality and overall efficiency of nucleic acid separation from impurities, such as protein, polysaccharide, and so on. According to Hindash and Hindash (2022), the DNA absorbs light maximally at a wavelength of 260 nm. Originating from the Beer-Lambert law, the amount of light absorbed at 260 nm was proportional to the concentration of nucleic acids in the solution (Koetsier and Cantor, 2019). The DNA purity is crucial to prevent smearing in electrophoresis results (Yulis and Isda, 2021).

DNA amplification

DNA amplification is a technique used to develop the copies of a specific DNA sequence, allowing for the generation of a larger amount of genetic material. The number of DNA bands produced by each primer and sample can be diverse, depending on the movement of molecules influenced by their shape, charge, and size. The DNA amplification results are visible in Figure 1. The polymerase chain reaction (PCR) is a method of DNA amplification that utilizes a pair of oligonucleotides, also known as primers, to produce a copy of the target DNA segment (Karunanathie *et al*., 2022). These primers specifically bind to complementary nucleotide sequences on the target DNA, thereby serving as the template for the DNA polymerase enzyme to synthesize the desired DNA copy. This process is particularly useful in analyzing DNA from small initial samples (Xu *et al*., 2021).

Each primer produced the DNA bands with different fragment sizes. Based on the visualization results, some primers were less compatible with pineapple accessions, such as primers OPA-14 and OPD-01, which failed to amplify DNA bands. The primer OPA-02 only produced one monomorphic band that could not be applicable to distinguish the pineapple accessions. Based on these results, primer selection proceeded by eliminating the primers OPA-14, OPA-02, and OPD-01 and keeping the five primers OPA-07, OPA-15, OPB-10, OPB-15, and OPC-05 to analyze the genetic variability in pineapple accessions.

The five RAPD primers OPA-07, OPA-15, OPB-10, OPB-15, and OPC-05 revealed the genetic diversity in the pineapple accessions, viz., Blitar, Indralaya, Jambi, Siak Riau, and Madu Subang (Table 2). With the primer OPA-07, the accession Blitar Pineapple produced eight bands, Indralaya Pineapple produced 10 bands, Jambi Pineapple produced eight bands, Siak Riau Pineapple produced 10 bands, and Madu Subang Pineapple produced seven bands, all at 370–1600 bp. With the primer OPA-15, the accessions Blitar Pineapple, Indralaya Pineapple, Jambi Pineapple, and Siak Riau Pineapple produced four bands at 220–900 bp, except for Madu Subang Pineapple, which produced five bands at 220–900 bp.

With primer OPB-10, the accessions Blitar Pineapple, Indralaya Pineapple, and Siak Riau Pineapple all produced four bands at 560–1800 bp. Meanwhile, Jambi Pineapple produced two bands at 730–800 bp, and Madu Subang Pineapple produced five bands at 430–1800 bp. With primer OPB-15, the accessions Blitar Pineapple and Madu Subang Pineapple both produced five bands at 400–1400 and 320–1400 bp, respectively. Other accessions, Indralaya Pineapple, produced four bands at 480–1400 bp; Jambi Pineapple produced four bands at 420–1400 bp; and Siak Riau Pineapple also produced four bands at 480–1400 bp. With primer OPC-05, the accessions Blitar Pineapple, Jambi Pineapple, and Madu Subang Pineapple all produced six bands at 180–1000 bp. Meanwhile, Indralaya Pineapple and Siak Riau Pineapple produced seven bands at 180-1000 bp.

The study revealed each RAPD primer produced a different number of bands. The variation in the number of bands produced by each primer was due to each primer having different target DNA sequences, resulting in

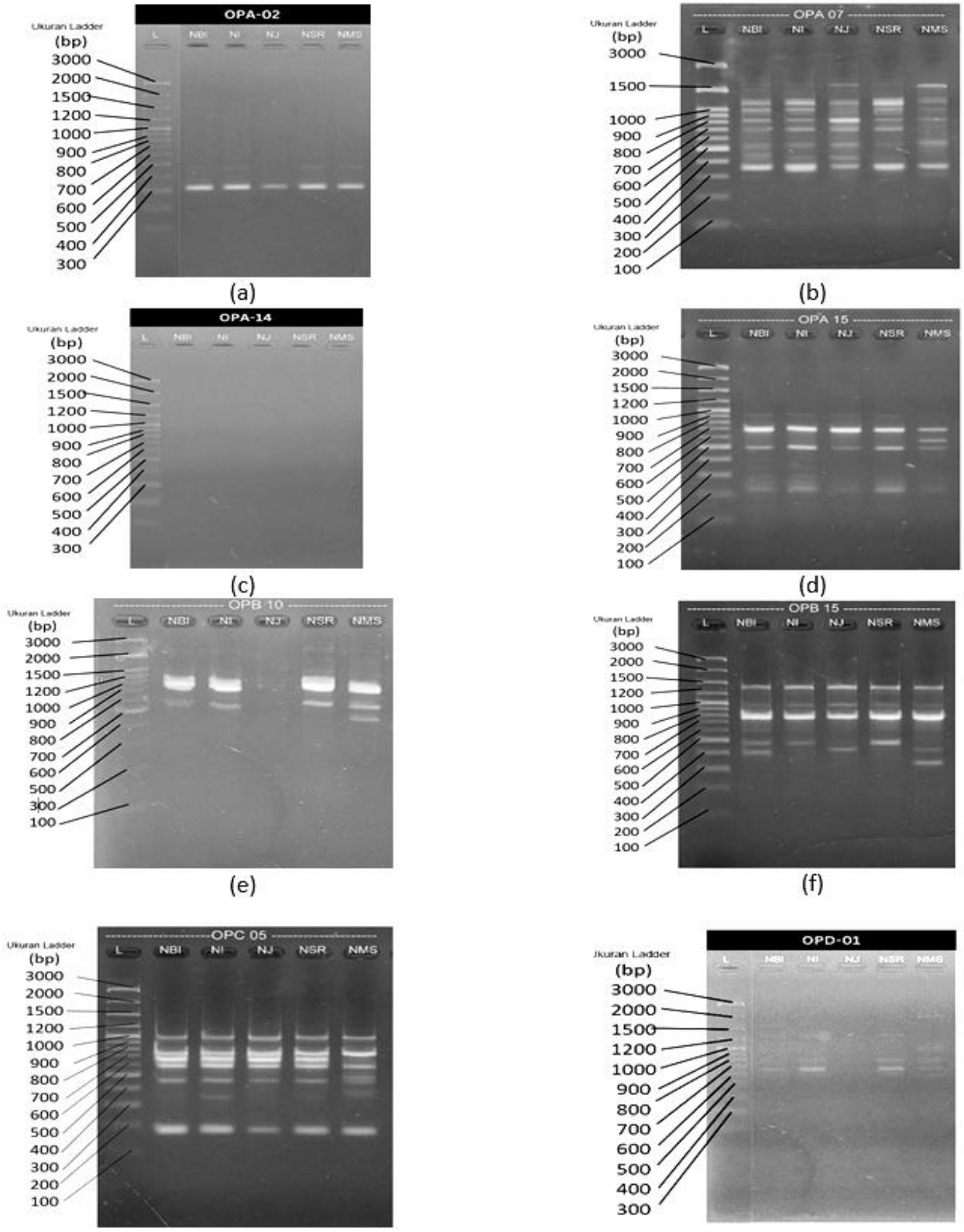

Figure 1. DNA amplification results: OPA-02 (a), OPA-07 (b), OPA-14 (c), OPA-15 (d), OPB-10 (e), OPB-15 (f), OPC-05 (g), and OPD-01 (h).

Table 2. The fragment sizes appearing with five RAPD primers in five pineapple accessions.

Primer	Sample Code	Fragments (bp)
	NBl	370, 530, 700, 820, 1000, 1200, 1270, 1600
	NI	370, 420, 530, 700, 820, 930, 1000, 1200, 1270, 1600
OPA 07	NJ	370, 420, 530, 700, 820, 1200, 1270, 1600
	NSR	370, 420, 530, 700, 820, 930, 1000, 1200, 1270, 1600
	NMS	370, 530, 820, 1000, 1200, 1270, 1600
	NBl	220, 500, 700, 900
	NI	220, 500, 700, 900
OPA 15	NJ	220, 500, 700, 900
	NSR	220, 500, 700, 900
	NMS	220, 500, 570, 700, 900
	NBl	560, 730, 800, 1800
	NI	560, 730, 800, 1800
OPB 10	NJ	730, 800
	NSR	560, 730, 800, 1800
	NMS	430, 560, 730, 800, 1800
	NBl	400, 480, 880, 970, 1400
	NI	480, 880, 970, 1400
OPB 15	NJ	420, 880, 970, 1400
	NSR	480, 880, 970, 1400
	NMS	320, 420, 880, 970, 1400
	NBl	180, 450, 590, 650, 740, 1000
	NI	180, 350, 450, 590, 650, 740, 1000
OPC 05	NJ	180, 450, 590, 650, 740, 1000
	NSR	180, 350, 450, 590, 650, 740, 1000
	NMS	180, 450, 480, 590, 740, 1000

NBl = Blitar Pineapple; NI = Indralaya Pineapple; NJ = Jambi Pineapple; NSR = Siak Riau Pineapple; and NMS = Madu Subang Pineapple.

diverse fragments. RAPD produces varying patterns due to the combination of genetic diversity, sequence polymorphisms, technical variables, and sometimes, environmental influences. The pineapple accession Madu Subang has unique DNA bands that are unavailable in other genotypes, with the unique DNA bands amplified by the primers OPA-15 (320 bp), OPB-10 (430 bp), OPB-15 (570 bp), and OPC-05 (480 bp). Amom *et al.* (2020) reported that the use of multiple primers provides an opportunity to find more genetic variation. Additionally, it offers a more comprehensive picture of genetic polymorphism in the studied genotypes. These results can gain support from other analytical methods, such as karyotyping. The karyotype composition can materialize as a karyogram, a photomicrograph showing a single image of somatic metaphase cells arranged according to their similar size and shape (Yuniastuti *et al.,* 2018).

Polymorphism

The scoring can determine how many bands appeared in each primer. The percentage of polymorphic bands in each primer can also be calculated (Table 3). The polymorphic bands produced by the five primers OPA-07, OPA-15, OPB-10, OPB-15, and OPC-05 were four, one, three, four, and three bands, respectively, with corresponding percentages of 40%, 20%, 60%, 57.14%, and 37.5%. Among the five primers, the highest percentage of polymorphic bands resulted from the primer OPB-10 (60%). The percentage of polymorphic bands, when calculated, determined the proportion of polymorphic loci formed by each primer. According to Terletskaya *et al.* (2023), the data based on the polymorphism served as an indicator to assess the genetic diversity in a population.

With primer OPB-10, the highest percentage of polymorphic bands indicated that the five pineapple samples tested have a high level of genetic diversity (Table 3). Conversely, a low percentage of polymorphic bands implies low genetic diversity, while a high percentage of polymorphic bands signifies the ultimate genetic variation among the studied populations (Zheng *et al.,* 2017). The variation in the number of bands amplified by different primers may acquire influences from varying factors, such as primer structure, template quantity, and the number of annealing sites in the genome (Ibrahem and Ajmi, 2021).

The level of polymorphism observed during the RAPD analysis can be because of the genetic flow due to population isolation and intraspecific diversity constraints. The greater number of polymorphic DNA fragments indicating a wide range of genetic variations allowed each individual to be distinct from the other (Ifah *et al.,* 2018). In this context, the geographic isolation that limits gene exchange among the populations can lead to the accumulation of genetic differences, increasing the level of polymorphism observed in the RAPD analysis. Additionally, disturbances in natural habitats can disrupt migration patterns and interactions between the individuals, reducing the likelihood of gene transfer among the different populations, thereby reinforcing genetic differences among them.

Another parameter used to assess the genetic diversity in different populations is the polymorphic information content (PIC). The PIC is a quantitative measurement used to assess the informativeness and discriminative power of a genetic marker (Serrote *et al.,* 2020). This parameter provides information about the diversity and variability of a genetic marker within a population. Based on the presented results, it is noticeable that the PIC values generated by the five primers ranged from 0.13 (OPA-15) to 0.3 (OPB-10) (Table 4). Higher PIC values indicate greater polymorphism. According to Rohini *et al.* (2020), higher PIC values also specify that the primer can distinguish the individuals more effectively. Moreover, PIC values can help in selecting the most informative and diverse genetic markers for research based on genetic diversity within a population. The results indicated that overall, the primer OPB-10 has a higher efficiency level than the other primers.

Table 3. Polymorphism of five RAPD primers in five pineapple accessions.

No.	Primer	Fragment Size (bp)	Total Bands	Polymorphic Bands	Polymorphic (%)	PIC
1	OPA-07	370-1600	10	4	40.00	0.16
2	OPA-15	220-900	5	1	20.00	0.13
3	OPB-10	430-1800	5	3	60.00	0.30
4	OPB-15	320-1400	7	4	57.14	0.23
5	OPC-05	180-1000	8	3	37.50	0.14
	Total		35	15		
	Average		7	3	42.93	0.19

Table 4. Genetic similarity matrix using Dice coefficient in five pineapple accessions.

Accessions	NBl	NI	NJ	NSR	NMS
NBl	1.00				
NI	0.929	1.000			
NJ	0.863	0.868	1.000		
NSR	0.945	0.982	0.885	1.000	
NMS	0.821	0.828	0.792	0.807	1.000

NBl = Blitar Pineapple; NI = Indralaya Pineapple; NJ = Jambi Pineapple; NSR = Siak Riau Pineapple; and NMS = Madu Subang Pineapple.

Genetic similarity

The genetic similarity coefficient is a statistical measurement used to evaluate the genetic similarity among the individuals within a species. The genetic similarity of five pineapple accessions appears in Table 4. The genetic similarity coefficient ranges from zero to one, where values closer to zero indicate low genetic similarity, and values closer to one signify a greater genetic similarity (Agustin *et al.,* 2023). Based on the genetic matrix using the Dice coefficient from five RAPD primers, it is notable that a considerable variation occurred in the level of genetic similarity among the pineapple accessions.

The pineapple accessions showing the highest level of genetic similarity with each other were Indralaya Pineapple (NI) and Siak Riau Pineapple (NSR), with a genetic similarity level of 98%. Meanwhile, the lowest value was evident between the accessions Madu Subang Pineapple (NMS) and Jambi Pineapple (NJ), with a genetic similarity level of 79%. These lower values indicated that Madu Subang Pineapple and Siak Riau Pineapple have a greater genetic variation than the other samples. Furthermore, it revealed that a correlation existed between genetic variation and the level of genetic similarity. The group rankings of different pineapple accessions can depend on the genetic similarity level, as follows: NBl and NI (0.929), NSR and NJ (0.885), NBl and NSR (0.945), NBl and NJ (0.863), NI and NSR (0.982), NI and NJ (0.868), NSR and NMS (0.807), NI and NMS (0.828), NBl and NMS (0.821), and NJ and NMS (0.792).

The UPGMA cluster analysis of the five primers, visualized through the dendrogram, gave two main clusters (Figure 2). Cluster 1 has a genetic similarity of 81%, comprising the pineapple accessions Blitar Pineapple, Indralaya Pineapple, Siak Riau Pineapple, and Jambi Pineapple. Cluster 1 further broke into subcluster 1, with a genetic similarity of 81%, separating the accession Blitar Pineapple from two other genotypes, Indralaya Pineapple and Siak Riau Pineapple. In subcluster 2, only one accession (Jambi Pineapple) appeared. Cluster 2 only contains the pineapple accession Madu Subang Pineapple. The UPGMA cluster analysis provides a clear overview of the relationships among the five pineapple accessions.

The presence of Indralaya Pineapple and Siak Riau Pineapple in the same cluster indicated a higher similarity between them (Figure 2). Furthermore, the appearance of the accession Madu Subang Pineapple in a separate cluster proves that this accession has a lower similarity with the other four accessions. The separation of accession Madu Subang Pineapple in a different cluster was also due to the appearance of unique DNA bands that were not evident in other pineapples, with the primers OPA-15 (570 bp),

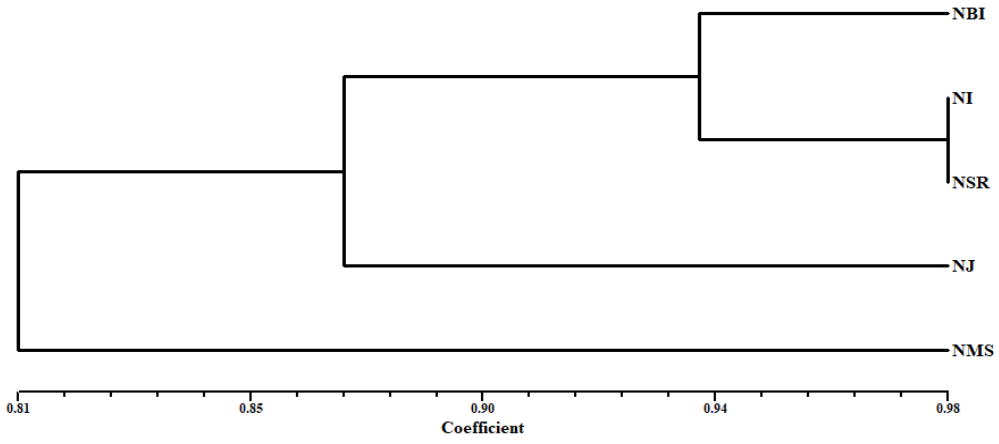

Figure 2. UPGMA dendrogram of genetic similarity among five pineapple accessions based on RAPD markers.

OPB-10 (430 bp), OPB-15 (320 bp), and OPC-05 (480 bp). Likewise, the presence of unique bands in the accession Madu Subang Pineapple signified that this genotype has specific characteristics unavailable in other pineapple accessions. One possible characteristic was the presence of honey in Madu Subang Pineapple not occurring in other pineapple accessions. Knowing these specific traits can serve as a basis in plant breeding to develop superior pineapple cultivars with desirable fruit and quality traits.

CONCLUSIONS

In pineapple accessions, the genetic diversity indicated by RAPD primers varies based on amplified bands, ranging from five to 10 bands, with DNA fragment sizes of 220–1800 bp, polymorphic bands (20%–60%), and PIC values (0.13 to 0.3). The specific primers OPA-15 at 570 bp, OPB-10 at 430 bp, OPB-15 at 320 bp, and OPC-05 at 480 bp can be effective as specific primers to differentiate the pineapple accession Madu Subang Pineapple from other accessions. The study revealed two main clusters, and cluster 1 comprised the accessions Blitar Pineapple, Indralaya Pineapple, Siak Riau Pineapple, and Jambi Pineapple, revealing the highest genetic similarity. Conversely, cluster 2 only consists of the accession Madu Subang Pineapple.

ACKNOWLEDGMENTS

The authors are grateful for the completion of the research, which would not have been possible without Prof. Endang Yuniastuti (yuniastutisibuea@staff.uns.ac.id) for supporting and funding this research.

REFERENCES

Agustin L, Ernawati E, Sulistyaningsih LD (2023). Genetic diversity of bananas (*Musa* spp.) in Pesawaran District, Lampung Province based on sequence-related amplified polymorphism (SRAP) marker. *J. Biol. Tropis*. 23(4): 680–690. https://doi.org/10.29303/jbt.v23i4.5493.

Akçali GRR (2020). Investigation of the genetic variation of anise (*Pimpinella anisum* L.) using RAPD and ISSR markers. *Genet. Res. Crop Evol.* 67: 763–780. https://doi.org/10.1007/s10722-019-00861-y.

Amda PP, Hanafiah DS, Kardhinata EH (2020). Karakterisasi morfologis dan hubungan kekerabatan tanaman nanas (*Ananas comosus* (L.) MERR.) di Kabupaten Kampar dan Siak Provinsi Riau. Rhizobia: *J. Agrotek.* 2(2):32–43. https://doi.org/10.36985/jra.v2i2.230.

Amom T, Tikendra L, Apana N, Goutam M, Sonia P, Koijam AS, Potshangbam AM, Rahaman H, Nongdam P (2020). Efficiency of RAPD, ISSR, iPBS, SCoT and phytochemical markers in the genetic relationship study of five native and economical important bamboos of North-East India. *Phytochemistry* 174: 1–12. https://doi.org/10.1016/j.phytochem.2020.112330.

Baum DA, Oginuma K (1994). A review of chromosome numbers in Bombacaceae with new counts for Adansonia. *J. Taxon.* 43(1):11–20.

Boomibalagan P, Subramanian SR, Rajasekharan PE, Karpakal S, Veeranan U, Saminathan E, Narayanan V, Kathiresan D (2021). Genetic relationship and polymorphism of selected medicinal plants of *Asclepiadaceae* using RAPD molecular analysis method. *Ecol. Genet. Genomics* 1–8. https://doi.org/10.1016/j.egg.2021.100101.

Bunu SJ, Otele D, Alade T, Dodoru RT (2020). Determination of serum DNA purity among patients undergoing antiretroviral therapy using NanoDrop-1000 spectrophotometer and polymerase chain reaction. *Biomed. Biotechnol. Res. J.* 4(3): 214-9. https://doi.org/10.4103/bbrj.bbrj_68_20.

Costa L, Oliveira Á, Sobrinho JGC, Souza LGR (2017). Comparative cytomolecular analyses reveal karyotype variability related to biogeographic and species richness patterns in Bombacoideae (Malvaceae). *J. Plant. Syst. Evol.* 303(9): 1131–1144.

Czekanowski J (1932). Coefficient of racial likeness and average difference. *Anthropologischer Anzeiger*. 9: 227–249.

Dice LR (1945). Measures of the amount of ecological association between species. *Ecology.* 26: 297–302.

Doyle JJ, Doyle JL, Brown AH (1990). Chloroplast DNA polymorphism and phylogeny in the B

genome of Glycine subgenus Glycine (Leguminosae). *Am. J. Bot*. 77(6): 772–782.

Foo PC, Nurul Najian AB, Muhamad NA, Muhamad NA, Ahamad M, Mohamed M, Yean C, Lim BH (2020). Loop-mediated isothermal amplification (LAMP) reaction as viable PCR substitute for diagnostic applications: A comparative analysis study of LAMP, conventional PCR, nested PCR (nPCR) and real-time PCR (qPCR) based on *Entamoeba histolytica* DNA derived from faecal sample. *BMC Biotechnol.* 20(34):1–15. https://doi.org/10.1186/s12896-020-00629-8.

Harahap F, Suriani C, Silaban SS (2013). Induksi pertumbuhan nanas (*Ananas comosus* L.) asal Sipatuhar secara in vitro. In *Prosiding Seminar Hasil Penelitian Lembaga Penelitian Unimed.* 1(1): 156–161. https://doi.org/10.22437/biospecies.v14i2.14817.

Hayati R, Kasiamdari RS (2023). Morphological characters and phenetic relationship of pineapple (*Ananas comosus*) from Central Java and Riau Provinces, Indonesia. *Int. J. Agric. Biol.* 30(6): 405–414. https://doi.org/10.17957/IJAB/15.2102.

Hindash DA, Hindash A (2022). Quantitative analysis of DNA samples. *Adv. Sci. Eng. Tech. Int. Conf. (ASET).* 1–3. IEEE. https://doi.org/10.1109/ASET53988.2022.9735102.

Ibrahem AG, Ajmi RN (2021). Forensic assessment of genotoxic effects of lead metal on leaf *Phragmites australis* using random amplified polymorphic DNA (RAPD-PCR) markers. *Indian J. Forensic Med. Toxicol.* 15(3). https://doi.org/10.37506/ijfmt.v15i3.15520.

Ifah AA, Yuniastuti E, Parjanto P (2018). Analysis of breadfruit plant diversity (*Artocarpus altilis* P.) by random amplified polymorphic DNA (RAPD) in DIY. In: *AIP Conf. Proc.* 2021(1). AIP Pub. https://doi.org/10.1063/1.5062802.

Karunanathie H, Kee PS, Ng SF, Kennedy MA, Chua EW (2022). PCR enhancers: Types, mechanisms, and applications in long-range PCR. *Biochimie.* 197: 130–143. https://doi.org/10.1016/j.biochi.2022.02.009.

Khoiriyah N, Yuniastuti E, Purnomo D (2018). Genetic diversity of pigeon pea (*Cajanus cajan* (L.) Millsp.) based on molecular characterization using randomly amplified polymorphic DNA (RAPD) markers. In: *IOP Conf. Ser.: Earth Environ. Sci.* 129(1). https://iopscience.iop.org/article/10.1088/1755-1315/129/1/012016.

Koetsier G, Cantor E (2019). A practical guide to analyzing nucleic acid concentration and purity with microvolume spectrophotometers. *New England Biolabs Inc.* pp. 1–8.

Li P, Li M, Zhang F, Wu M, Jiang X, Ye B, Chen H (2021). High-efficient nucleic acid separation from animal tissue samples via surface modified magnetic nanoparticles. *Separation and Purification Technology 262*, 118348.

Lima MFD, Miranda DP, Karsburg IV (2012). Characterization of the chromosomes of *Pachira aquatica* Aubl, (Malvaceae). *J. Goiânia* 39(3): 337–344.

Passos ML, Saraiva ML (2019). Detection in UV-visible spectrophotometry: Detectors, detection systems, and detection strategies. *Measurement* 135: 896–904. https://doi.org/10.1016/j.

Prasetyo HI, Wijana G, Pradnyawathi NLM (2023). Identifikasi dan karakterisasi tanaman nanas (*Ananas comosus* (L.) Merr) di daerah Bali dalam upaya pengembangannya. *J. Agric. Sci.* 3(1): 113–123. https://doi.org/10.24843/AJoAS.2023.v13.i01.p10.

Rohini MR, Sankaran M, Rajkumar S, Prakash K, Gaikwad A, Chaudhury R, Malik SK (2020). Morphological characterization and analysis of genetic diversity and population structure in *Citrus* × *jambhiri* Lush. using SSR markers. *Genet. Res. Crop Evol.* 67: 1259–1275. https://doi.org/10.1007/s10722-020-00909-4.

Russo A, Mayjonade B, Frei D, Potente G, Kellenberger RT, Frachon L, Copetti D, Studer B, Frey JE, Grossniklaus U, Schlüter PM (2022). Low-input high-molecular-weight DNA extraction for long-read sequencing from plants of diverse families. *Front. Plant Sci.* 13:1494: 1–12. https://doi.org/10.3389/fpls.2022.883897.

Salgotra RK, Chauhan BS (2023). Genetic diversity, conservation, and utilization of plant genetic resources. *Genes*. 14(1):174. https://doi.org/10.3390/genes14010174.

Serrote CM, Reiniger LR, Silva KB, dos Santos Rabaiolli SM, Stefanel CM (2020). Determining the polymorphism information content of a molecular marker. *Gene*s 726: 144175. https://doi.org/10.1016/j.gene.2019.144175.

Shahabadi N, Zendehcheshm S, Khademi F (2022). Exploring the ct-DNA and plasmid DNA binding affinity of the biogenic synthesized Chloroxine-conjugated silver nanoflowers: Spectroscopic and gel electrophoresis

methods. *Process Biochem.* 121: 360–370. https://doi.org/10.1016/j.procbio.2022.07.024.

Sørensen TJ (1948). A method of establishing groups of equal amplitude in plant sociology based on similarity of species content and its application to analyses of the vegetation on Danish commons. *Royal Danish Academy Sci Letters, Bio Ser.* 5: 1–34.

Swarup S, Cargill EJ, Crosby K, Flagel L, Kniskern J, Glenn KC (2021). Genetic diversity is indispensable for plant breeding to improve crops. *Crop Sci.* 61(2): 839–852. https://doi.org/10.1002/csc2.20377.

Terletskaya NV, Khapilina ON, Turzhanova AS, Erbay M, Magzumova S, Mamirova A (2023). Genetic polymorphism in the *Amaranthaceae* species in the context of stress tolerance. *Plants* 12(19): 1–14. https://doi.org/10.3390/plants12193470.

Xu L, Duan J, Chen J, Ding S, Cheng W (2021). Recent advances in rolling circle amplification-based biosensing strategies. *Anal. Chimica Acta.* 1148: 1–16. https://doi.org/10.1016/j.aca.2020.12.062.

Yin J, Khayatnezhad M, Shakoor A (2021). Evaluation of genetic diversity in Geranium (*Geraniaceae*) using RAPD marker. *Genetika* 53(1): 363–378. https://doi.org/10.2298/GENSR2101363Y.

Yulis PA, Isda MN (2021). Molecular characterization of Riau's Mascot Flora (*Oncosperma tigillarium*). In: *7th Int. Conf. Res. Implementation and Edu. Math. Sci. (ICRIEMS).* 528: 23–27. Atlantis Press. https://doi.org/10.2991/assehr.k.210305.004.

Yuniastuti E, Parjanto, Yullianingsih E, Delfianti MN (2018). Cytogenetic and karyotype analysis of sapodilla (*Achras zapota*). *Bulgarian J. Agric. Sci.* 24(3):421–426.

Yuniastuti E, Rahmawati ER, Sukaya (2022). Karyotype analysis of Pachira (*Pachira aquatica*). *SABRAO J. Breed. Genet*. 54(5): 1141–1148.

Yuniastuti E, Yuliana A, Sukaya N (2023). Genetic diversity of matoa (*Pometia pinnata*) species based on RAPD markers. *SABRAO J. Breed. Genet*. *55*(6): 1941–1949. http://doi.org/10.54910/sabrao2023.55.6.8.

Zheng Y, Xu S, Liu J, Zhao Y, Liu J (2017). Genetic diversity and population structure of Chinese natural bermuda grass [*Cynodon dactylon* (L.) Pers.] germplasm based on SRAP markers. *PLoS One* 12(5): 1–15. https://doi.org/10.1371/journal.pone.0177508.