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Research Article

Phenetic Analysis of Cultivated Black Pepper (*Piper nigrum* L.) in Malaysia

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Phenetic analysis of all the black pepper cultivars in Malaysia is crucial to determine the morphological difference among them. The objective of this study is to ascertain the morphological distinctness and interrelationships among the cultivars to ensure registration of each variety under the Plant Variety Protection Act, as a prerequisite toward implementation of a monovarietal farm policy in the future. Cluster analysis revealed that cultivars “Semongok Aman” and “Semongok 1” have high distinctness values for identification; thus, varietal diagnosis for the two cultivars is easy. Cultivars “Nyerigai,” “India,” “Semongok Perak,” and “Semongok Emas” were grouped in the most diverse clusters among the ten cultivars studied. The four cultivars have a similarity index as high as 92%; however, investigation of leaf width, leaf width-length ratio, seed weight, and conversion rate (fresh to black pepper) gives the ability to determine the characteristic differences. Cultivars “Lampung Daun Lebar” and “Yong Petai” have a similarity of 96%; however, the two showed distinctive differences in leaf width, leaf length-width ratio, spike thickness, and spike length characteristics. On the contrary, cultivars “Kuching” and “Sarikei” showed the highest similarity index, at 98%, and thus are among the most difficult cultivars to diagnose the morphological difference. However, the principle component analysis showed that the fruit size and seed diameter were the important diagnostic key characteristics. Overall, the leaf width, leaf width-length ratio, fruit spike, and conversion rate characteristics are among the key characteristics to differentiate among cultivars of black pepper in Malaysia. At the same time, the principle component analysis carried out has enlightened some interrelationships on the morphological characteristics between cultivars. This information is crucial for the future of the plant varietal improvement program in Malaysia.

1. Introduction

Black pepper, scientifically called *Piper nigrum* L. from the family of Piperaceae, is the most important spice in the world. In Malaysia, the crop has been highlighted as one of the national commodities based on its substantial contribution to the economy of the country. However, the production of black pepper has been diminishing since the early 1980s mainly due to pest and disease occurrence and labour constraints [1]. Thus, the government strategized a new policy to ensure sustainability of the industry by strengthening the quality of peppercorn. A monovarietal farm concept was believed to be able to strengthen the quality of peppercorn.

Black pepper germplasm assemblage has been established in Sarawak, Malaysia, since the 1980s. Since that time, there have been 47 accessions of black pepper varieties and 46 accessions of unidentified species of *Piper* [2]. In Malaysia's current black pepper farms, most are multivarietal and planted because farmers are unaware of monovarietal importance and lack knowledge on varietal identification. Based on a manual entitled “Pepper Production Technology in Malaysia,” released by the Malaysian Pepper Board in 2011 [3], seven cultivated varieties have been described as common cultivars, including cv. “Semongok Aman,” cv. “Semongok Emas,” cv. “Kuching,” cv. “Semongok Perak,” cv. “Uthirancotta,” cv. “Nyerigai,” and cv. “PN129.” However, in 2007, Sim reported the existence of other cultivars in a Malaysian farm, namely, cv. "Lampung Daun Lebar" and cv. "Lampung Daun Kecil" [4]. In 2000, Ravindran also reported three cultivars in Malaysia, namely, cv. "Kuching," cv. "Sarikei," and cv. "Miri" [5]. The number of cultivars existing in a Malaysian black pepper farm is thus unidentified.

A morphological study on Malaysian cultivated black pepper has been reported by Sim in 1979 [6]. The preliminary identification of the collection was based on Ridley's The Flora of the Malay Peninsula [7]. In her study, morphological descriptions include dioecious and monoecious classification, branching behavior, and leaf, stem, and flower spikes. The assessment on fruit development of three selected cultivars, that is, cv. "Kuching," cv. "Semongok Emas," and cv. "Hybrid 10," has also been reported by Sim et al. in 1996 [8], while Chen in 2011 reported the floral biology study on *P. nigrum* [9] and also an apomixis study in 2013 which is related to floral morphology [10]. In India, Ravindran [5], George et al. [11], Parthasarathy et al. [12], and Krishnamoorthy and Parthasarathy [13] have reported the description of the morphology of black pepper. Besides, morphometrical analysis of forty-four cultivars [14] and multivariate analysis of fifty cultivars have been carried out in India [15].

Phenetic analysis of black pepper cultivars is novel in Malaysia. To date, none of the existing cultivars have been registered under the Plant Variety Protection (PVP) Act. This study ascertains the morphological distinctness and interrelationships among cultivars to ensure registration of each variety under the PVP Act, as a prerequisite toward implementation of a monovarietal farm policy in the future.

2. Materials and Methods

2.1. Sampling Site and Experimental Design. This experiment was initiated in January 2015 with the aim of collecting morphological data on important cultivars of black pepper in Malaysia. The field-grown vine was established at three locations, namely, Kampung Jagoi, Serikin; Kampung Karu, Padawan; and Kampung Belawan, Sri Aman; and one potted vine experiment was carried out under the controlled environment at the Agriculture Research Center (ARC) Semongok, Department of Agriculture Sarawak.

The field experiment was laid out in the Randomized Complete Block Design (RCBD) having ten treatments with 5 replications, which are T1: "Semongok Aman" vine; T2: "Kuching" vine, T3: "Semongok Emas" vine; T4: "Semongok Perak" vine; T5: "Semongok 1" vine; T6: "Nyerigai" vine; T7: "India" vine; T8: "Lampung Daun Lebar" vine; T9: "Sarikei" vine; and T10: "Yong Petai" vine. Each trial plot at different locations containing ten treatments consists of 50 vines. The planting procedure followed the standard practice as described by Paulus et al. [3] in "Pepper Production Technology in Malaysia." The planting material used is pepper cutting of 5 nodes, planted with a spacing of $1.8\,\text{m} \times 2.0\,\text{m}$ (between vine × between row). Whilst, the pot experiment was based on the Completely Randomized Design (CRD) that consists of a total of 50 potted vines, with 10 replicates for each treatment, that is, T1: "Semongok Aman" vine; T2: "Kuching" vine, T3: "Semongok Emas" vine; T4: "Semongok Perak" vine; T5: "Semongok 1" vine; T6: "Nyerigai" vine; T7: "India" vine; T8: "Lampung Daun Lebar" vine; T9: "Sarikei" vine; and T10: "Yong Petai" vine. The pot was arranged $1\,\text{m} \times 1\,\text{m}$ (between vine × between row). The data collection was initiated on a 2-year-old vine.

Vine growing morphology or vigour was assessed on field-grown vines in the three field experimental plots, while leaf, inflorescence, fruit, and seed morphology studies were based on samples collected from potted plants grown under the controlled environment. Data collection was carried out from January to December 2017. Microscopy assessment and data analysis were performed at the Malaysian Pepper Board.

2.2. Data Collection and Measurement. Ten cultivated varieties were selected in this study as the operational taxonomic unit (OTU) for the phenetic analysis. The ten OTUs were the cultivars "Semongok Aman" (SA), "Kuching" (KCH), "Semongok Emas" (SE), "Semongok Perak" (SP), "Semongok 1" (S1), "Nyerigai" (NYE), "India" (IND), "Lampung Daun Lebar" (LDL), "Sarikei" (SAR), and "Yong Petai" (YP). A total of 35 characteristics covering important parts of the plant were targeted for assessment. The characteristics listed in the Black Pepper Test Guideline in 2009 were mostly included in the assessment [2]. Details of characteristics and data collection methods are listed in Table 1.

2.3. Data Analysis. Measurement of morphological characteristics as variables is performed in this analysis. Characteristics used for phenetic analysis all tested to be significantly different between at least two cultivars under the ANOVA test, using SPSS, and are further analyzed by the Duncan test for significant character state differences ($P < 0.05$). Two analyses were performed in this study, cluster analysis and principle component analysis (PCA). The cluster analysis measured the similarity indexes between the OTUs using the Pearson correlation and average linkage with phenogram as the final output [16], while the PCA helped to extract the valuable information from a multivariate data table and express this information as new variables [17].

3. Results and Discussion

3.1. Cluster Analysis. Morphological characteristics used in the phenetic analysis of black pepper cultivars include all parts of the black pepper plant, including the leaf, inflorescence, fruit, seed, and shoot tips. The components of the characteristics used are shown in Table 2, while the data matrix for phenetic character states is shown in Table 3. For all quantitative data, the mean, range, and standard deviation were estimated (Figures 1–19). Characteristics used were all tested and showed significant differences between at least two cultivars under the ANOVA test using SPSS. The Duncan test proved that the characteristics of leaf length-width ratio (Figure 4) and number of flowers per

Table 1: Morphological characteristics used in the phenetic analysis of black pepper cultivars.

Morphological characteristics	Measurement methods
Leaf characteristics	
(1) Leaf shape, leaf apex, and leaf base	Description based on the UPOV standard
(2) Leaf area (cm^2), blade width (w, mm), blade length (L, mm), and blade length-width ratio (Lw^{-1})	Measured by using the WinFOLIA image analysis system
(3) Leaf colour (fully expanded leaf)	RHS colour codes used
Inflorescence characteristics	
(1) Inflorescence length at the stigma withering stage (cm) and inflorescence thickness at the stigma withering stage (mm)*	Measured by using a vernier calliper
(2) Inflorescence colour	RHS colour codes used
(3) Number of flowers per inflorescence	Counted via stereomicroscope
(4) Number of inflorescences (spikes) per branch per node	Counted manually
Fruit characteristics	
(1) Fruit spike length (cm) and fruit size in diameter (mm)	Measured by using a vernier calliper
(2) Fruit weight (single fresh berry) (g)	Measured by using an analytical balance
(3) Fruit colour (hard dough stage)	RHS colour codes used
(4) Percent fruit set	Counted manually. Percent = (number of developed fruits)/(number of developed fruits + number of underdeveloped fruits) × 100
(5) Conversion rate (fresh to black pepper) (%)	Measured by using an analytical balance (drying specification: oven-drying at 40°C; moisture content ≤12%)
(6) Conversion rate (fresh to white pepper) (%)	Measured by using an analytical balance (drying specification: oven-drying at 40°C; moisture content ≤12%)
(7) Pericarp thickness (mm)	Measured by using a vernier calliper (horizontal diameter of a fresh berry–horizontal diameter of the seed)
Seed characteristics	
(1) Seed diameter (mm)	Measured by using a vernier calliper (horizontal diameter of the seed)
(2) Seed weight (g)	Measured by using an analytical balance
Vigour	
(1) Branch column	By observation
(2) Internode length (cm)	Measurement by using a ruler (node-to-node distance)
(3) Number of nodes/foot of the stem	Counted manually
Shoot tips	
(1) Anthocyanin: absent or present	By observation of shoot tip colouration. Green colour = absence of anthocyanin; purple colour = presence of anthocyanin

*Withering stage [6].

inflorescence (Figure 7) have seven significant difference groups, among the characteristics with highest distinctness values. Characteristics such as leaf area (cm^2) (Figure 1), leaf width (cm) (Figure 2), inflorescence or spike thickness (mm) (Figure 6), number of inflorescences or spikes per branch per node (Figure 8), seed weight (g) (Figure 17), percent fruit set (Figure 12), internode length (cm) (Figure 18), and number of nodes per foot of the stem (Figure 19) have six significant groups among ten cultivars. Meanwhile, characteristics such as leaf length (cm) (Figure 3), inflorescence length (cm) (Figure 5), number of flowers per spike/inflorescence (Figure 7), conversion rate from fresh to dried white pepper (Figure 14), pericarp thickness (mm) (Figure 15), and seed diameter (mm) (Figure 16) have five significant groups. The Duncan test also showed that characteristics such as single berry size in diameter (mm) (Figure 10), conversion rate from fresh to dried black pepper (Figure 13), and fruit weight (single berry) (g) (Figure 11) have only four significant groups. The assessment of characteristics such as number of branches per node, hilum-micropyle distance, petiole length (mm), blade thickness (mm), colour of an immature fruit, colour of a ripened fruit, seed shape, and venation pattern do not show a significant difference and thus are not included in the analysis.

Cluster analysis is based on measuring the similarity between the operational taxonomic units (OTUs). In this study, ten OTUs, the cultivars "Semongok Aman" (SA), "Kuching" (KCH), "Semongok Emas" (SE), "Semongok Perak" (SP), "Semongok 1" (S1), "Nyerigai" (NYE), "India" (IND), "Lampung Daun Lebar" (LDL), "Sarikei" (SAR), and "Yong Petai" (YP), were compared. Each OTU will be clustered into groups based on a similarity index of morphological characteristics. The greater the value of the similarity index, the closer the OTU unit or grouping, and vice versa [18].

Referring to the phenogram in Figure 20, black pepper cultivars were clustered into five groups, with a similarity index ≥85%. Cluster A is composed of two cultivars, "Kuching" and "Sarikei," with a similarity index as high as 99%. The two cultivars share 15 similar characteristics out of

Table 2: Characteristics and character states used for phenetic analysis.

Number	Characteristics and character states
	Leaf characteristics
1	Leaf shape: (1) lanceolate; (2) lanceolate-ovate; (3) ovate
2	Leaf apex: (1) acute; (2) obtuse; (3) rounded
3	Leaf base: (1) acute; (2) oblique; (3) rounded; (4) ovate
4	Leaf area (cm^2): (1) <40; (2) 40–60; (3) 60–80; (4) 80–100; (5) >100
5	Leaf colour (fully expanded leaf)* :(1) green group 137 series (moderate olive green); (2) green group NN137 series (greyish olive green); (3) green group 139 series (dark yellowish green)
6	Blade width (w, mm): (1) <6; (2) 6–8; (3) 8–10; (4) 10–12; (5) >12
7	Blade length (L, mm): (1) <10; (2) 10–11; (3) 11–12; (4) 12–13; (5) >13
8	Blade width-length ratio (Lw^{-1}): (1) <1.4; (2) 1.4–1.7; (3) 1.7–2.0; (4) 2.0–2.3; (5) >2.3
	Inflorescence characteristics
9	Inflorescence length at the withering stage (cm): (1) <7; (2) 7–8; (3) 8–9; (4) 9–10; (5) >10
10	Inflorescence colour* : (1) green group 144 series (strong yellowish green); (2) yellow-green group N144 series (strong yellowish green); (3) yellow-green group 145 series (strong yellowish green)
11	Inflorescence thickness at the withering stage (mm): (1) <2.8; (2) 2.8–3.2; (3) 3.2–3.6; (4) 3.6–4.0; (5) >4.0
12	Number of flowers per inflorescence: (1) <80; (2) 80–90; (3) 90–100; (4) >100
13	Number of inflorescences (spikes) per branch per node: (1) <20; (2) 20–30; (3) 30–40; (4) >40
	Fruit characteristics
14	Fruit spike length (cm): (1) <7; (2) 7–9; (3) 9–11; (4) >11
15	Fruit size (single berry) in diameter (mm): (1) <6; (2) 6–7; (3) >7
16	Fruit weight (single berry) (g): (1) <0.12; (2) 0.13–0.18; (3) >0.18
17	Fruit colour (hard dough stage)*: (1) green group NN137 series (greyish olive green); (2) green group 139 series (dark yellowish green); (3) green group 141 series (deep yellowish green)
18	Percent fruit set: (1) <60; (2) 60–70; (3) >70
19	Conversion rate (fresh to black pepper) (%): (1) <40; (2) 40–50; (3) >50
20	Conversion rate (fresh to white pepper) (%): (1) <20; (2) 20–30; (3) >30
21	Pericarp thickness (mm): (1) <1.6; (2) 1.6–1.8; (3) 1.8–2.0; (4) 2.0–2.2; (5) >2.2
	Seed characteristics
22	Seed diameter (mm): (1) <3.5; (2) 3.5–4.0; (3) >4.0
23	Seed weight (g): (1) < 4.8; (2) 4.8–5.0; (3) 5.0–5.2; (4) 5.2–5.4; (5) >5.4
	Vigour
24	Branch column types: (1) erect; (2) horizontal; (3) drooping
25	Internode length (cm): (1) <8; (2) 8–9; (3) 9–10; (4) 10–11; (5) >11
26	Number of nodes/foot of the stem: (1) 1; (2) 2; (3) 3; (4) 4; (5) 5
	Shoot tip
27	Anthocyanin: (1) absent; (2) present

*RHS colour codes used.

27 and 10 minor dissimilarity characteristics (one state difference). However, the distinctions are found in Characteristic 21 (pericarp thickness) and Characteristic 23 (seed weight). The second cluster (B) has the greatest population among all clusters, comprising four cultivars, "Nyerigai," "India," "Semongok Perak," and "Semongok Emas." The group was further clustered into two subgroups, with a similarity index of 97% for "Nyerigai" and "India" (Subgroup 1) and 95% for "Semongok Perak" and "Semongok Emas" (Subgroup 2). The distinctive characteristics that differentiate Subgroup 1 are Characteristic 8 (blade width-length ratio) and Characteristic 21 (pericarp thickness), while for Subgroup 2, Characteristic 8 (blade width-length ratio), Characteristic 17 (fruit colour at the hard dough stage), and Characteristic 23 (seed weight) are the important distinctive characteristics. Cluster C only consists of one cultivar, "Semongok Aman." This cultivar was clustered alone with plenty of distinct characteristics, even when compared to the closest cluster (Cluster B), especially in Characteristic 2 (leaf apex), Characteristic 3 (leaf base), Characteristic 10 (inflorescence colour at the withering stage), and Characteristic 18 (percent fruit set). Cluster D has two cultivars in its group, "Lampung Daun Lebar" and "Yong Petai." The two cultivars share a 94% similarity; however, they show dissimilarity in characteristics such as Characteristic 8 (blade width-length ratio), Characteristic 10 (inflorescence colour at the withering stage), Characteristic 11 (inflorescence thickness at the withering stage), and Characteristic 14 (fruit spike length). Cultivar "Semongok 1" is another cultivar clustered alone. This cultivar showed great morphologically distinct values in Characteristic 3 (leaf base), Characteristic 6 (blade width), Characteristic 23 (seed weight), and Characteristic 25 (number of nodes per foot of the stem), if compared to the nearest cluster (Cluster D).

Ho et al. [19] support the outcome of this analysis in their research on evaluation of genetic relatedness among black pepper (*Piper nigrum* L.) accessions using direct amplification of the minisatellite-region DNA (DAMD). That team included the same ten cultivars in their study, and the DAMD-based clustering is tallied with the phenetic-based clustering in this study.

3.2. Principle Component Analysis (PCA)

In this study, principle component analysis was performed using 27 morphological characteristics. Seven components were

Table 3: Data matrix of phenetic character states corresponding to Table 1.

Cultivars	Characteristics and character states																										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
SA	2	3	3	2	3	2	2	3	2	1	3	2	1	2	2	3	1	3	1	2	3	3	5	2	5	4	2
KCH	2	1	1	1	3	1	2	4	2	2	3	1	4	2	2	2	1	2	2	3	4	3	3	2	2	5	2
SE	2	2	1	2	1	1	5	5	2	2	3	2	1	2	2	2	3	2	2	3	4	3	5	3	5	4	2
SP	2	2	1	1	2	2	5	3	1	3	4	1	2	1	2	3	1	2	1	2	5	3	2	2	4	3	2
S1	3	2	4	5	3	5	5	2	3	2	4	4	1	4	3	3	1	2	2	2	5	3	5	1	2	4	1
NYE	2	2	1	2	3	2	4	3	2	2	2	1	3	2	2	2	1	2	2	3	5	3	1	1	3	4	2
IND	1	1	1	2	3	1	5	5	2	2	2	2	2	2	2	2	1	2	2	2	3	2	1	2	3	4	2
LDL	2	2	2	4	2	3	5	2	2	1	4	4	1	2	2	2	2	1	1	2	4	3	1	3	5	4	2
SAR	2	1	1	1	3	1	2	4	1	2	3	1	3	1	1	3	1	2	1	2	2	2	1	2	3	4	2
YP	2	2	1	4	3	2	5	4	3	3	2	3	1	4	3	3	1	1	1	2	5	3	2	2	5	3	2

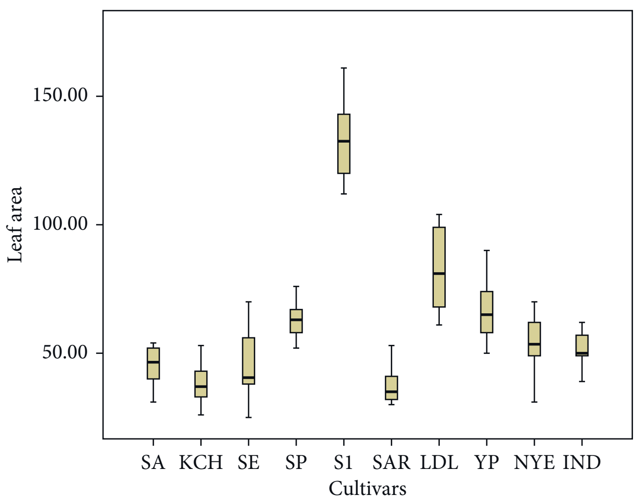

Figure 1: Leaf area (cm^2).

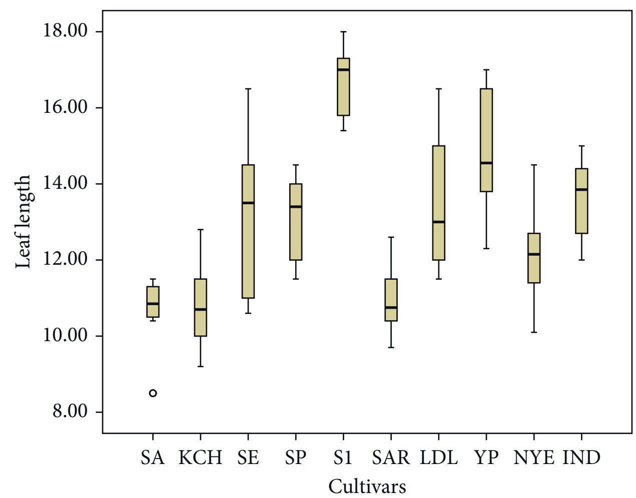

Figure 3: Leaf length (cm).

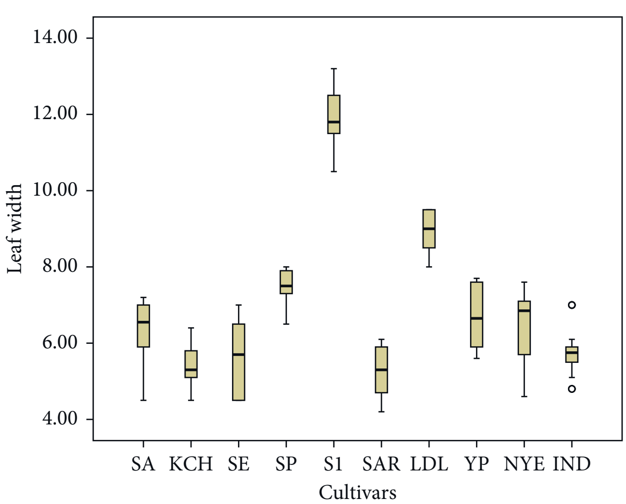

Figure 2: Leaf width (cm).

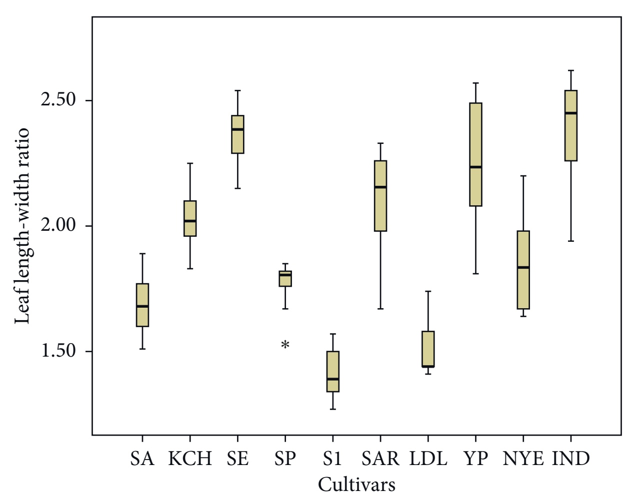

Figure 4: Leaf length-width ratio.

extracted as meaningful factors, with eigenvalues >1 (Figure 21). These components explained 95.79% of the total variance, fulfilling the 95% confidence level mentioned by Jackson [20] (Table 4). The first principle component (PC1) explained 33.71% of the total variation, the second component (PC2) explained 17.85% of the variation, and the third component (PC3) explained 13.43% of the variation. The other principle components (PC4–PC7) explained the additional 30.80% of the variation. Pasagi et al. [21] explained that the PCA value can be categorized into three levels: the first level, with the component value $X \geq 0.75$, has a very strong influence on the grouping; the second level,

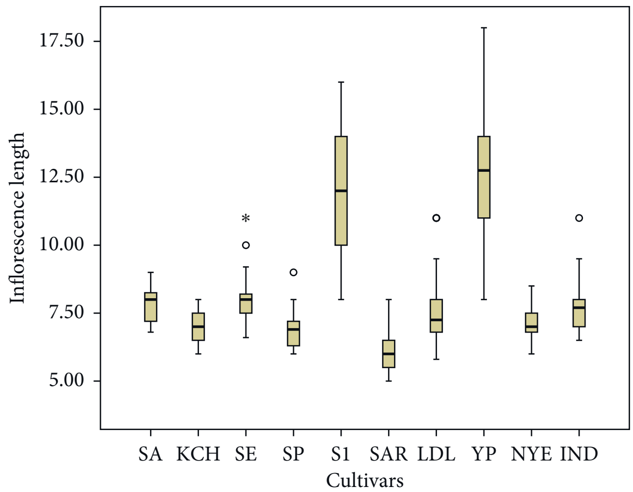

Figure 5: Inflorescence length (cm).

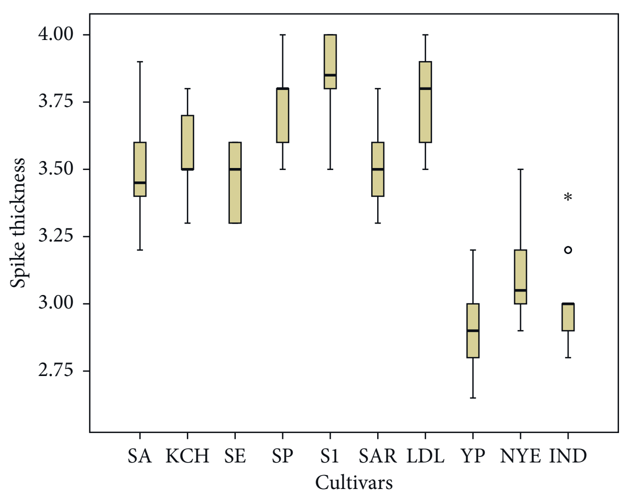

Figure 6: Spike or inflorescence thickness (mm).

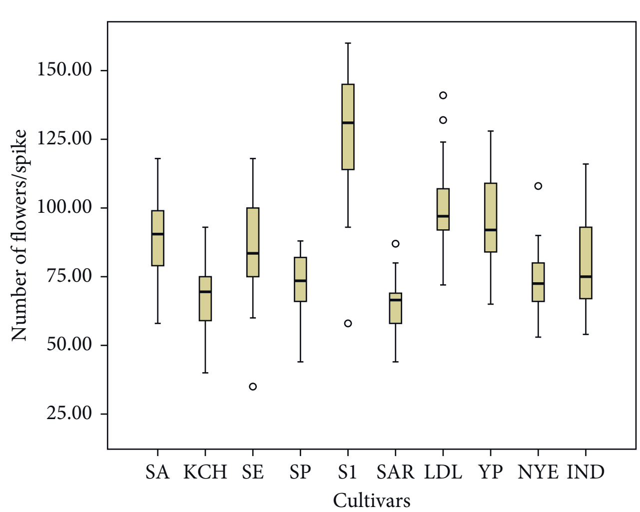

Figure 7: Number of flowers per inflorescence.

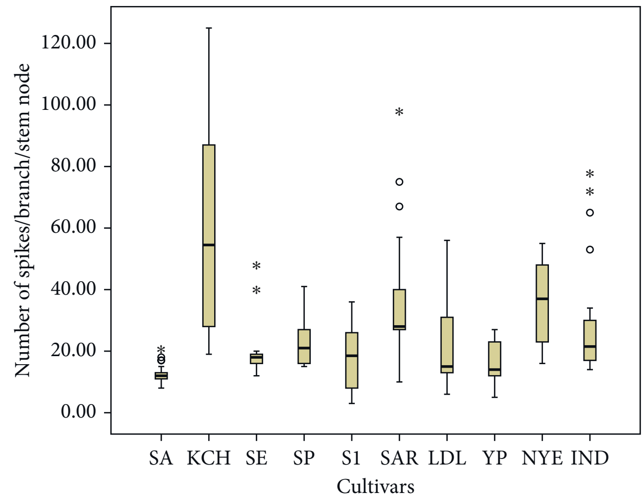

Figure 8: Number of inflorescences (spikes) per branch per node.

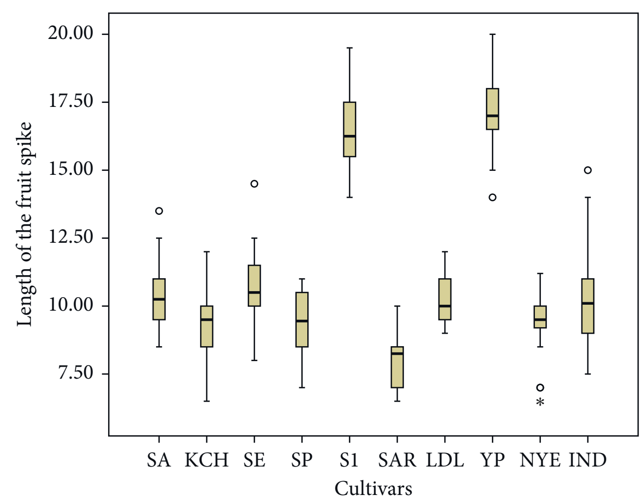

Figure 9: Fruit spike length (cm).

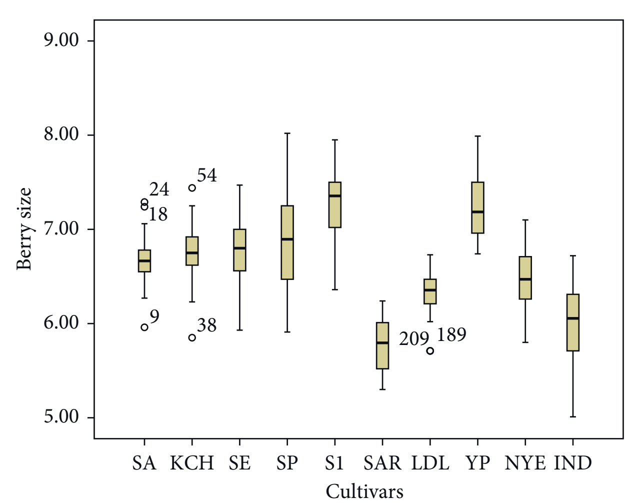

Figure 10: Single fresh berry size in diameter (mm).

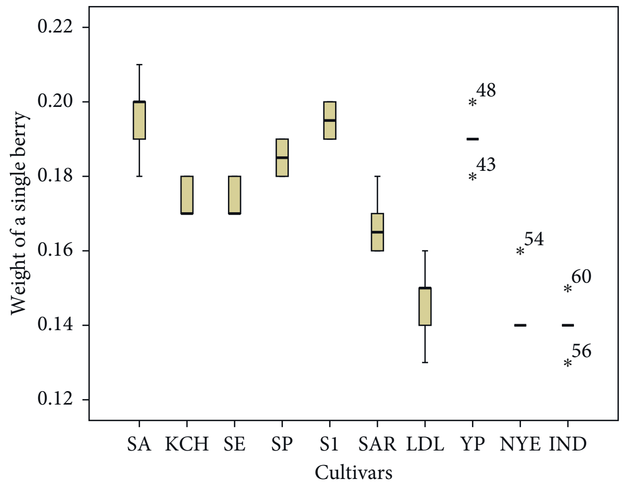

Figure 11: Fruit weight (single berry) (g).

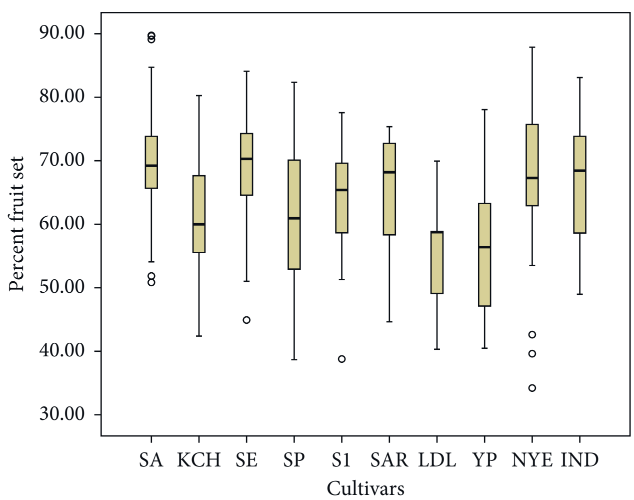

Figure 12: Percent fruit set.

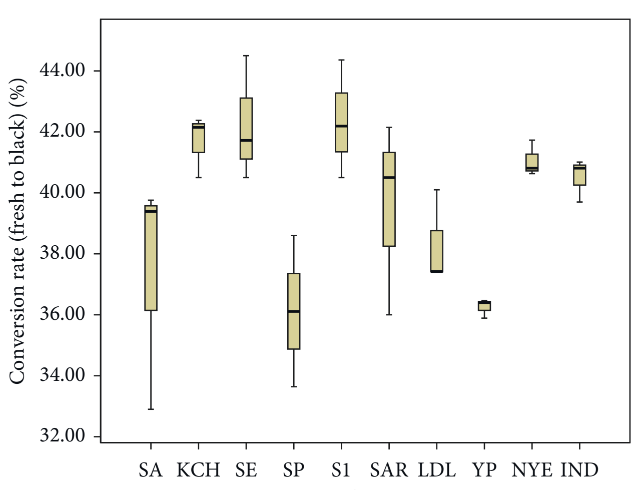

Figure 13: Conversion rate (fresh to dried black pepper) (%).

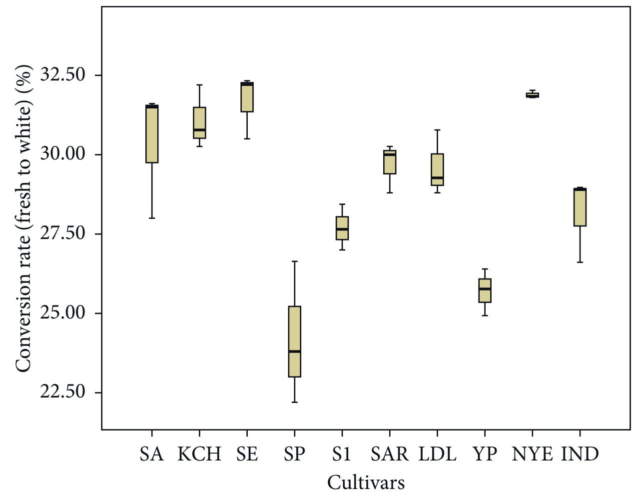

Figure 14: Conversion rate (fresh to dried white pepper) (%).

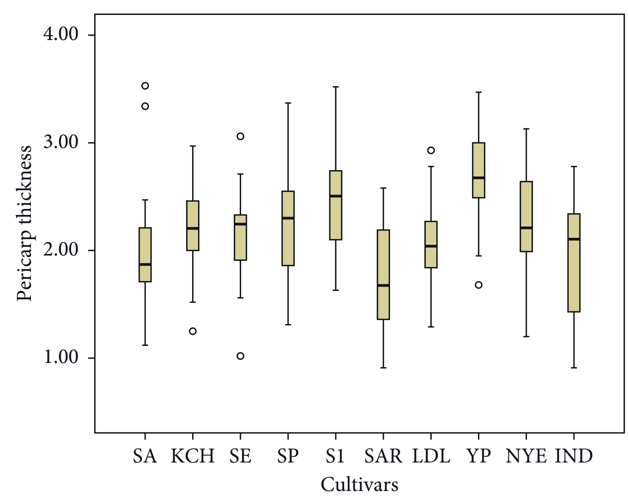

Figure 15: Pericarp thickness (mm).

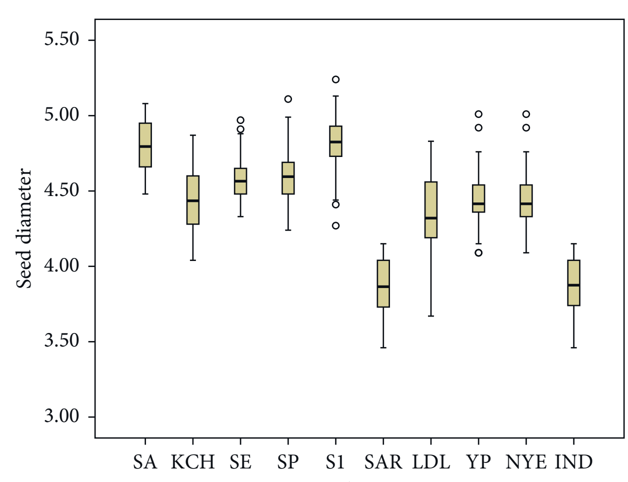

Figure 16: Seed diameter (mm).

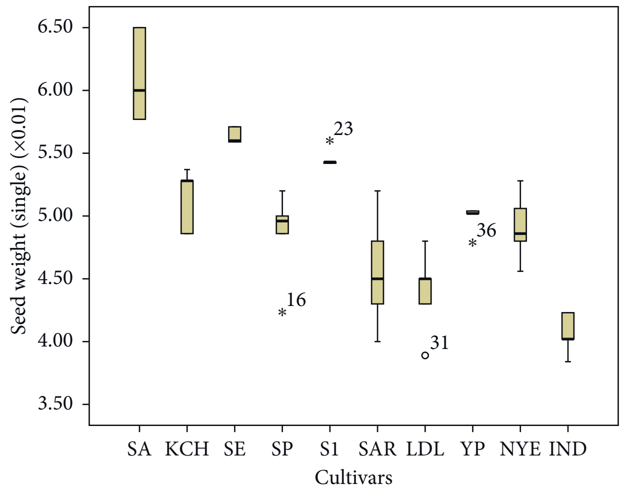

Figure 17: Seed weight (10^{-2}) (g).

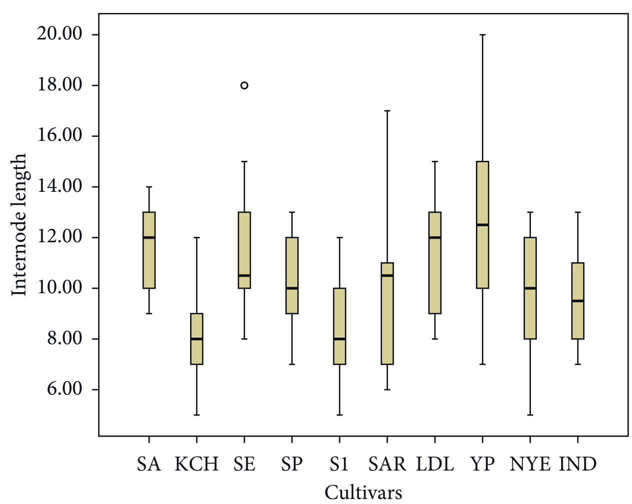

Figure 18: Internode length (cm).

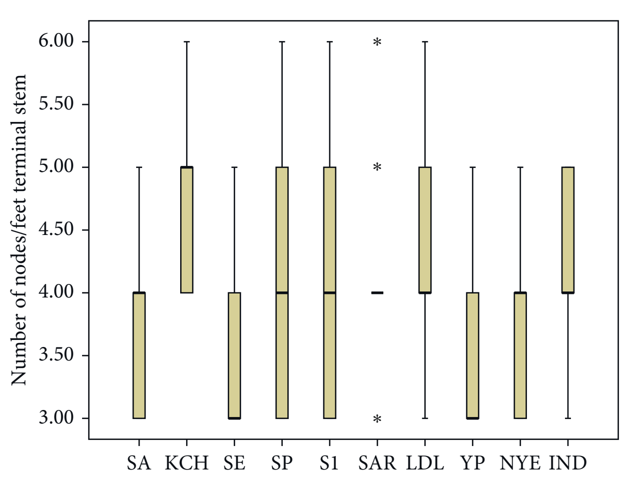

Figure 19: Number of nodes per foot of the stem.

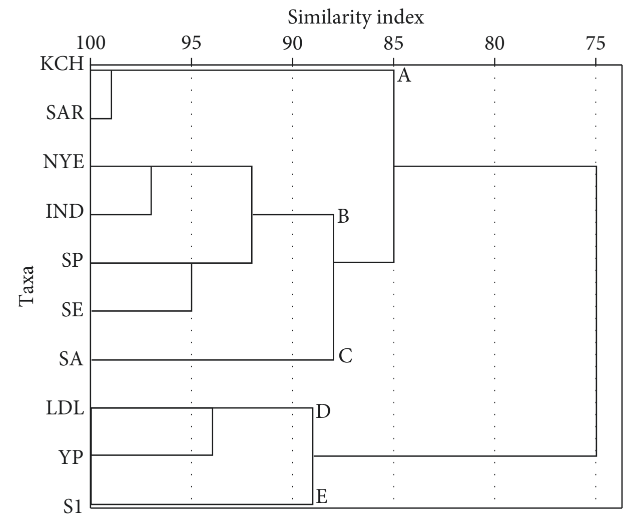

Figure 20: Phenogram presented based on the average linkage (between groups) using the squared Euclidean distance method.

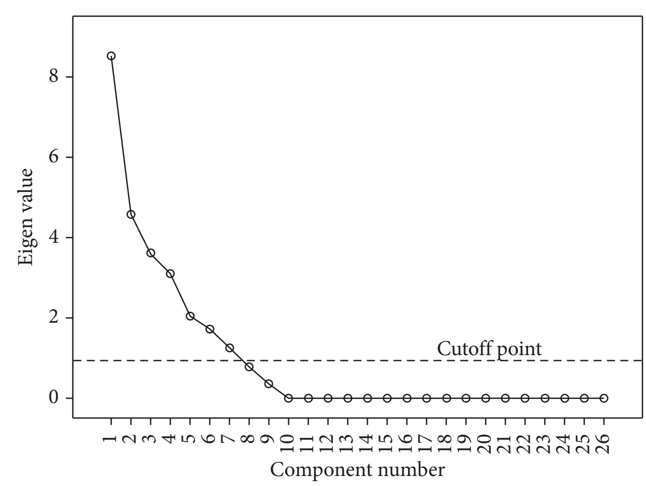

Figure 21: Scree plot showing the cutoff point of extracted components for PCA.

with a component value $0.50 \leq X < 0.75$, has a secondary influence on the separation of OUT; the third level, with the component value less than 0.50, has the least influence on separation at a minimal level. Based on PCA (Table 5), among the 27 characteristics used for analysis, 18 characteristics have a strong influence on the grouping of black pepper cultivars, with the PCA value ranging from 0.78 to 0.96. These characteristics include leaf shape, leaf area, blade width, leaf colour, inflorescence length, inflorescence colour, inflorescence thickness, number of flowers/inflorescence, fruit spike length, fruit size in diameter, fruit colour (hard dough stage), percent fruit set, conversion rate percent of fresh to black pepper, conversion rate percent of fresh to white pepper, pericarp thickness, seed weight, number of nodes per foot of the stem, and anthocyanin colouration. This analysis also revealed that leaf, inflorescence, and fruit

Table 4: Total variance obtained using principle component analysis (PCA).

PC	Initial eigenvalues			Extraction sums of squared loadings			Rotation sums of squared loadings		
	Total	Variance (%)	Cumulative (%)	Total	Variance (%)	Cumulative (%)	Total	Variance (%)	Cumulative (%)
1	9.102	33.713	33.713	9.102	33.713	33.713	6.213	23.011	23.011
2	4.820	17.850	51.563	4.820	17.850	51.563	5.176	19.169	42.180
3	3.626	13.431	64.994	3.626	13.431	64.994	3.967	14.693	56.873
4	3.133	11.602	76.596	3.133	11.602	76.596	3.789	14.034	70.907
5	2.048	7.587	84.183	2.048	7.587	84.183	2.457	9.099	80.006
6	1.805	6.684	90.867	1.805	6.684	90.867	2.393	8.862	88.868
7	1.329	4.921	95.788	1.329	4.921	95.788	1.869	6.921	95.788
8	0.785	2.907	98.696	—	—	—	—	—	—
9	0.352	1.304	100.000	—	—	—	—	—	—

Table 5: Matrix component value for all the distinguishing characteristics.

	Principle component (PC)						
	1	2	3	4	5	6	7
Leaf shape	0.204	**0.807**	−0.101	0.029	0.078	0.402	0.043
Leaf apex	0.279	0.159	0.213	−0.455	−0.151	**0.632**	0.432
Leaf base	0.453	**0.696**	−0.107	−0.167	−0.367	0.047	0.360
Leaf area (cm^2)	**0.868**	0.380	0.072	−0.166	−0.080	0.039	−0.219
Leaf colour	0.167	−0.060	**−0.955**	−0.058	−0.172	−0.154	0.017
Blade width (cm)	**0.538**	**0.793**	−0.090	−0.150	−0.037	0.113	−0.036
Blade length (cm)	**0.531**	0.102	0.497	−0.056	0.485	−0.125	−0.212
Blade width-length ratio	−0.119	**−0.737**	0.167	0.296	0.261	−0.337	0.179
Inflorescence length (cm)	**0.948**	0.048	−0.126	0.154	−0.026	0.195	−0.029
Inflorescence colour	−0.011	−0.125	−0.171	−0.033	**0.957**	0.019	−0.150
Inflorescence thickness (mm)	−0.220	**0.849**	0.334	−0.191	−0.093	0.057	−0.099
Number of flowers/inflorescence	**0.780**	0.393	0.258	−0.233	−0.233	−0.070	−0.234
Number of inflorescences/branch	**−0.621**	−0.155	**−0.523**	**0.515**	0.020	−0.045	−0.220
Fruit spike length (mm)	**0.923**	0.150	−0.193	0.036	0.132	0.153	−0.057
Fruit size in diameter (mm)	**0.848**	0.189	−0.080	−0.009	0.298	0.294	−0.027
Fruit weight (single berry) (g)	0.011	0.343	−0.355	**−0.689**	0.300	0.035	0.277
Fruit colour (hard dough stage)	0.050	−0.048	**0.932**	0.219	−0.098	0.105	−0.023
Percent fruit set	−0.340	0.060	−0.196	0.103	−0.203	0.020	**0.881**
Conversion rate (fresh to black pepper) (%)	0.240	0.012	−0.002	**0.922**	0.084	−0.136	0.209
Conversion rate (fresh to white pepper) (%)	−0.189	−0.231	0.132	**0.824**	0.024	0.439	0.029
Pericarp thickness (mm)	0.236	0.276	0.187	0.056	0.043	**0.890**	−0.033
Seed diameter (mm)	0.423	0.032	0.092	0.059	0.477	**0.566**	**−0.500**
Seed weight (g)	−0.169	−0.279	**0.791**	−0.180	−0.246	−0.039	−0.256
Branch column types	0.122	−0.357	**0.601**	**−0.604**	−0.099	0.341	0.023
Internode length (cm)	−0.167	0.065	−0.129	**0.713**	**−0.628**	−0.076	0.019
Number of nodes/foot of the stem	−0.496	**−0.789**	0.177	−0.161	−0.109	0.148	−0.185
Anthocyanin	0.496	**0.789**	−0.177	0.161	0.109	−0.148	0.185

Note. Extraction method: principle component analysis; rotation method: varimax with Kaiser normalisation.

are three important parts in diagnosing the morphological difference among black pepper cultivars. Meanwhile, the most dominant characteristic influencing the grouping is the inflorescence colour, with a PCA value as high as 0.957. On the contrary, the less important characteristics assisting in grouping are blade length and seed diameter, both with a PCA value lower than 0.60.

This analysis also revealed some interesting interactions among morphology characteristics of black pepper cultivars. In PC1, the positive loading of characteristics, such as leaf area, inflorescence length, number of flowers per inflorescence, fruit spike length, and fruit size, proves the proportional relationship among these characteristics, while the characteristic of number of inflorescences per branch has a negative loading in this PC. This explains that if the black pepper vine has a bigger leaf area, the inflorescence length, number of flowers per inflorescence, fruit spike length, and fruit size will have a greater value, while the number of inflorescences per branch will perform the opposite. This reveals that greater leaf areas do not contribute to better cultivar yield, even though the flower intensity per spike, inflorescence length, and fruit spike have positive relationship with fruit sizes. Gazzoni and Moscardi support this finding in their soybean study [22], as do Subedi and Ma in their maize study [23]. On the contrary, Heuvelink et al. reported a reduction of leaf area can contribute to the yield of tomato [24]. PC2 showed a positive loading in characteristics such as leaf shape, leaf base, blade width, inflorescence thickness, and anthocyanin, whilst a negative loading in blade width-length ratio and number of nodes per foot of the stem. This PC reveals that if the cultivars have a very distinct leaf shape compared to other cultivars, the cultivar also tends to have a distinct leaf base, distinct leaf width, greater inflorescence thickness, and anthocyanin present, but smaller width-length ratio and number of nodes per foot of the stem. This PC explains Cluster C and Cluster E (Figure 20), both with single cultivars in the cluster because of their high distinctive values. The identification of these two cultivars is easy. PC3 has a negative loading for two characteristics, leaf colour and number of inflorescences per branch, and a positive loading for fruit colour, seed weight, and branch column type. This explains that when the fruit colour is greener, the seed weight tends to increase and the column type becomes denser, but when the leaf colour seems to be lighter, the number of inflorescences per branch will respond negatively. PC4 to PC7 showed fewer interrelations among the morphology characteristics. In PC4, two types of conversion rate studies, fresh to dried black pepper and fresh to dried white pepper, show proportionate relationships. This result is partially supported by Paulus et al., who reported only on cultivars SA, KCH, and SE [3]. Fruit weight and branch column type also have proportionate relationships, both with negative loadings. This relationship could be interpreted as the less-dense column type of cultivar yielding less-weight fruit, whilst PC5 to PC7 do not show an interesting interrelationship.

4. Conclusions

Phenetic cluster analysis revealed that cultivars "Semongok Aman" and "Semongok 1" have high distinctive values for identification; thus, varietal diagnosis could be very easy. Cultivars "Nyerigai," "India," "Semongok Perak," and "Semongok Emas" were grouped in the most diverse cluster among all clusters. The four cultivars have a similarity index as high as 92%; however, investigation on leaf width, leaf width-length ratio, seed weight, and conversion rate (fresh to black pepper) can determine the characteristic differences. Cultivars "Lampung Daun Lebar" and "Yong Petai" have a similarity of 96%; however, the two showed distinctive differences in leaf width, leaf length-width ratio, spike thickness, and spike length characteristics. In Cluster A (Figure 20), cultivars "Kuching" and "Sarikei" showed the highest similarity index and thus are among the most difficult cultivars to diagnose morphological differences. However, the principle component analysis showed that the fruit size and seed diameter were the key diagnostic characteristics. Overall, the leaf width, leaf width-length ratio, fruit spike, and conversion rate characteristics are among the key characteristics to differentiate among cultivars of black pepper in Malaysia. At the same time, principle component analysis has been carried out, discerning some interrelationships within the morphological characteristics between cultivars. The overall phenetic analysis showed that all the selected cultivars have distinct values enabling registration under the Malaysian Plant Variety Protection Act. This information is also crucial for plant varietal improvement programs in the future.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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