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BIODIVERSITAS
Volume 25, Number 4, April 2024
Pages: 1771-1780

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250447

Diversity of *Salacca zalacca* (Gaertn.) Voss from Bali, Indonesia based on morphological and molecular characters

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Manuscript received: 10 March 2023. Revision accepted: 26 April 2024.

Abstract. *Sumantra IK, Listihani L, Ariati PEP. 2024. Diversity of* Salacca zalacca *(Gaertn.) Voss from Bali, Indonesia based on morphological and molecular characters. Biodiversitas 25: 1771-1780.* There are several salak (*Salacca zalacca* (Gaertn.) Voss) variations based on epicarp (skin) color, pulp aroma, and taste. The different characters are not only found among different production centers, but also among plants cultivated in the same area. This study aimed to identify the morphological and molecular characters of 16 salak cultivars from Bali, Indonesia. Morphological characters were described based on the individual test guideline for salak species and molecular characters were analyzed using four Random Amplified Polymorphic DNA (RAPD) primers, namely OPA3, OPC7, OPD4, and OPD20. The morphological characters of 16 salak cultivars are very diverse in plant height, number of leaflets, leaflets length and width, leaf sheath length, fruit skin color, fruit shape, fruit length, fruit diameter, number of fruits per bunch, number of seeds per fruit, and thick pulp. A total of 37 DNA bands ranging from 250-4000 bp was obtained and 28 bands of which are polymorphic. The dendrogram formed 4 clusters. Cluster 1 consists of Bingin, Kelapa, Gulapasir, Nangka, Muani, Gonong, Penyalin, Gondok, Layu, Sudamala, Merah, Jaka, and Putih salak cultivars. Cluster 2 consists of Pada cultivar, cluster 3 consists of Injin cultivar, and cluster 4 consists of Nenas cultivar. This research is the first diversity analysis report on *S. zalacca* from Bali based on morphological and molecular characters.

Keywords: Bali, molecular, morphological characters, RAPD, *Salacca zalacca*, salak cultivars

INTRODUCTION

The salak plant (*Salacca zalacca* (Gaertn.) Voss) belongs to the family Palmae or Arecaceae (Herawati et al. 2018; Ilmiah et al. 2021). It is a native species to Indonesia, especially in Java and Sumatra (Zumaidar et al. 2014; Hakim et al. 2019). Afterwards, salak is cultivated throughout tropical countries and mostly in Southeast Asia, including in Indonesia, Malaysia, Thailand, and Myanmar (Zumaidar et al. 2014; Bais 2016; Ismail and Baka 2018; Ritonga et al. 2018; Hakim et al. 2019; Cepkova et al. 2021).

There are three kinds of salak flowers, namely female, male, and mixed flowers. Male flowers are wrapped in spadix with long peduncle. In salak plantations, male flowers are often sold in the market as a source of males. The female flowers are wrapped in a sheath with short peduncle. Inflorescences emerge from the axils of the leaf (Wiangsamut et al. 2017). The fruit has scales skin to a snake, and thus it is called the snake fruit (Saleh et al. 2018; Mazumdar et al. 2019). Salak is preferred for the fruit's texture, taste, and high nutritional value (Ritonga et al. 2018; Saleh et al. 2018). Salak fruit contains bioactive compounds as antioxidants (Tan et al. 2020), and is also a source of vitamins, minerals, and dietary fiber (Ritonga et al. 2018; Mazumdar et al. 2019; Cepkova et al. 2021). The fruit's pulp is used for anti-inflammatory, anti-cancer, anti-diabetes (Saleh et al. 2018), and anti-aging agents (Girsang et al. 2019). *Salacca zalacca* is also used for traditional medicine and as a food ingredient by local communities (Eddy et al. 2023).

Sibetan Village, Karangasem Regency is the center of salak plantation in Bali, Indonesia (Sumantra et al. 2012; Sumantra et al. 2014; Tamba and Sumantra 2022). According to Darmadi et al. (2002), salak in Bali consists of 12 cultivars, while Gari (2011) reported that it has 13 cultivars, namely Boni, Bingin, Selem, Embad, Nangka, Salinan, Maong, Nyuh, Putih, Muani, Gondok, Nanas, dan Gula. Diversity of salak Bali is categorized based on pulp texture, epicarp (skin) color, aroma, and pulp taste (Darmadi 2002; Sumantra et al. 2014; Sumantra and Martiningsih 2016; Tamba and Sumantra 2022; Sumantra et al. 2023). Morphological differences in salak were found not only in different production centers but also among plants cultivated within the same area or region (Zumaidar et al. 2014; Mazumdar et al. 2019).

Salak cultivars are differentiated based on the place of origin, fruit epicarp (skin) color, fruit pulp color, aroma, and taste. Morphological, anatomical, molecular characteristics in salak are crucial for germplasm conservation and to develop salak superior cultivars through breeding programs and agronomy studies on the desired attributes (Budiyanti et al. 2015). Morphological characterization based on leaf micromorphology of 13 cultivars of salak Bali has not shown accurate results (Gari 2011). Utilization of morphology characters is a fast and simple method, but the problem is environmental factors can visually influence the characterization result. Molecular analysis, such as Random Amplified Polymorphic DNA (RAPD) method which detects variation at the DNA level overcomes most of the limitations of morphological characters. It is not affected by environmental conditions and the developmental stage of plants and is repeatable in data (Williams et al. 1990). Elly et al. (2018) reported genetic diversity analysis of *Salacca edulis* from West Seram District, Maluku, Indonesia based on morphological characters and RAPD profiles and showed moderate genetic diversity based on RAPD profiles but low morphological variations.

According to Sumantra et al. (2012), identification of salak Bali cultivars based on leaf micromorphology was less accurate. Therefore, it is very important to identify salak Bali cultivars based on morphological and molecular characters. This study aimed to analyze 16 salak cultivars from Bali, Indonesia based on morphological and molecular characters.

MATERIALS AND METHODS

Plant materials

The plant materials were obtained from Karangasem Regency, Bali, Indonesia. A total of 16 cultivars were used for the observation of morphological and molecular characters, namely S1 (Bingin), S2 (Pada), S3 (Layu), S4 (Kelapa), S5 (Sudamala), S6 (Gulapasir), S7 (Merah), S8 (Jaka), S9 (Muani), S10 (Gonong), S11 (Putih), S12 (Penyalin), S13 (Injin), S14 (Gondok), S15 (Nangka), and S16 (Nenas). The samples observed in each cultivar were 20 samples, so the total samples observed in the 16 cultivars were 320 samples. Several cultivars used in the study are presented in Figure 1.

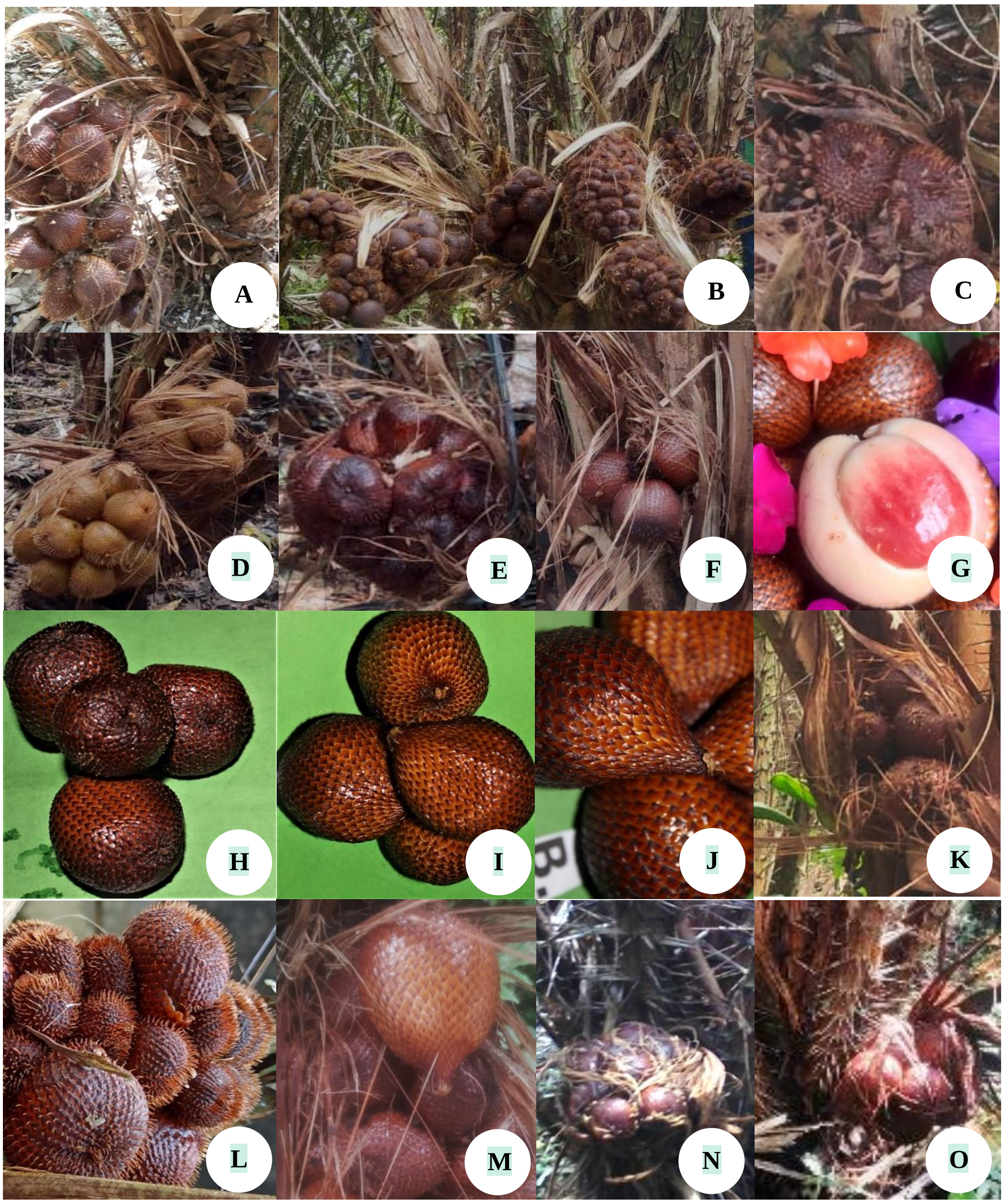

Figure 1. The shape and epicarp (skin) color of several cultivars of salak Bali used in the study. A. Nenas, B. Gonong, C. Sudamala, D. Putih, E. Kelapa, F. Injin, G. Merah, H. Gondok, I. Nangka, J. Gulapasir, K. Jaka, L. Bingin, M. Pada, N. Layu, O. Penyalin

Morphological characters

The 18 morphological characters were observed, including 8 qualitative and 10 quantitative characters of salak cultivars based on the Individual Testing Guide (Ministry of Agriculture Republic Indonesia 2006). Qualitative characters observed are young leaf color, leaf sheath color, flower sheath color, flower crown color, filament color, fruit skin color, fruit shape, and spine color. Meanwhile, quantitative colors observed are plant height, number of leaflets, leaflets length, leaflets width, leaf sheath length, fruit length, fruit diameter, number of fruits per bunch, number of seeds per fruit, and thick pulp.

Molecular characters

DNA extraction

The DNA was extracted using a method described by Doyle and Doyle (1987) which was modified to deal with secondary metabolite brought along inside the salak leaf samples. Young salak leaves were chopped and weighed into 0.5 g and then homogenized by mortar and pestle using liquid nitrogen. Once a fine consistency, 1 mL CTAB (cetyltrimethyl ammonium bromide) extract buffer containing 1% µL β-mercaptoethanol was added after incubation at 65°C for 15 minutes. The homogenate was then incubated at 65°C for 30 minutes, then centrifuged at 12000 rpm for 7 minutes. The supernatant was separated from the pellet and added PCI (phenol: chloroform: isoamyl alcohol) with a ratio of 25:24:1 and then centrifuged at 12000 rpm for 7 minutes. The supernatant was put inside a new 1.5 mL tube and added CI (chloroform: isoamyl alcohol) with a ratio of 1:1 and then centrifuged at 12000 rpm for 7 minutes. The CI working steps were repeated two times to obtain the product purified from secondary metabolites. The supernatant was put inside a new 1.5 mL tube and added by 50 µL NaCl 5 M and 6 µL isopropanol; before incubating for 1 hour at room temperature. About 500 µl ethanol 80% was added into supernatant and then it was incubated at -20°C for 1 hour. The DNA obtained was separated by centrifugation at 12000 rpm for 7 minutes. The supernatant was discarded and into the pellet, 500 µL ethanol 70% was added before it was centrifuged at 12000 rpm for 5 minutes. The supernatant was discarded again and the pellet was air-dried at room temperature until the smell of ethanol was gone. The pellet was added 50 µL TE buffer pH 7.6 and the DNA sample was stored at -20°C.

DNA amplification

The DNA extraction product from the 16 salak cultivars was amplified with PCR in a total volume of 25 µL consisting of 12.5 µL 2x PCR Master Mix Solution (TianGen), 2 µL primer RAPD (10 µM), 1 µL DNA, and 9.5 µL ddH_2O, then put inside PCR tube (Axygene). Amplification was performed using a Gene Amp. PCR System 9700 PE (Applied Biosystem) with the following program as follows: (i) Pre-denaturation at 94°C for 5 minutes, (ii) 45 cycles for denaturation at 94°C for 1 minute, annealing at 36°C for 1 minute, extension at 72°C for 2 minutes, (iii) final extension at 72°C for 5 minutes, and soaking at 4.0°C. The RAPD primers used were OPA3 (AGTCAGCCAC), OPA17 (GACCGCTTGT), OPA19 (CAAACGTCGG), OPC7 (GTCCCGACGA), OPD4 (TCTGGTGAGG), OPD20 (ACCCGGTCAC) (Operon Tech. Inc.).

DNA visualization

Electrophoresis in agarose gel 1.5% was performed at 60 V for 30 minutes and the tracking dye was placed on two lines from under the plate. The agarose gel dye used was FlouroVue TM (Smobio, Taiwan). The visualization of electrophoresis results used a UV transilluminator (LETY) and was captured by a digital camera DSLR Canon EOS 60D.

Data analysis

Morphological data were analyzed using the Bartlett test and the comparison between variance value with deviation standard (Ministry of Agriculture Republic Indonesia 2006). Bartlett test was used to determine the homogeneity of variety in samples obtained from two or more populations. Analysis was performed using Minitab version 14 program. The decision-making was made based on the P value obtained. If the P value >0.05, phenotype character is homogenous, whereas if P value <0.05, phenotype character is varied (Andrade 2019). Data analysis through phenotype variant comparisons with deviation standards was performed on the measured phenotype variables. The value of the phenotype variants is calculated based on the following equation described by Wientjes et al. (2016) as follows:

$$\sigma^2 f = \frac{\sum X_i^2 - (\sum X_i)^2/n}{(n-1)}$$

Where: $\sigma^2 f$: phenotype variants, Xi: average value of phenotype I, and n: the number of phenotypes tested.

The phenotype variant deviation standard was calculated based on the equation:

$$Sd\sigma_2 f = \frac{\sqrt{\sigma 2f}}{(n-1)}$$

The measurement criteria on how wide or narrow was calculated based on the following equation (Australian Department of Agriculture 2014):

If $\sigma^2 f > 2\ Sd\sigma_2 f$, phenotype variability is wide,

If $\sigma^2 f < 2\ Sd\sigma_2 f$, phenotype variability is narrow

The decision was made based on the two tests and performed with phenotype variability criteria based on the Bartlett test, variant comparison, and deviation standard.

The RAPD bands were scored for their presence (1) or absence (0). To determine the correlation between primers, correlation analysis was performed by the NTSYS program via the use of the comparison analysis equation MXCOMP. The genotype similarity matrix was calculated based on the Dice coefficient using the equation described by Soengas et al. (2006) as follows:

$$S = \frac{2\ n\ ab}{na + nb}$$

Where: S: similarity coefficient, a and b: the compared two individuals, n ab: the number of DNA bands in the same position for individual a or b, na: the number of DNA bands in individual a, and nb: the number of DNA bands in individual b.

Cluster analysis used NTSYSpc (Numerical Taxonomy and Multivariate Analysis) version 2.1 program. Genetic similarity among cultivars was calculated according to the Dice coefficient using Similarity for Qualitative Data (SIMQUAL). The similarity coefficients were then used to construct a dendrogram using Unweighted Pair-group Method with Arithmetical Averages (UPGMA) through Sequential, Agglomerative, Hierarchical, and Nested Clustering (SAHN).

RESULTS AND DISCUSSION

Morphological characters

Eighteen morphological characters observed in 16 salak cultivars were presented in Tables 1 and 2. Within the 16 salak cultivars, qualitative character differences were found in fruit epicarp (skin) color and fruit shape (Table 1). Epicarp (skin) color in Pada and Putih cultivars is brownish red and yellowish brown respectively (Figure 1; Table 1). Salak Bali with blackish brown skin color is Bingin, Gulapasir, Gonong, and Injin cultivars. Layu, Kelapa, Sudamala, Merah, Jaka, Penyalin, Gondok, Nangka, and Nenas cultivars have reddish brown skin color (Figure 1). Salak fruit is oval-shaped, round, and ovate with an elongated tapering tip (Table 1; Figure 1). Of all the salak plants observed, only the Muani salak never bore fruit. Farmers call it "muani" or male salak. Muani salak contains sterile pollen so it cannot produce fruit (Tables 1 and 2).

The morphological character did not show a specific character as a differentiator. Until now, no information on specific morphological characters to differentiate this plant. Budiyanti et al. (2019) stated that measurement and analysis based on statistical rules are required to determine the presence of variations in a population. Other than environmental influence, individual qualitative characteristic appearance is also controlled by genes or an interaction of both. In plants, many genes control various parts. Each gene has a special function in controlling an individual's characteristics. The psbA, psbK, and psbI genes found in *Salacca zalacca* chloroplast produce products in the form of photosystem II proteins D1, K, l which are useful in the process of photosynthesis (Chen et al. 2022). These characteristics can be in the form of morphological characters, such as plant height, length, width, color, and shape of fruit, and also habitus (Elly et al. 2018; Ilmiah et al. 2021; Wahyudi et al. 2021).

According to Matatula et al. (2021), elevation factor influences fruit epicarp (skin) color and the plant height in salak cultivars. High elevation will influence the sunlight intensity and temperature. Moreover, the CO_2 concentration in lower elevations will be higher than in higher elevations. Such conditions will directly influence photosynthesis which can cause differences in the leaf color (Simin et al. 2022). In this research, altitude of all salak cultivation in Karangasem Regency is similar, so altitude has no effect in this research and the leaf color found in all locations was the same, which was green. The similarities in several morphological characters among salak cultivars in Bali do not mean that the cultivars are identical to each other (Adelina et al. 2021). According to Elly et al. (2018), the similarities and differences can be influenced by environmental and genetic factors. The basic theory of genetics states that the interaction between environment and genotype will result in phenotype.

Table 1. Qualitative variability of 8 morphological characters from 16 salak cultivars

Cultivar	Qualitative character							
	Young leaf color	Leaf sheath Color	Flower sheath color	Flower crown Color	Filament color	Fruit skin color	Fruit shape	Spine color
Bingin	Brown	Green	Brown	Pink	Pink	BB	O	Black
Pada	Brown	Green	Brown	Pink	Pink	BR	O	Black
Layu	Brown	Green	Brown	Pink	Pink	RB	O	Black
Kelapa	Brown	Green	Brown	Pink	Pink	RB	OE	Black
Sudamala	Brown	Green	Brown	Pink	Pink	RB	OE	Black
Gulapasir	Brown	Green	Brown	Pink	Pink	BB	OE	Black
Merah	Brown	Green	Brown	Pink	Pink	RB	OE	Black
Jaka	Brown	Green	Brown	Pink	Pink	RB	OE	Black
Muani	Brown	Green	Brown	Pink	Pink	-	-	Black
Gonong	Brown	Green	Brown	Pink	Pink	BB	R	Black
Putih	Brown	Green	Brown	Pink	Pink	YB	OE	Black
Penyalin	Brown	Green	Brown	Pink	Pink	RB	OE	Black
Injin	Brown	Green	Brown	Pink	Pink	BB	OE	Black
Gondok	Brown	Green	Brown	Pink	Pink	RB	R	Black
Nangka	Brown	Green	Brown	Pink	Pink	RB	OR	Black
Nenas	Brown	Green	Brown	Pink	Pink	RB	O	Black

Note: R: round; OE: ovate; O: oval; BB: blackish brown; BR: brownish red; RB: reddish brown; YB: yellowish-brown; (-): plants do not bear fruit

Table 2. Quantitative variability of 10 morphological characters of 16 salak cultivars

Cultivar	Plant height (m)	Number of leaflets	Leaflets length (cm)	Leaflets width (cm)	Sheath length (cm)	Fruit length (cm)	Fruit diameter (cm)	Number of fruits per bunch	Number of seeds per fruit	Thick pulp (cm)
Bingin	1	27	16	1	12	3	2	9	1	0.8
Pada	2	30	25	2	16	4	2	13	1	0.9
Layu	2	30	25	2	16	3	2	8	1	0.8
Kelapa	4	54	40	2	29	6	4	8	1	1.3
Sudamala	3	54	42	2	25	4	4	9	1	0.8
Gulapasir	4	77	60	4	28	5	5	20	1	1.1
Merah	4	75	65	4	28	5	5	21	1	1.1
Jaka	4	70	60	2	25	5	5	16	1	1.0
Muani	5	75	65	3	25	-	-	-	-	-
Gonong	3	41	37	3	26	4	4	48	1	0.8
Putih	4	67	41	3	26	6	4	18	1	1.1
Penyalin	3	50	40	2	25	4	4	14	1	0.8
Injin	4	60	65	3	28	5	5	10	1	1.3
Gondok	4	76	59	4	27	5	5	17	1	0.9
Nangka	5	77	68	3	26	6	5	19	1	1.1
Nenas	4	60	50	3	26	6	5	19	2	0.6
σ_{2f}	1.54	324.54	247.92	0.52	23.96	0.73	1.46	97.49	0.04	0.03
Sdσ_{2f}	0.083	1.201	1.050	0.048	0.326	0.061	0.086	0.705	0.014	0.014
2Sdσ_{2f}	0.166	2.402	2.099	0.096	0.653	0.122	0.173	1.411	0.028	0.031
Variability	Wide	Wide	Wide	Wide	Wide	Wide	Wide	Wide	Narrow	Narrow

The comparison of variance value and deviation standard with a wide variety ($\sigma^2 f > 2Sd\sigma_{2f}$) could be found on 8 quantitative characters observed. In addition, two characters showed narrow variation ($\sigma^2 f < 2Sd\sigma_{2f}$) in seed numbers and fruit pulp thickness characters (Table 2). Among 16 salak Bali cultivars, Nangka and Nenas cultivars have the largest length and fruit diameter of 6 and 5 cm, respectively. Nenas cultivar has 2 seeds per fruit, while other cultivars only have 1 seed. The most number of fruits per infructescence belongs to Gonong cultivar with 48 fruits, while the least number of fruits belongs to Layu and Kelapa cultivars with 8 fruits. Salak Bali with the thickest fruit pulp is Kelapa and Injin cultivars of 1.3 cm. Nenas cultivar has a lower fruit pulp thickness of 0.6 cm when compared with the other 15 cultivars.

According to Pacheco-Hernández (2021), similarity and different morphological characters were influenced by environmental and genetic factors. They explain the basic theory of genetic science, which states that the interaction between environment and genotype will give rise to a phenotype. The morphological character that distinguish the features of salak Bali based on the Ministry of Agriculture Republic Indonesia characterization guide (2006) are the young leaf color, leaf sheath color, flower sheath color, flower crown color, filament color, fruit skin color, fruit shape, spine color, plant height, number of leaflets, leaflets length, leaflets width, sheath length, fruit length, fruit diameter, number of fruits per bunch, number of seeds per fruit, and thick flesh.

Gari (2011) reported that anatomical characteristics can be used to identify salak Bali cultivars. Cultivar division is influenced by the periclinal cell wall pattern. Guna, Boni, Maong, Bingin, and Muani cultivars have flat periclinal cell wall patterns, while the other 8 cultivars have convex periclinal cell wall patterns. Herawati et al. (2018) reported that the number of trichomes can also used for identifying salak cultivars. The number of trichomes in the Kedung Paruk and Kalisube cultivars is 1 mm^2 per leaf area unit, while Candinegara cultivar has no trichomes.

Molecular characters

DNA polymorphisms

Polymorphism is characterized by the presence and absence of the band in the sample and the difference in the size of the band produced by each sample. According to Herawati et al. (2018), Nandariyah et al. (2021), and Prihatini et al. (2022), polymorphism is an illustration of amplification obtained from DNA fragment differences that are observed and scored as whether there is any sequence difference to show the presence of variation. The results showed that four of six RAPD primers present clear and reproducible bands. The polymorphism of *S. zalacca* among 16 cultivars is 76%, based on polymorphic band percentage (Table 3). OPA3 produces the highest polymorphic DNA bands (Figure 2). It produces 9 DNA bands, and all of them are polymorphic (Table 3). DNA polymorphisms are the different DNA sequences among individuals, groups, or populations. Polymorphism occurs in the same population of two or more alleles at one locus, each with a significant enough frequency, where the minimum frequency is usually 1% (Sukhumsirichart 2018).

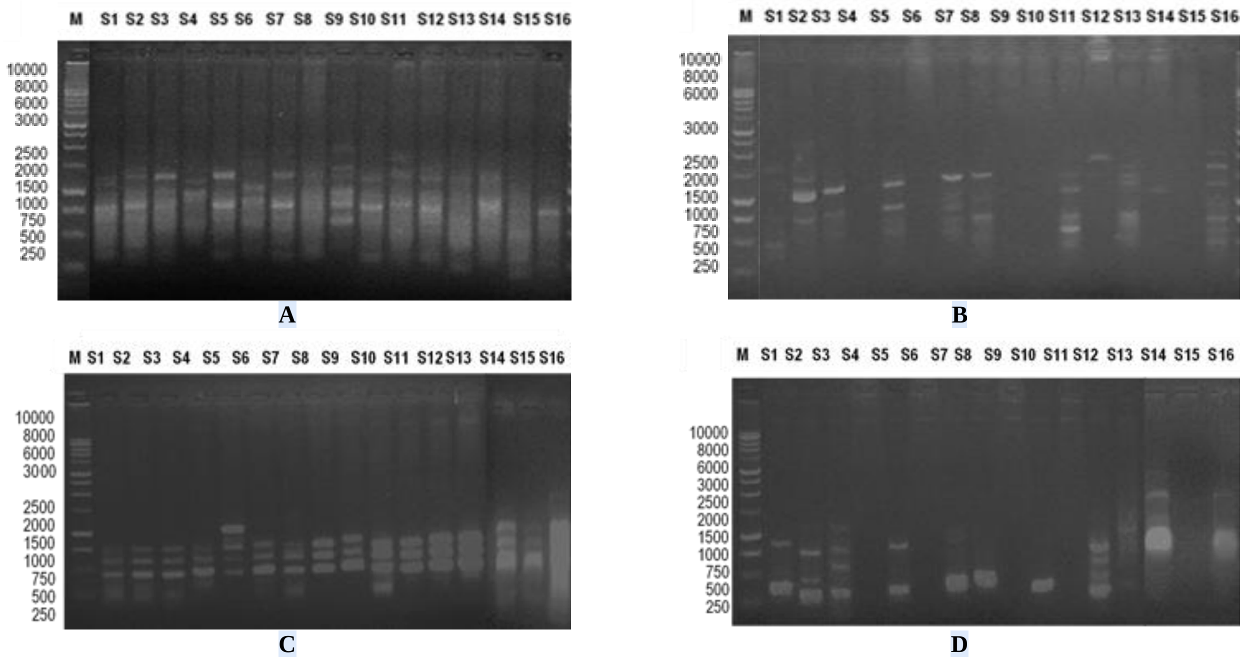

Figure 2. Visualization of the DNA bands of 16 cultivars of salak Bali using four RAPD primers. A. OPA 3, B. OPC 7, C. OPD 20, D. OPD 4. Blue arrow: the polymorphic band, green arrow: monomorphic band, M: Marker 1 kb, S1: Bingin, S2: Pada, S3: Layu, S4: Kelapa, S5: Sudamala, S6: Gulapasir, S7: Merah, S8: Jaka, S9: Muani, S10: Gonong, S11: Putih, S12: Penyalin, S13: Injin, S14: Gondok, S15: Nangka, S16: Nenas

Based on the interpretation of amplification by OPA 3, the number of bands appearing are nine bands from 250 bp to 4000 bp in which samples of Muani (S9), Gonong (S10), Penyalin (S12) and Injin (S13) produced the most number of bands (5 bands) (Figure 2A). The DNA band pattern diversity interpretation showed that band pattern diversity exists in Nenas (S16) with a DNA band of 750 bp in length, whereas a DNA band of 600 bp in length is only owned by Injin (S13), and the band size of 400 bp is produced from Pada (S2). According to Ediwirman et al. (2017) and Elly et al. (2018), DNA amplification occurs when primer attaches in two complementary sites located close together with reverse orientation to each other. The distance of this amplification site will produce DNA fragments in various base pair sizes. Generally, the number of base pairs that can be amplified by plant DNA genome is up to 2000 bp or even reaches 5000 bp. DNA bands located in the same base pair (bp) showed that the DNA bands have the same migration and are assumed as homologous locus.

The number of bands appearing on each location with 300-3000 bp in length are presented by primer OPC7 (Figure 2B). The interpretation of DNA band pattern diversity showed that there is a band in Nenas (S16) of 2100 bp in length, Penyalin (S12) of 2000 bp, Layu (S2) of 1400 bp, and Injin (S13) of 1300 bp. Kelapa (S4), Gulapasir (S6), Muani (S9), Gonong (S10), and Nangka (S15) did not produce amplification products with size under 1500 bp. This is possibly due to the nucleotide base sequence constructing the primer not complementing the base pair constructing the DNA of the five salak samples.

Amplification with OPD 20 showed that seven band patterns appeared, with the most number of bands produced by Nenas (S16) with 5 bands ranging around 580-1500 bp, followed by Bingin (S1), Pada (S2), Layu (S3), Sudamala (S5), Merah (S7), and Gonong (S10) with four bands ranging around 250-1100 bp (Figure 2C). Amplification with OPD 4 showed that Gonong (S10) produced one band of 400 bp in length (Figure 2D). Pada (S2), Merah (S7), Jaka (S8), and Putih (S11) did not produce amplification products with size 500 bp. This is probably due to the nucleotide base sequence constructing the primer not complementing the base pairs constructing the five salak sample's DNA (Wang et al. 2021).

Cluster analysis

The genetic similarity among cultivars ranged from 0.49 to 0.97 (Table 4). A higher genetic similarity level of 0.97 in Kelapa (S4) and Gulapasir (S6) means these cultivars have a low genetic diversity of 0.03. The lowest genetic distance was found in samples of Penyalin and Nenas cultivars with a similarity percentage of 0.49. High genetic similarities indicate low genetic variety, and vice versa (Listihani et al. 2020, 2021, 2022; Selangga and Listihani 2022; Selangga et al. 2023). Bingin cultivar has a high genetic similarity with Nangka, Kelapa, and Gulapasir cultivars ranging from 0.81 to 0.84, with low genetic similarity with Merah, Nenas, and Injin cultivars ranging from 0.65 to 0.68. This shows that Bingin cultivar has a high genetic diversity with Merah, Nenas, and Injin cultivars. Hakim et al. (2021) stated that high genetic variation is a gene source that can be utilized to form recombination, so there is a chance to fix the character of a plant and form a superior new cultivar.

Table 3. The level of polymorphism of the four primers used was based on the DNA banding patterns of 16 salak cultivars

Primer	Nucleotide sequence (5'-3')	DNA amplification size (bp)	TNB	NPB	PB (%)
OPA 3	AGTCAGCCAC	250-4000	9	9	100
OPC 7	CACACTCCAG	300-3000	11	7	64
OPD 20	CAAACGTCGG	580-1500	7	3	43
OPD 4	TCTGGTGAGG	660-3570	10	9	90
Total			37	28	76

Note: TNB: total number of amplified bands, NPB: number of polymorphic bands, PB: percentage of polymorphic band, PIC: polimorphic information content

Table 4. Genetic similarity among salak Bali cultivars based on Dice coefficient

Cult.	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16
S1	1.00															
S2	0.73	1.00														
S3	0.76	0.70	1.00													
S4	0.81	0.70	0.68	1.00												
S5	0.73	0.57	0.81	0.70	1.00											
S6	0.84	0.73	0.70	0.97	0.73	1.00										
S7	0.65	0.59	0.73	0.68	0.81	0.70	1.00									
S8	0.76	0.70	0.78	0.78	0.81	0.81	0.84	1.00								
S9	0.76	0.65	0.73	0.89	0.76	0.92	0.68	0.78	1.00							
S10	0.76	0.59	0.73	0.78	0.76	0.81	0.73	0.78	0.89	1.00						
S11	0.76	0.65	0.78	0.78	0.81	0.81	0.84	0.84	0.78	0.73	1.00					
S12	0.78	0.73	0.76	0.76	0.68	0.78	0.70	0.76	0.76	0.81	0.70	1.00				
S13	0.68	0.57	0.65	0.65	0.68	0.62	0.65	0.65	0.59	0.59	0.76	0.68	1.00			
S14	0.76	0.65	0.73	0.78	0.76	0.81	0.78	0.78	0.78	0.78	0.78	0.81	0.70	1.00		
S15	0.81	0.70	0.68	0.95	0.70	0.92	0.68	0.78	0.84	0.84	0.78	0.81	0.70	0.78	1.00	
S16	0.65	0.54	0.68	0.62	0.65	0.59	0.62	0.68	0.57	0.62	0.68	0.49	0.54	0.62	0.62	1.00

Note: S1: Bingin, S2: Pada, S3: Layu, S4: Kelapa, S5: Sudamala, S6: Gulapasir, S7: Merah, S8: Jaka, S9: Muani, S10: Gonong, S11: Putih, S12: Penyalin, S13: Injin, S14: Gondok, S15: Nangka, S16: Nenas

The high genetic similarity between Gulapasir and Kelapa cultivars will make it harder for character improvement if the two cultivars are hybridized. High genetic similarities in a plant showed that the genetic variation of the plant is low, and vice versa. According to Elly et al. (2018), genetic similarity is the opposite of genetic distance. Genetic diversity is the key to plant breeding. The plant breeding program is one of the ways to improve genetic variability in a population. The study of genetic diversity is important in supporting artificial selection, preparing test provenance, and controlling crossings. Selection is the initial step in obtaining a new superior cultivar dan requires high genetic diversity to be effective (Klee and Tieman 2018; Cockerton et al. 2021; Anisa et al. 2022). Genetic resource conservation is required to protect genetic diversity. Cultivars with higher genetic diversity have adaptability and can avoid extinction. High genetic diversity can possibly produce several individuals resistant to extreme environmental conditions and to several diseases (Klee and Tieman 2018; Colantonioa et al. 2022).

The genetic variation of *S. zalacca* in this study may be caused by animals (types of pollinators and herbivores). Besides, as in general fruit plants, these plants do cross-breeding which is assisted by the wind to provide a higher likelihood for random pollination that allows the process of gene transfer or migration through pollen displacement can cover a reasonably widespread area (Kamper et al. 2021). Fruit plants such as *S. zalacca* have considerable variability for interbreeding. Besides, species that are more widely distributed with large and close together populations have high productivity and large genetic variability (Hahn et al. 2017).

High genetic diversity in Penyalin salak populations with Nenas salak of 0.49 can arise due to natural mutations. The large differences in genetic diversity in populations can be caused by several factors such as isolation by distance, geography, ecology, and reproduction. If this happens, a new type of plant will emerge that is able to adapt to its environment (Yulita and Rahmat 2019). Genetic diversity is also influenced by the markers used and also the number of primers. In this study, RAPD markers are quite sensitive methods in detecting the genetic structure of populations (Rahiman et al. 2015). The results of this study can be a reference to support plant breeding activities. Besides, high genetic diversity is also beneficial in the implementation of conservation of salak Bali. In addition, species with high genetic diversity is also capable of occupying new areas and is likely to have an excellent opportunity to escape natural selection and survive extinction (Hahn et al. 2017).

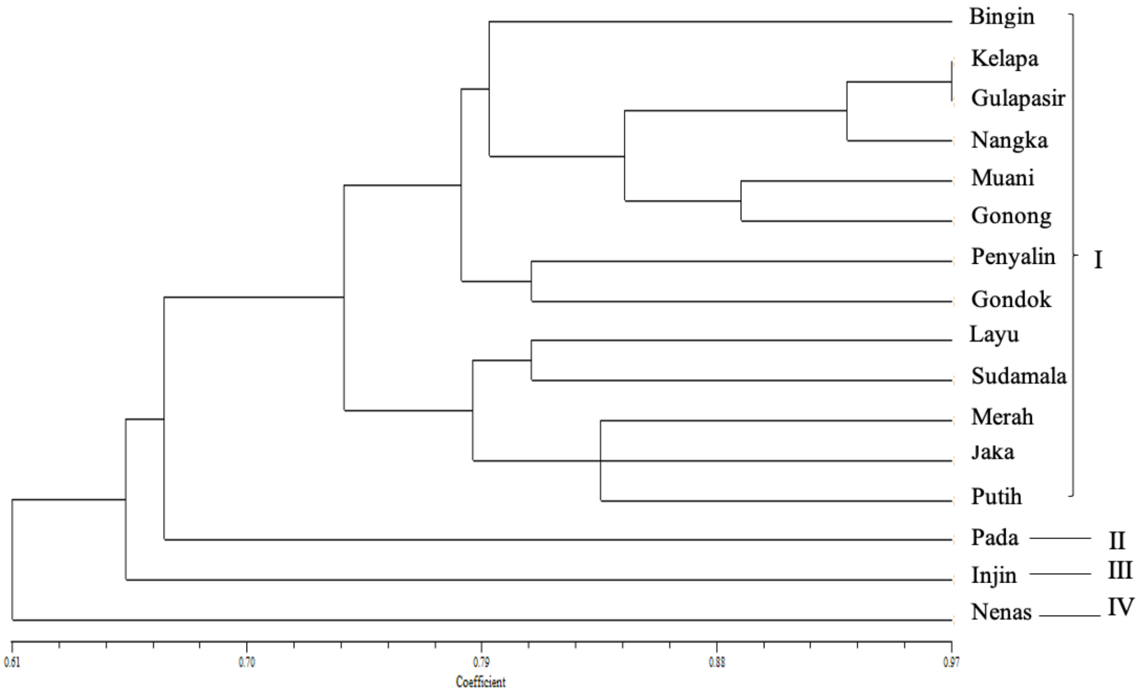

Figure 3. Dendrogram of DNA banding patterns of 16 cultivars of the salak Bali based on genetic character

The 16 salak cultivars are grouped and divided into 4 clusters (Figure 3). Cluster 1 consists of S1 (Bingin), S3 (Layu), S4 (Kelapa), S5 (Sudamala), S6 (Gulapasir), S7 (Merah), S8 (Jaka), S9 (Muani), S10 (Gonong), S11 (Putih), S12 (Penyalin), S14 (Gondok), and S15 (Nangka). Cluster 2 consists of S2 (Pada), cluster 3 consists of S13 (Injin), and cluster 4 consists of SNN 16 (Nenas). This clustering only shows plant similarities based on the four RAPD primers used. Cluster analysis revealed three cultivars (Pada, Injin, and Nenas) showed unique quantitative and qualitative characteristics combination. Pada is a very different cultivar based on several qualitative characters, which are fruit epicarp (skin) color and fruit shape, while Injin cultivar is different based on several qualitative characters, which are fruit epicarp (skin) color and fruit shape, as well as quantitative character fruit pulp thickness which is larger than other cultivars. Nenas cultivar has a unique quantitative characteristic that is not in other cultivars, which is the number of seeds per fruit being two seeds per fruit. These prove that several qualitative and qualitative characteristics chosen are beneficial in differentiating cultivars and the result is equal to the result of their genetic analysis.

The Dendrogram grouped salak cultivars based on their genetic relationship. Pada, Injin, and Nenas cultivars form their separate groups far from other cultivars. Babu et al. (2021) and Al-Khayri et al. (2022) stated that grouping is unrelated to geographical location because the RAPD markers used show DNA variations on both coding and non-coding regions. To obtain an accurate grouping, DNA analysis with more primers and samples is required. The further the distance between samples, the smaller the success of crossing, although it does not close the possibility of obtaining a superior genotype in a successful attempt. Crossing between individuals of close genetic relationships will elevate homozygosity due to the meeting of bad alleles. Crossing between individuals with large genetic distances will improve heterozygosity. Genetic relationship information can be useful during the developing process of good-quality seeds (Elly et al. 2018; Ilmiah et al. 2021).

The 18 morphological characteristics chosen during the research showed that between the individuals or populations, genetic relationship exists with the assumption that genetic variation can cause different morphological characteristics. In the study of plant breeding, information on the description, relationship, and genetic distance is crucial in determining and selecting ancestors. A large genetic distance between crossing parent candidates will grant a better chance of producing hybrid plants with high compatibility and fertility (Martínez-Fortún et al. 2022). Thus, high genetic variety is a supporting factor in the success of plant breeding programs. This research is very beneficial in selecting salak Bali ancestors to produce superior cultivars.

In conclusion, 16 salak cultivars from Bali, Indonesia are varied in morphological and molecular characters. Twelve of 18 morphological characters observed, namely fruit skin color, number of leaflets, leaflets length and width, leaf sheath length, fruit skin color, fruit shape, fruit length, fruit diameter, number of fruits per bunch, number of seeds per fruit, and thick pulp are diverse among cultivars. A total of 37 DNA bands were obtained, of which 28 bands are polymorphic. The dendrogram formed 4 clusters. Cluster 2 consists of Pada cultivar, cluster 3 consists of Injin cultivar, cluster 4 consists of Nenas cultivar, and cluster 1 consits of the remaining cultivars.

ACKNOWLEDGEMENTS

2 The authors thank the Head of the Regional Research and Innovation Agency of Bali Province, Indonesia for the funding provided for this research with contract number: B.17.027/3220/Bid.II/BaRI.

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