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Numeric–Phenetic Study of *Lablab purpureus* from East Java Based on Morphology Character and DNA Fingerprinting

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Abstract. This research aims to conduct a numeric–phenetic study of *Lablab purpureus* (L) Sweet based on morphology character and DNA fingerprinting with cophenetic–correlation algorithm confirmation. DNA genome extraction is based on the Blood Animal Plant DNA Preparation Kit. Ten RAPD primers were chosen for the amplification DNA genome, including OPA-6, OPA-8, OPA-10, OPA-20, OPC-19, OPD-8, OPD-12, OPE-8, OPE-15, and OPE-16. Cophenetic correlation is used to analyze three clustering algorithms (Nearest Neighbor, UPGMA, Farthest Neighbor) and five similarity indexes (Simple Matching, Jaccard, Nei & Li, Sorensen, Yule). *Lablab purpureus* (L) Sweet samples have shown morphology variation, mostly on flowers, pods, and seeds. DNA fingerprinting reveals a variety of band numbers ranging from 300BP to 1000BP. RAPD analysis revealed a total of 87 bands, including 18 polymorphic bands, with an average percentage polymorphism of 31.15%. The UPGMA technique was developed as a result of cophenetic-correlation analysis, and the Jaccard similarity index is the optimal combination for dendrogram generation. The topology of a morphology character dendrogram differs slightly from that of a DNA fingerprinting dendrogram. It concluded that the UPGMA algorithm and Jaccard similarity index is the optimum combination to analyze the diversity of *L. purpureus* (L) Sweet accession from East Java, resulting in the accession of this species having high diversity. This research can give a novel approaching method in the taxonomy field, which is combining morphological and molecular data.

Keywords: DNA Fingerprinting; *Lablab purpureus*; Morphology Character; Numeric-Phenetic

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INTRODUCTION

The hyacinth bean (*Lablab purpureus* (L) Sweet) is a woody climbing herb that can grow up to 5 meters in length. The leaves are pinnate and 3-foliolate in general. The leaflets are sharp or blunt, measuring 6-12 cm by 5-9 cm. The blossoms are white or purple in hue. Fruits are green pods that are 6 cm long by 2 cm wide, flattened, and contain 4-5 seeds (Al-Snafi, 2017). The Indian subcontinent, Africa, and Southeast Asia all have large populations of hyacinth bean. This tropical legume is a versatile crop that can be used as a vegetable, pulse, fodder, and green manure crop (Vaijayanthi et al., 2019). Technically, this crop can be distributed all across Indonesia. However, L. purpureus is reported to be well developed in Java Island and West Nusa Tenggara as an essential food crop (Purwanti et al., 2019)

Hyacinth bean (*L. purpureus*) has been used as a raw material for several Indonesian traditional foods through ethno-botanical studies (Maulidan et *al*., 2022). The seed legume is the organ primarily utilized by this species, known as koro komak. The koro komak seed was boiled and fermented into traditional tempeh (Fazrin et al., 2020). Koro komak could also be mixed with several Indonesian traditional sambals to produce koro komak spicy products, mostly sold as gifts

for tourists (Majdi *et al*., 2019).

The seed legume of *L. purpureus* includes a variety of health-promoting chemicals that may supplement daily nutrition intake (Habib et al., 2017). The seed contains carbs, protein, dietary fiber, and essential fatty acids, suggesting that it could be a good source of quality food components (Hossain et al., 2016). However, this species is less common for food items than other legume species. One of the most potentially underutilized crops for food security is *L. purpureus* (Minde et al., 2021; Naeem et al., 2020).

Underutilized crops such as hyacinth beans have been prioritized in Indonesian food security policy (Limenta& Chandra, 2017). East Java is one Indonesian province that has produced this neglected crop. In this province, the hyacinth bean is used to replace soybean commodities (Diniyah et al., 2013). This crop commodity's development could begin with a diversity analysis for raw material selection utilizing a taxonomical approach (Dhivyabharathi et al., 2019).

Several taxonomic approaches were utilized to explore the diversity of *L. purpureus*. Morphological and molecular characters are the two basic techniques (Singh & Kudesia, 2020). Morphological characteristics are used in a traditional taxonomic analysis of *L. purpureus* using simple methods (Jayanti &Harisanti, 2013). A contemporary taxonomical investigation of this species is undertaken mostly using DNA fingerprinting analysis (Saravanan et al., 2013). Several markers, including the RAPD marker (Sanaullah et al., 2012) and the SSR marker (Rai et al., 2016), have been created for DNA fingerprinting study of *L. purpureus*. ISSR and RAPD marker has advantages in genetic analysis, which can be easily used to examine the genetic variation in the population (Poyraz, 2016). However, to explore whether genetic variation was expressed in the metabolomic stage, additional analysis should be conducted. One of the further analyses is the morphological characterization. Research combining morphological characteristics with DNA fingerprinting to build a strong Numeric-phenetic approach method is still required (Wahyuni et al., 2019).

A clustering analysis numeric-phenetic investigation requires an algorithm and a similarity index. These two parameters controlled how selected organism taxonomical unit (OTU) features were employed in the clustering procedure to generate a dendrogram. The dendrogram should not be built using the random clustering method as a primary result of the numeric-phenetic study. The clustering approach should be adjusted in order to produce the best representative dendrogram based on the selected characters (Bouyer &Hatamlou. 2018). One method that might be employed for that optimization process is cophenetic correlation (Carvalho et al., 2019).

Numeric-phenetic is one of the greatest ways to modern taxonomical inquiry since it compares organism taxonomical units using as many characters as feasible. The use of numeric-phenetics and cophenetic-correlation has improved the accuracy and dependability of this method of approach. A combination approach like this, however, has been rare in the taxonomic study of *L. purpureus*.

The purpose of this research is to perform a Numeric-phenetic analysis of *L. purpureus* based on morphological and DNA fingerprinting, with cophenetic-correlation method confirmation. This research can give a robust approaching method in the taxonomy field area that will be useful for another researcher to combine the morphological and the molecular data when studying a particular plant.

METHODS

Study area

This investigation focused on four districts in East Java, where *L. purpureus* samples were collected. Sumber (7°57'37.2"S 113°06'54.3" E), Lumbang (7°50'34.5"S 113°07'20.9"E), Bantaran (7°52'11.9"S 113°11'02.6"E), and Gunungtugel (7°54'10.4"S 113°12'16.3"E) are the districts. Weather variables at the collection site are used to determine the diversity of *L. purpureus* accessions. Each district's *Lablab purpureus* (L) Sweet samples were submitted to a selection process based on the diversity of leaves, flowers, and pods. For the Numeric-phenetics study, *L. purpureus* samples were employed as an organism taxonomical unit.

Obtaining morphology character

The morphology of the chosen *L. purpureus* samples was collected from four regions by studying the stem, leaves, blossom, and pods. The branching type is a morphological property of the stem. The dimensions and form of the leaf are morphological characteristics. Dimension, corolla, and coloring are the morphological qualities of the flower. Dimension, form, and seed

coat color are morphological properties of pods. All morphological features were photographed and measured for Numeric-phenetic analysis.

DNA extraction

Leaves of *L. purpureus* were used as a DNA extraction sample with the Blood Animal Plant DNA Preparation Kit (Jena Bioscience) according to the manufacturer's recommendation with some modifications (Simon-Oke et al., 2018). *L. purpureus* leaves were frozen powder after being pulverized with liquid nitrogen. Eighty milligrams of frozen powder samples were introduced to the extraction kit column, which contained all of the extraction ingredients and reagents. During extraction, additional Proteinase K and RNase reagents were mixed together to destroy all RNA and protein contamination in the sample (Wangiyana et al., 2022).

Genomic DNA concentration and purity analysis were carried out by measuring absorbance at wavelengths 260 nm, 280 nm, and 230 nm using UV-1601PC Shimadzu (Lucena-Aguilar et al., 2016). Genomic DNA visualization was conducted by electrophoresis on a 0.8% agarose gel with ethidium bromide staining. Ladder 1000bp (Invitrogen) was used as a marker for molecular weight estimation of genomic DNA (Aboul-Maaty &Oraby, 2019).

DNA fingerprinting

Several random primers developed by Operon Technologies were used for DNA fingerprinting analysis of *L. purpureus* genomic DNA. Those primers were arbitrarily selected from the RAPD primers commonly used for *L. purpureus* (Rai et al., 2011). Ten RAPD primers that used in this research are OPA-6, OPA-8, OPA-10, OPA-20, OPC-19, OPD-8, OPD-12, OPE-8, OPE-15, and OPE-16.

For DNA fingerprinting analysis, RAPD-PCR was used. PCR mixes contained 12.5 l 2 x KAPA 2G PCR mix (KAPA Biosystems), 8.5 l ddH2O, 2 l of each primer (10 pmol/ l), and 2 l *L. purpureus* DNA template (40 ng/ l). PCR amplification was carried out on a Labcycler thermocycler with the following parameters: initial denaturation at 95ºC for 3 minutes, followed by 40 cycles of denaturation at 95ºC for 15 seconds, annealing at 37ºC for 1 minute, extension at 72ºC for 2 minutes, and final extension at 72ºC for 5 minutes (Wangiyana et al., 2024). Electrophoresis on 1.2% agarose gels with ethidium bromide labeling was used to visualize the amplified DNA fragments. Ladder 1000bp (Invitrogen) was used as a marker for molecular weight estimation of RAPD bands.

Data analysis

Samples of *L. purpureus* from 4 districts in East Java were used in the Organism Taxonomical Unit (OTU) for numeric-phenetic analysis. The key basis data for similarity analysis was morphology features and DNA fingerprinting. For clustering analysis, the presence or absence of numerous characters was converted into binary data (1 = presence, 0 = absence). Three algorithms (Nearest Neighbor, UPGMA, Farthest Neighbor) and five similarity indices (Simple Matching, Jaccard, Nei & Li, Sorensen, Yule) were utilized in the clustering study. The similarity index was distorted before and after clustering using cophenetic-correlation analysis (Saracli et al., 2013). Co-Stat for Windows was used to calculate the coefficient of R-value from the cophenetic-correlation study. The best algorithm and similarity index for building the dendrogram were chosen based on the best R-value (Werme et al., 2022; Sukri et al., 2022). The MVSP 3.1A application was used to create the dendrogram.

RESULTS AND DISCUSSION

Numeric – Phenetic Based on Morphology Characters

Weather conditions vary across four Hyacinth bean (*L. purpureus*) sampling locations (Table 1). Each area has unique *L. purpureus* additions: Sumber's black seed-purple flower, Lumbang's black seed-white flower, Bantaran's white seed-purple flower, and Gunungtugel's brown seed-white flower. Weather conditions have an impact on soil water resources. The hyacinth bean (*L. purpureus*) is a legume species that has been observed to respond to drought conditions (Robotham & Chapman, 2017). The morphology variation (eco-morphology) of this species has revealed its many adaptations. This study indicates that diverse weather circumstances resulted in different *L. purpureus* accession features as an adaptation mechanism.

Table 1. Weather Conditions on the Different Sampling Locations in East Java, Indonesia

Weather conditions	Sampling Location			
	Sumber	Lumbang	Bantaran	Gunungtugel
Average temperature(°C)	23	29	31	31
Precipitation (%)	14	3	3	3
Air humidity (%)	76	57	58	58
Average Wind Speed (kph)	13	16	16	16

Table 2. Different Distinctive Morphology Characters of *L. purpureus* Accession from Sampling Location

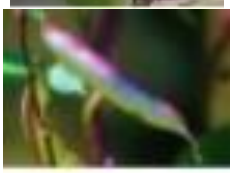
Character	**Brown Seed White Flower (BrS-WhF)**	**Black Seed Purple Flower (BlS-PrF)**	**Black Seed White Flower (BlS-WhF)**	**White Seed Purple Flower (WhS-PrF)**
Corolla				
Young Pods				
Mature Pods				
Average Flower Length (cm)	1.1	1.3	1.0	1.1
Average Flower Width (cm)	0.8	1.0	0.8	0.9
Average Flower Thickness (cm)	0.9	0.7	0.5	0.5
Average Leaf Length (cm)	10.5	11.5	8.9	9.5
Average Leaf Width (cm)	7.1	7.2	7.5	6.5
Average Pod Length (cm)	8.5	10	8.0	5.5
Average Pod Width (cm)	1.5	2	1.5	2
Pods Edge	Acute	Acute	Acute	Blunt
Seed Coat	Brown	Black	Black	Black
Seed Shape	Narrow-Ovate	Elliptic	Circular	Circular

Table 2 shows the tabulation result of *L. purpureus* morphology characters from 4 districts in East Java. This table shows variations of this species' character from different sampling locations, including the characteristics of leaves, flowers, pods, and seeds. This distinctive morphology characteristic of each variety can be found in four different sampling locations. Different distinctive characteristics are the primary consideration in classifying the *L. purpureus* variety into 4 different groups, which can be found in each of 4 different sampling locations.

Morphology diversity has been seen in L. purpureus samples, primarily on flowers, pods, and seeds. Pigmentation differs among various organs and can be easily detected without the use of special equipment. The most prevalent variation among L. purpureus accessions is a change in the color of the flowers, pods, and seed (Bahadur et al., 2016). As a result, it is critical to establish standards for analyzing this qualitative aspect of numeric-phenetic research (Modha et al., 2019). The different distinctive morphology characteristics can be related to the topography and environmental conditions of each sampling location. Precipitation and air humidity are the main factors in the environment that contributed to the morphological variation in the group. The different distinctive morphology characteristics can be related to the topography and environmental conditions of each sampling

location. Precipitation and air humidity are the main factors in the environment that contributed to the morphological variation in the group. Flowering and pod production in legumes mostly depend on humidity conditions (Cayetano-Marcial et al., 2021).

There are no statistically significant differences in the diameters of leaves and pods grouped into quantitative morphological features. *L. purpureus* should be divided into many categories based on the length and width of its leaves and pods. That range could provide useful information for numeric-phenetic investigations using this measurement (Khatun et al., 2022).

DNA Fingerprinting Based on RAPD-PCR

The electrophoresis results of *L. purpureus* from four districts in East Java revealed a variety of banding patterns from several RAPD primers (Figure 1). The bands with differing molecular weights are positioned differently in the agarose gel. These bands show RAPD loci that can be monomorphic or polymorphic. If the band is present in all OTUs, the locus is monomorphic. A polymorphic locus is one in which a band is missing in at least one OTU (Wangiyana et al., 2021). The number of polymorphic bands demonstrates the RAPD primer's capacity to distinguish OTUs.

DNA fingerprinting revealed a variety of bands with varying molecular weights produced by the RAPD primer (Table 3). The band's smallest molecular weight is 200 BP, while its greatest molecular weight is 1400 BP. The majority of bands have molecular weights ranging from 300 to 1000 BP, which is the most common fragment molecular weight of the *L. purpureus* RAPD primer (Dholakia et al., 2019). Most primers in the OPE series, however, produce a rather narrow range of molecular weight bands, such as OPE-8 (800 bp - 1000 bp) and OPE-16 (700 bp - 800 bp).

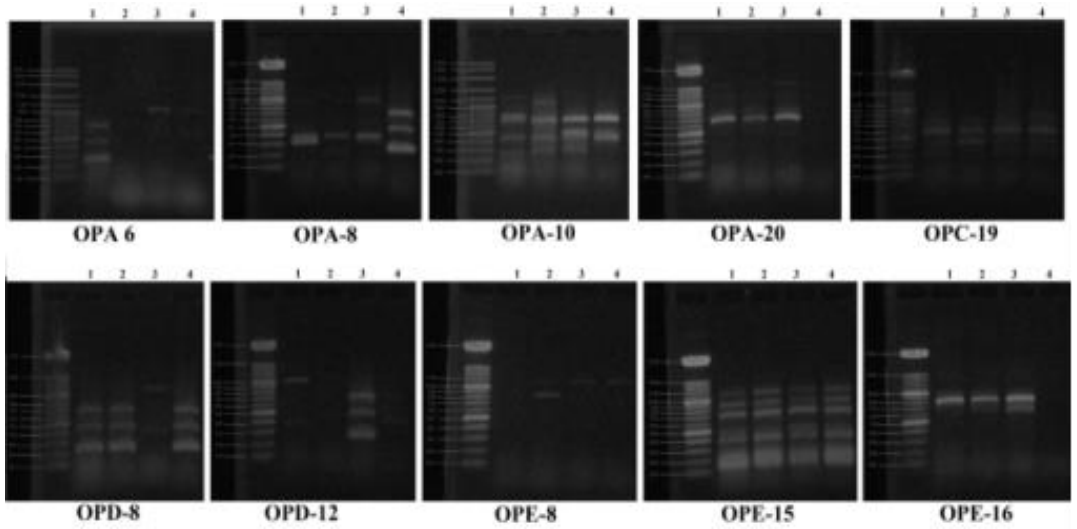


Figure 1. RAPD result of *L. purpureus* using 10 random primers including: OPA-6, OPA-8, OPA-10, OPA-20, OPC-19, OPD-8, OPD-12, OPE-8, OPE-15, and OPE-16 (1: Brown Seed White Flower, 2: Black Seed Purple Flower, 3: Black Seed White Flower, 4: White Seed Purple Flower)

Table 3. Banding Patern of Different RAPD primer

Primers	Primers Sequences (5'→3')	Molecular Weight (BP)	Number of all Band	Number of Polymorphic Bands	Polymorphic Band (%)
OPA-6	GGTCCCTGAC	300 – 1000	5	3	60
OPA-8	GTGACGTAGG	300 – 1000	6	4	66.67
OPA-10	GTGATCGCAG	300 – 1000	12	1	8.3
OPA-20	GTTGCGATCC	700 – 1400	5	0	0
OPC-19	GTTGCCAGCC	500 – 900	12	1	8.3
OPD-8	GTGTGCCCCA	300 – 1100	10	1	10
OPD-12	CACCGTATCC	300 – 1000	6	6	100
OPE-8	TCACCACGGT	800 – 1000	3	1	33.3
OPE-15	ACGCACAACC	200 – 1100	24	0	0
OPE-16	GGTGACTGTG	700 – 800	4	1	25
	Total		87	18	-
	Average		8.7	1.8	31.15

RAPD analysis revealed a total of 87 bands, 18 of which were polymorphic. According to the statistics, the average percentage of polymorphic bands produced by each RAPD primer is 31.15%. This is less than the typical percentage of polymorphic bands produced by RAPD primers, which can reach 74% (Singh et al., 2020). This data means that *L. purpureus* samples have low genetic variation that can be distinguished by the common RAPD primers. It could happen due to the low Polymorphism Information Content (PIC) of molecular markers that have been used (Serrote et al., 2020). The number of polymorphic bands in DNA fingerprinting analysis reflects the diversity of the Organism's Taxonomical Unit (Ellegren & Galtier, 2016). Several primers with no polymorphic band (OPA-20 and OPE-15) are primarily responsible for the low average polymorphism band %. These primers are not suggested for *L. purpureus* DNA fingerprinting analysis.

The number of total bands and polymorphic bands in RAPD primers varies. Among the other primers, OPE-15 has the most total bands. However, because this primer and OPA-20 lack a polymorphic band, they are underutilized for RAPD analysis. OPD-12 contains the most polymorphic bands. Despite the fact that this primer only produced six bands, all of them were polymorphic, resulting in a 100% polymorphism rate. This polymorphic proportion was greater than the average single RAPD primer polymorphism for *L. purpureus* (Rai et al., 2010). This finding suggests that OPD-12 primers could be useful in carrying out a routine DNA fingerprinting investigation of *L. purpureus*.

Cophenetic – Correlation Analysis and Dendrogram Construction

The r-value of all clustering algorithms and the similarity index was greater than 0.7, according to a cophenetic-correlation study. Because the R-value is the correlation coefficient that defines the interpretation of the analysis (Schober et al., 2018), this data suggests that the clustering approach used has excellent accuracy and precision. The relevance of the R-value was further investigated to evaluate the analysis's inaccuracy. Except for Yule, the similarity index has a significant r value. Yule Similarity Index for all clustering algorithms has a non-significant r-value based on DNA fingerprinting data. This finding suggests that Yule is not a suitable similarity index for *L. purpureus* clustering.

Table 4. Cophenetic-correlation Analysis of Algorithm and Similarity Index

Character	Algorithm	Similarity Index	r	SE. of r	P (r=0)	significant
Morphological	Nearest Neighbor	Simple Matching	0.968	0.126	0.0015	**
		Jaccard	0.970	0.121	0.0013	**
		Nei & Li	0.968	0.125	0.0015	**
		Sorensen	0.968	0.125	0.0015	**
		Yule	0.966	0.130	0.0017	**
	UPGMA	Simple Matching	0.980	0.100	0.0006	**
		Jaccard	0.982	0.095	0.0005	**
		Nei & Li	0.980	0.099	0.0006	**
		Sorensen	0.980	0.099	0.0006	**
		Yule	0.979	0.102	0.0007	**
	Farthest Neighbor	Simple Matching	0.966	0.129	0.0017	**
		Jaccard	0.969	0.123	0.0014	**
		Nei & Li	0.967	0.127	0.0016	**
		Sorensen	0.967	0.127	0.0016	**
		Yule	0.964	0.132	0.0019	**
DNA Fingerprinting	Nearest Neighbor	Simple Matching	0.829	0.280	0.0414	*
		Jaccard	0.927	0.187	0.0077	**
		Nei & Li	0.900	0.218	0.0144	*
		Sorensen	0.900	0.218	0.0144	*
		Yule	0.752	0.329	0.0843	ns
	UPGMA	Simple Matching	0.855	0.260	0.0301	*
		Jaccard	0.935	0.177	0.0062	**
		Nei & Li	0.912	0.205	0.0114	*
		Sorensen	0.912	0.205	0.0114	*
		Yule	0.771	0.319	0.0729	ns
	Farthest Neighbor	Simple Matching	0.840	0.271	0.0364	*
		Jaccard	0.923	0.193	0.0088	**
		Nei & Li	0.897	0.221	0.0153	*
		Sorensen	0.897	0.221	0.0153	*
		Yule	0.721	0.346	0.1056	ns

Note:

r	:	Correlation value
SE of r	:	Error Standard of Correlation Value
P (r=0)	:	P critical value (α = 0.05)
**	:	Very significant (P critical value < 0.01)
*	:	Significant (P critical value < 0.05)
ns	:	Non-Significant (P critical value > 0.05)

In the cophenetic-correlation analysis, the UPGMA method paired with the Jaccard similarity index yielded the highest r-value. This approach clustering and similarity index is the ideal combination for the numeric-phenetic investigation of *L. purpureus*. This finding validates recent numeric-phenetic investigations of *L. purpureus* that mostly employ the UPGMA algorithm and the Jaccard similarity index (Jayanti et al., 2016; Dholakia et al., 2019; Pidigam et al., 2021). It is suggested that a further numeric-phenetic analysis of *L. purpureus* be developed using the UPGMA-Jaccard combination.

The UPGMA method was used to create a dendrogram based on morphology characters, and the Jaccard similarity matrix showed three clusters. BrS - WhF accession is immediately grouped with BlS - WhF accession. With a Jaccard coefficient smaller than 0.2, WhS - PrF accession connects with the BlS-WhF - BlS-PrF cluster. BlS - PrF is the last addition to join the cluster. Based on morphology, this accession has the lowest degree of similarity to another accession.

A dendrogram produced using the UPGMA method and the Jaccard Similarity Index has a different topology than a dendrogram constructed using morphological characters. In the morphology character dendrogram, BrS - WhF accession was associated with BlS - PrF rather than BlS - WhF. In the morphology character dendrogram, WhS - PrF is the final entry to join all clusters rather than BlS - PrF. This result demonstrates a distinct pattern of accession with the least match between the character morphology-based dendrogram and DNA fingerprinting-based dendrogram.

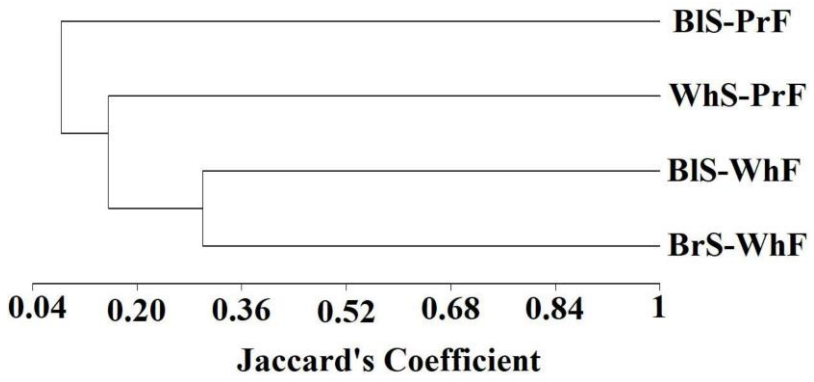


Figure 2. Dendrogram Based on Morphology Character Using UPGMA Algorithm and Jaccard Similarity Index (BlS-PrF: Black Seed Purple Flower, WhS-PrF: White Seed Purple Flower, BlS-WhF: Black Seed White Flower, BrS-WhF: Brown Seed White Flower)

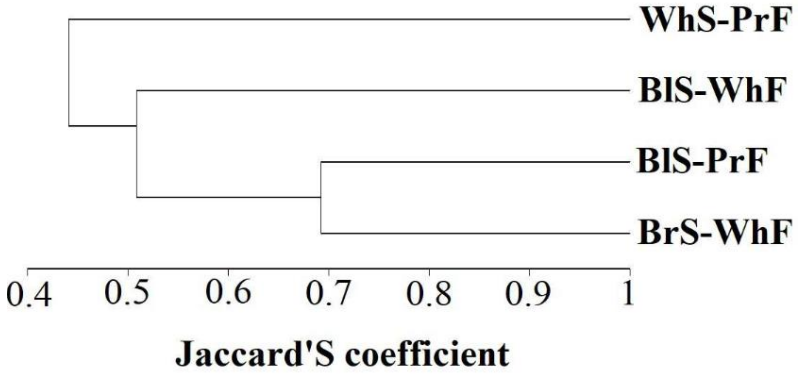


Figure 3. Dendrogram Based on DNA Fingerpriting Using UPGMA Algorithm and Jaccard Similariy Index (BlS-PrF: Black Seed Purple Flower, WhS-PrF: White Seed Purple Flower, BlS-WhF: Black Seed White Flower, BrS-WhF: Brown Seed White Flower)

The disparity in Dendrogram topology and the Jaccard coefficient value is a common outcome of numeric-phenetic analysis employing character morphology and DNA fingerprinting (Kongjaimun et al., 2023). This result can occur due to the algorithm reading different similarity matrices, which are observed from morphology characters and DNA fingerprinting characters. However, this finding suggests that morphology could be used to support DNA fingerprinting in a polyphasic taxonomical investigation (Wangiyana et al., 2022).

The DNA fingerprinting dendrogram has a higher average Jaccard coefficient value than the morphological dendrogram. However, the Jaccard coefficient for both dendrograms is less than 0.7. The value of 0.7 means the similarity is 70%, which is the common threshold for different organism taxonomical units that can be classified as different species. This finding indicates that genetic variety is high among *L. purpureus* accession, which is supported by a morphological study (Ayub et al., 2020).

This is the first investigation of a taxonomical study on *L. purpureus* using a combination of morphology character and molecular character with optimization of the correlation coefficient. This research can give a better understanding of taxonomical analysis using different characters and the optimisation of the algorithm using correlation analysis. The combination of morphology data and molecular data can also give robust taxonomical analysis on a particular plant taxon.

CONCLUSION

Numeric–phenetic study based on morphology character and DNA fingerprinting confirmed by cophenetic–correlation analysis has shown that the UPGMA algorithm and Jaccard similarity index is the optimum combination to analyze the diversity of *L. purpureus* accession from East Java, which resulted in the accession of this species as having high diversity. It is suggested that in future research, more characters should be used to increase the fidelity of the analysis. A combination of morphology, chemical, and molecular data could be the best combination to create a more robust taxonomical study approach. Wider region sampling for L. purpureus is also essential to give better taxonomical analysis results of this species.

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