Pertanika J. Trop. Agric. Sci. 43 (3): 257 - 274 (2020)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Analysis of Qualitative and Quantitative Trait Variability among Black Pepper (*Piper nigrum* L.) Cultivars in Malaysia

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ABSTRACT

This project comprehensively documented the morphological characteristics of ten black pepper cultivars in Malaysia, focusing on diagnosing the morphological difference among the cultivars via qualitative traits. These cultivars are cv. 'Semongok Aman', cv. 'Kuching', cv. 'Semongok Emas', cv. 'Semongok Perak', cv. 'Semongok 1', cv. 'Nyerigai', cv. 'India', cv. 'Lampung Daun Lebar', cv. 'Sarikei', and cv. 'Yong Petai'. The morphological characteristics had been evaluated via field-grown vine where the randomized complete block design (RCBD) was adopted and potted vine evaluation via completely randomized design (CRD). Cv. 'Semongok 1' showed ovate shaped leaf and anthocyanin free shoot tip; cv. 'Semongok Aman' had rounded shape of leaf apex and base; cv. 'Lampung Daun Lebar' had an oblique shape in leaf base and 'Nyerigai' showed erect type branching; cv. 'Semongok Emas' had leaf colour of Green group 137 series (RHS code) and fruit colour of Green group 141 series (RHS); cv. 'India' had a lanceolate shaped leaf. At the same time, this study also revealed the key differences in quantitative traits that included leaf area, length-width ratio, inflorescent length, fruit spike length, and fresh to dried berry conversion rate. The study showed that cv. 'India' had a low length-width ratio (Lw-1) at 1.52 and lightest seed weight at 4.07(x 10-2) g; cv. 'Sarikei' had the smallest leaf area (36.90 cm²), shortest inflorescence (6.06 cm), shortest fruit spike (8.07 cm), smallest fruit diameter (5.78 cm), smallest seed diameter (3.84 cm), and thinnest pericarp (1.73 cm); cv. 'Kuching' had the greatest number of inflorescence per branch per node

ARTICLE INFO

Article history:
Received: 8 January 2020
Accepted: 27 April 2020
Published: 28 August 2020

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ISSN: 1511-3701
e-ISSN 2231-8542

(ca.58.67) and the greatest number of node/ feet of the stem (ca.4.73); cv. 'Yong Petai' had the longest inflorescence (12.75 cm), longest fruit spike (17.07 cm), but thinnest fruit spike (2.90 mm); and, lastly, cv. 'Semongok Perak' had the conversion rate (from fresh to dried black) (36.12 %) and conversion rate (from fresh to dried white) (24.21 %). The comprehensive evaluation of both qualitative and quantitative traits of all the black pepper cultivars has ensured the efficiency of cultivar identification.

Keywords: Black pepper cultivar, qualitative and quantitative traits

INTRODUCTION

Black pepper, scientifically called *Piper nigrum* L., is known as the 'King of Spice' and is the most commonly used spice in the world. The plant is woody perennial climber required support, living or non-living to promote normal growth; leaves alternate and petiolate type, with shape commonly elliptical, lanceolate or ovate; inflorescence of catkin types and the flower is minute, bracteates, bisexual or unisexual and protogynous; the fruit of drupe type with thin pericarp and seed spherical shaped with diameter 3-5 mm; nodal stem with internode ranged from 8-13 cm when mature; shoot tip purplish-green or whitish green (Chen, 2011; Ravindran et al., 2000). In India, the morphological analyses of black pepper cultivars were comprehensively studied by Ravindran et al. (1997) through morphometric analysis. Whilst in Malaysia, Chen et al. (2018) had comprehensive analysis on the morphology of ten important cultivars while Noorasmah et al. (2018) had also recorded the inflorescence characteristics of some important pepper variety.

The plant was introduced to Malaysia as early as 1856 (Dalton, 1912), with cultivation focus in the state of Sarawak. However, the diversity of the black pepper cultivar in Malaysia is unidentified because varietal control is not practised. The most common black pepper cultivars are cv. 'Kuching' and cv. 'Sarikei', both widely planted throughout Sarawak (Sim, 1993), while Paulus (2007) reported three important cultivars in his publication, i.e. cv. 'Semongok Perak', cv. 'Semongok Aman', and cv. 'Kuching'. Through the International Pepper Community (IPC) exchange program, cv. 'Lampung Daun Lebar' and cv. 'Lampung Daun Kecil' was introduced to Malaysian farmers (Sim, 1993). A manual entitled 'Pepper production technology in Malaysia' was recently released by the Malaysian Pepper Board, mentioning the existence of seven cultivated varieties as common cultivars in Malaysia, including cv. 'Semongok Aman', cv. 'Semongok Emas', cv. 'Kuching', cv. 'Semongok Perak', cv. 'Uthirancotta', cv. 'Nyerigai', and cv. 'PN129' (Paulus, 2011). A total of 47 accessions of black pepper varieties and 46 accessions of wild *Piper* were reported, conserved in form of a living plant in the Agricultural Research Centre (ARC) in Sarawak, Malaysia, from 1957 until 1992 (Sim, 1993).

Black Pepper Test Guideline for Plant Variety Protection Act implementation has been established by the Department of Agriculture Malaysia (2009). This guideline listed the entire important characteristic for the diagnosis of black pepper variety. However, the existing documentation on cultivated black pepper in Malaysia is less comprehensive, and none of the cultivars is registered under the National Crop List of Malaysia. The importance of this study is to comprehensively document the morphological characteristics of all the important black pepper cultivars in Malaysia, focusing on the diagnosis of the distinctness among the cultivars. The Malaysian government strategized a new policy to ensure the sustainability of the industry by strengthening the quality of peppercorn. A mono-varietal farm concept is believed able to strengthen the quality of peppercorn. This can be achieved through varietal control, and a pre-requisite to this policy is comprehensive documentation on all important cultivars in the country.

MATERIALS AND METHODS

Extensive fieldwork has been undertaken by the first author since January 2014, to cover all the possible black pepper cultivation areas throughout Malaysia, to verify the diversity of black pepper cultivars. The black pepper farm distribution info was sourced from the Department of Crop, Extension, and Farmer's Development, from the Malaysian Pepper Board. Photography data particularly on the leaf, inflorescence, fruit spike, and shoot tip were comprehensively generated for the preliminary cultivar's diagnosis. The preliminary diagnosis must show at least one distinct character to be eligible for further cultivars verification study. The pepper germplasm centre situated at Agricultural Research Centre Semongok (ARC) Sarawak, Malaysia was referred for verification on the cultivar designation.

To develop a comprehensive guide for cultivar identification, a thorough assessment of morphological characteristics (qualitative or quantitative traits) had been conducted. Both potted peppers and field-grown vines were assessed in this study. Vine growing morphology or vigour was assessed on field-grown mature vines at the three field experimental plots while leaf, inflorescence, fruit, and seed morphology studies were based on samples collected from potted mature vines grown under a controlled environment. Data collection was carried out from January 2016 to December 2017. Microscopy assessment and data analysis were performed at the Malaysian Pepper Board, Kuching.

The field experiments were conducted at three locations, namely Kampung Jagoi, Serikin; Kampung Karu, Padawan and Kampung Belawan, Sri Aman. The plots were laid out in a Randomized Complete Block Design (RCBD) (Figure 1) having ten treatment 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 a different location containing ten treatments consists of 50 vines. Whilst, a pot experiment was conducted under a controlled environment at the Agriculture Research Center (ARC) Semongok, Department of Agricultural Sarawak, using Completely Randomized Design (CRD) (Figure 2) that consist of a total of 50 potted vines, with 5 replicates for each treatment, i.e. 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 1m x 1m (between vine x between row). The data collection was initiated at 2 years old vine.

T2	T8	T6	T7	T1
T1	T3	T7	T6	T10
T10	T4	T1	T5	T9
T9	T7	T2	T1	T8
T8	T6	T9	T2	T3
T3	T5	T8	T4	T7
T4	T1	T3	T3	T2
T7	T2	T10	T8	T4
T6	T9	T4	T10	T5
T5	T10	T5	T9	T6

Figure 1. Randomized complete block design (RCBD) for the field-grown vine of ten cultivars. 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 block is differenced by topography elevation

T1	T4	T2	T4	T7	T6	T7	T5	T7	T2
T3	T8	T3	T8	T8	T2	T8	T5	T8	T6
T10	T10	T1	T6	T1	T2	T10	T1	T3	T8
T2	T10	T8	T3	T5	T4	T6	T3	T1	T10
T4	T5	T6	T8	T5	T8	T7	T8	T4	T7

Figure 2. Completely randomized design (CRD) pot arrangement for experimental plot under a controlled environment. 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

A total of 26 morphology characteristics, consisting of both qualitative and quantitative traits, had been assessed in this study, as listed in Table 1. A dichotomous key for diagnosing the cultivars was constructed as the outcome for this study.

Table 1

Morphological characteristic used for diagnosis of cultivar distinctness

	Morphological character	Measurement methods
1.	**Leaf characters**	
	Leaf shape; leaf apex and leaf base	Description based on UPOV standard
	Leaf area (cm^2); blade width (w) mm; blade length (L) mm and blade length-width ratio (Lw^{-1})	Measured by WinFOLIA image analysis system
	Leaf colour (fully expanded leaf)	RHS colour codes used
2.	**Inflorescence characters**	
	Inflorescence length at stigma withering stage (cm) and Inflorescence thickness at stigma withering stage (mm)*	Measured by Vernier calliper
	Inflorescence colour	RHS colour codes used
	Number of flowers per inflorescence	Counted via stereomicroscope
	Number of inflorescence (spike) per branch per node	Counted manually
3.	**Fruit characters**	
	Fruit spike length (cm) and fruit size in diameter (mm)	Measured by Vernier calliper
	Fruit weight (single fresh berry) (g)	Measured by analytical balance
	Fruit colour (hard dough stage)	RHS colour codes used
	Per cent fruit set (%)	Counted manually. Percent = (Number of developed fruit)/ (Number of developed fruit + number of underdeveloped fruit) x 100%.
	Conversion rate % (fresh to black pepper)	Measured by analytical balance (Drying specification: Oven dry at 40°C; moisture content ≤12%)
	Conversion rate % (fresh to white pepper)	Measured by analytical balance (Drying specification: Oven dry at 40°C; moisture content ≤12%)
	Pericarp thickness (mm)	Measured by Vernier calliper (Horizontal diameter of fresh berry - Horizontal diameter of seed)
4.	**Seed characters**	
	Seed diameter (mm)	Measured by Vernier calliper (Horizontal diameter of seed)
	Seed weight (g)	Measured by analytical balance

Table 1 *(Continued)*

	Morphological character	Measurement methods
5.	**Vigour**	
	Branch column	By observation
	Internode length (cm)	Measurement by a ruler (Node to node distance)
	Number of node /1feet stem	Counted manually
6.	**Shoot tips**	
	Anthocyanin: Absent or present	By observation on shoot tip colouration Greenish colour = Absent of anthocyanin; Purplish colour = Present of anthocyanin

Note. UPOV- International Union for the Protection of New Varieties of Plants; RHS - Royal Horticultural Society

RESULTS AND DISCUSSIONS

In this study, a total of ten black pepper cultivars have been assessed, including 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). Comprehensive assessment consisting of both qualitative and quantitative traits had been carried out to reveal key diagnostic morphology for each of the cultivars. The results of the assessment are shown in Table 2.

Table 2

Qualitative and quantitative traits used to diagnose the differences among black pepper cultivars

No.	Morphological characteristic	Cultivars				
		SA	KCH	SE	SP	S1
	Leaf (Refer to Figure 3)					
1.	Leaf shape	Elliptical	Ovate	Elliptical	Elliptical	Cordate
2.	Leaf apex	Mucronate	Acute	Acute	Acute	Acute
3.	Leaf base	Acute	Rounded	Acute	Oblique	Cordate
4.	Leaf area (cm^2)	45.40^{abc}	37.70^{ab}	46.60^{bc}	62.80^{d}	132.60^{f}
5.	Blade width (w)	6.36^{b}	5.37^{a}	5.67^{a}	7.47^{c}	11.87^{e}
6.	(cm)	10.70^{a}	10.83^{a}	13.31^{c}	13.20^{c}	16.67^{e}
7.	Blade length (L) (cm)	1.70^{b}	2.02^{d}	2.35^{ef}	1.77^{bc}	1.41^{a}
8.	Blade length-width ratio (Lw^{-1})	Green group 139 series	Green group 139 series	Green group 137 series	Green group NN137	Green group 139 series
	Leaf colour (fully expanded leaf)					

Table 2 *(Continued)*

No.	Morphological characteristic	Cultivars				
		NYE	IND	LDL	SAR	YP
	Leaf (Refer to Figure 3)					
1.	Leaf shape	Elliptical	Lanceolate	Ovate	Elliptical	Elliptical
2.	Leaf apex	Acute	Acuminate	Acute	Acute	Acute
3.	Leaf base	Oblique	Rounded	Oblique	Acute	Acute
4.	Leaf area (cm^2)	53.60^{c}	50.90^{c}	81.40^{e}	36.90^{a}	66.50^{d}
5.	Blade width (w)	6.49^{b}	5.75^{a}	8.85^{d}	5.26^{a}	6.62^{b}
6.	(cm)	12.07^{b}	13.63^{c}	13.50^{c}	10.93^{a}	14.75^{d}
7.	Blade length (L) (cm)	1.86^{c}	2.39^{f}	1.52^{a}	2.10^{d}	2.24^{e}
8.	Blade length-width ratio (Lw^{-1})	Green group 139 series	Green group 139 series	Green group NN137	Green group 139 series	Green group 139 series
	Leaf colour (fully expanded leaf)					

No.	Morphological characteristic	Cultivars				
		SA	KCH	SE	SP	S1
	Inflorescence (Refer to Figure 4)					
9.	Inflorescence length (cm)	7.84^{d}	7.03^{bc}	7.95^{d}	6.93^{b}	12.40^{e}
10.	Inflorescence thickness (mm)	3.50^{d}	3.56^{d}	3.47^{d}	3.73^{e}	3.85^{f}
11.	Inflorescence colour	Green group 144 series	Green group N144	Green group N144	Green group 145 series	Green group N144
12.	Number of flowers per	88.30^{de}	67.57^{ab}	86.33^{de}	72.70^{abc}	127.90^{g}
13.	inflorescence (average)	12.47^{a}	58.67^{f}	19.57^{abc}	22.20^{bc}	17.60^{ab}
	Number of inflorescence (spike) per branch per node (average)					

Table 2 *(Continued)*

No.	Morphological characteristic	Cultivars				
		NYE	IND	LDL	SAR	YP
	Inflorescence (Refer to Figure 4)					
9.	Inflorescence length (cm)	7.06^{bc}	7.80^{d}	7.68^{cd}	6.06^{a}	12.75^{e}
10.	Inflorescence thickness (mm)	3.10^{c}	3.00^{b}	3.75^{e}	3.54^{d}	2.90^{a}
11.	Inflorescence colour	Green group N144	Green group N144	Green group 144 series	Green group N144	Green group 145 series
12.	Number of flowers per inflorescence (average)	73.77^{bc}	80.40^{cd}	100.53^{f}	65.10^{a}	93.57^{ef}
13.	Number of inflorescence (spike) per branch per node (average)	35.83^{e}	27.37^{cd}	21.47^{bc}	34.73^{de}	16.20^{ab}

No.	Morphological characteristic	Cultivars				
		SA	KCH	SE	SP	S1
	Fruit (Refer to Figures 5 and 6)					
14.	Fruit spike length (cm)	10.38^{c}	9.37^{b}	10.62^{c}	9.27^{b}	16.48^{d}
15.	Fruit size in diameter (mm)	6.68^{e}	6.75^{e}	6.76^{e}	6.86^{e}	7.27^{f}
16.	Fruit weight (single fresh berry) (g)	0.20^{d}	0.17^{b}	0.17^{b}	0.19^{c}	0.20^{d}
17.	Fruit colour (hard dough stage)	Green group NN137 series	Green group NN137 series	Green group 141 series	Green group NN137 series	Green group NN137 series
18.	Per cent fruit set (%)	70.68^{f}	61.10^{bc}	68.75^{ef}	61.51^{bc}	64.24^{cde}
19.	Conversion rate (%) (fresh to black pepper)	37.35^{ab}	41.68^{cd}	42.24^{cd}	36.12^{a}	42.35^{d}
20.	Conversion rate (%) (fresh to white pepper)	30.37^{de}	31.08^{e}	31.68^{e}	24.21^{a}	27.70^{bc}
21.	Pericarp thickness (mm)	2.00^{bc}	2.20^{c}	2.16^{bc}	2.22^{cd}	2.46^{de}

Table 2 *(Continued)*

No.	Morphological characteristic	Cultivars				
		NYE	IND	LDL	SAR	YP
	Fruit (Refer to Figures 5 and 6)					
14.	Fruit spike length (cm)	9.39^{b}	10.38^{c}	10.22^{c}	8.07^{a}	17.07^{d}
15.	Fruit size in diameter (mm)	6.48^{d}	6.02^{b}	6.30^{c}	5.78^{a}	7.27^{f}
16.	Fruit weight (single fresh berry) (g)	0.14^{a}	0.14^{a}	0.15^{a}	0.17^{b}	0.19^{cd}
17.	Fruit colour (hard dough stage)	Green group NN137 series	Green group NN137 series	Green group 139	Green group NN137 series	Green group NN137 series
18.	Per cent fruit set (%)	66.93^{def}	65.76^{cdef}	55.10^{a}	64.28^{cde}	56.26^{ab}
19.	Conversion rate (%) (fresh to black pepper)	41.06^{bcd}	40.51^{bcd}	38.31^{abc}	39.55^{abcd}	36.25^{a}
20.	Conversion rate (%) (fresh to white pepper)	31.89^{e}	28.16^{cd}	29.62^{cde}	29.69^{cde}	25.70^{ab}
21.	Pericarp thickness (mm)	2.25^{cd}	1.94^{ab}	2.06^{bc}	1.73^{a}	2.69^{e}

No.	Morphological characteristic	Cultivars				
		SA	KCH	SE	SP	S1
	Seed (Refer to Figure 6)					
22.	Seed diameter (mm)	4.80^{e}	4.44^{c}	4.60^{d}	4.60^{d}	4.82^{e}
23.	Seed weight (g) (x 10^{-2})	6.11^{g}	5.13^{e}	5.40^{f}	4.85^{d}	5.46^{f}

No.	Morphological characteristic	Cultivars				
		NYE	IND	LDL	SAR	YP
	Seed (Refer to Figure 6)					
22.	Seed diameter (mm)	4.44^{c}	3.90^{a}	4.32^{b}	3.84^{a}	4.45^{c}
23.	Seed weight (g) (x 10^{-2})	4.91^{d}	4.07^{a}	4.30^{b}	4.56^{c}	4.98^{de}

Table 2 *(Continued)*

No.	Morphological characteristic	Cultivars				
		SA	KCH	SE	SP	S1
	Vigour					
24.	Branch column types	Horizontal	Horizontal	Drooping	Horizontal	Horizontal
25.	Internode length (cm)	11.42^{e}	8.33^{a}	11.40^{e}	10.10^{cd}	8.73^{ab}
26.	Number of node /feet of stem (average)	3.67^{ab}	4.73^{f}	3.33^{a}	4.13^{cdf}	4.17^{cdf}

No.	Morphological characteristic	Cultivars				
		NYE	IND	LDL	SAR	YP
	Vigour					
24.	Branch column types	Erect	Horizontal	Drooping	Horizontal	Horizontal
25.	Internode length (cm)	9.77^{bc}	9.57^{bc}	11.23^{de}	9.83^{bc}	12.7^{f}
26.	Number of node /feet of stem (average)	3.83^{bc}	4.30^{de}	4.43^{ef}	3.97^{bcd}	3.33^{a}

No.	Morphological characteristic	Cultivars				
		SA	KCH	SE	SP	S1
	Shoot tips (Refer to Figure 7)					
27.	Anthocyanin: Absent or present	Present	Present	Present	Present	Absent

No.	Morphological characteristic	Cultivars				
		NYE	IND	LDL	SAR	YP
	Shoot tips (Refer to Figure 7)					
27.	Anthocyanin: Absent or present	Present	Present	Present	Present	Present

Note. SA - 'Semongok Aman'; KCH - 'Kuching'; SE - 'Semongok Emas'; SP - 'Semongok Perak'; S1 - 'Semongok 1'; NYE - 'Nyerigai'; IND - 'India'; LDL - 'Lampung Daun Lebar'; SAR - 'Sarikei', and YP - 'Yong Petai'. Means followed by the different superscript letter within the same row are significantly different at $P \leq 0.05$

The dichotomous key for cultivar diagnosis was constructed by considering both the qualitative and quantitative traits of the ten black peppers.

Identification key to black pepper cultivars in Malaysia:

1a	Leaf area <80 cm² (fully developed leaf from matured and vigorous vine); Number of flowers per inflorescence less than 90	2
1b	Leaf area >80 cm² (fully developed leaf from matured and vigorous vine); Number of flowers per inflorescence more than 90	8
2a	Blade length less than 11 cm long; Blade width-length ratio (Lw^{-1}) ranged from 2.0 to 2.3	3
2b	Blade length more than 12 cm long; Blade width-length ratio (Lw^{-1}) ranged from 1.7 to 2.0 or >2.3	4
3a	Pericarp thickness 2.0-2.2 mm thick; Seed weight 5.0-5.2 (x 10^{-2}) g	'Kuching'
3b	Pericarp thickness 1.6-1.8 mm thick; Seed weight <4.8 (x 10^{-2}) g	'Sarikei'
4a	Leaf base acute; Percent fruit set >70%	'Semongok Aman'
4b	Leaf base rounded; Percent fruit set 60-70%	5
5a	Inflorescence thickness 2.8-3.2 mm thick; Internode 9-10 cm long	6
5b	Inflorescence thickness 3.2-4.0 mm thick; Internode 10-11 cm long	7
6a	Leaf shape lanceolate; Leaf apex acuminate; Plagiotropic branching horizontal type	'India'
6b	Leaf shape elliptical; Leaf apex acute; Plagiotropic branching erect type	'Nyerigai'
7a	Mature leaf, blueish-green colour (RHS colour code: Green group137 series); Mature unripe fruit, pale green (RHS colour code: Green group 141 series)	'Semongok Emas'
7b	Mature leaf, greyish green colour (RHS colour code: Green group NN137); Mature unripe fruit, dark green (RHS colour code: Green group NN137 series)	'Semongok Perak'
8a	Anthocyanin absent in shoot tip (green whitish colour); Seed weight, >5.4(x 10^{-2}) g; internode length, 8-9 cm	'Semongok 1'

8b	Anthocyanin present in shoot tip (Purplish green colour); Seed weight, <4.8(x 10^{-2}) g or 4.8-5.0(x 10^{-2}) g; internode length, >11 cm	9
9a	Fruit spike length, 7-9 cm; Fruit size in diameter, 6-7 mm	'Lampung Daun Lebar'
9b	Fruit spike length, >11 cm; Fruit size in diameter, >7 mm	'Yong Petai'

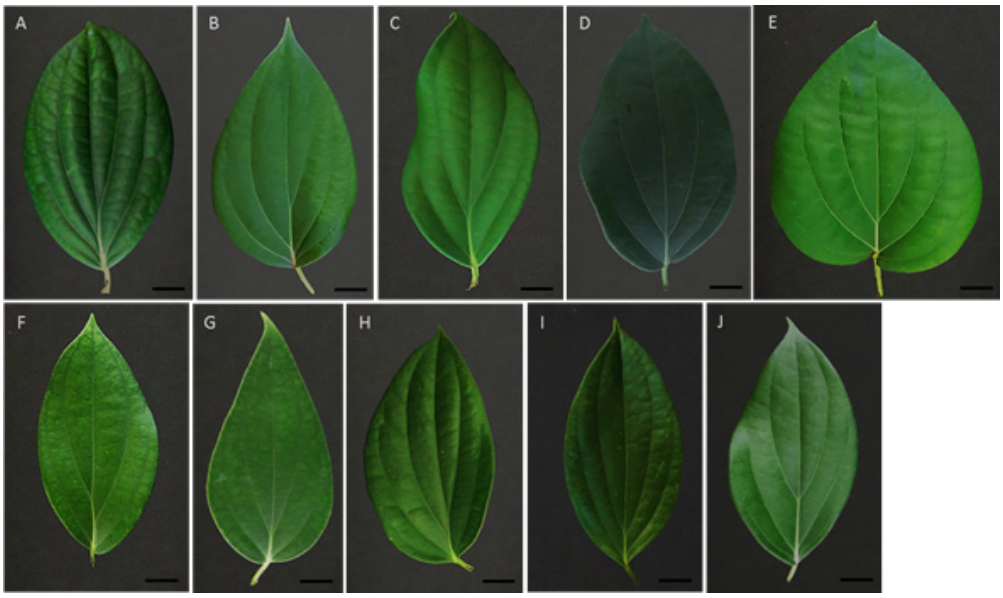

Figure 3. Cultivar designation and leaf shape. A. cv. 'Semongok Aman'- Elliptical; B. cv. 'Kuching'- Ovate; C. cv. 'Semongok Emas'- Elliptical; D. cv. 'Semongok Perak'- Elliptical; E. cv. 'Semongok 1'- Cordate; F. cv. 'Nyerigai'- Elliptical; G. cv. 'India'- Lanceolate; H. cv. 'Lampung Daun Lebar'- Ovate; I. cv. 'Sarikei'- Elliptical; J. cv. 'Yong Petai'- Elliptical. Scale bar: 1cm

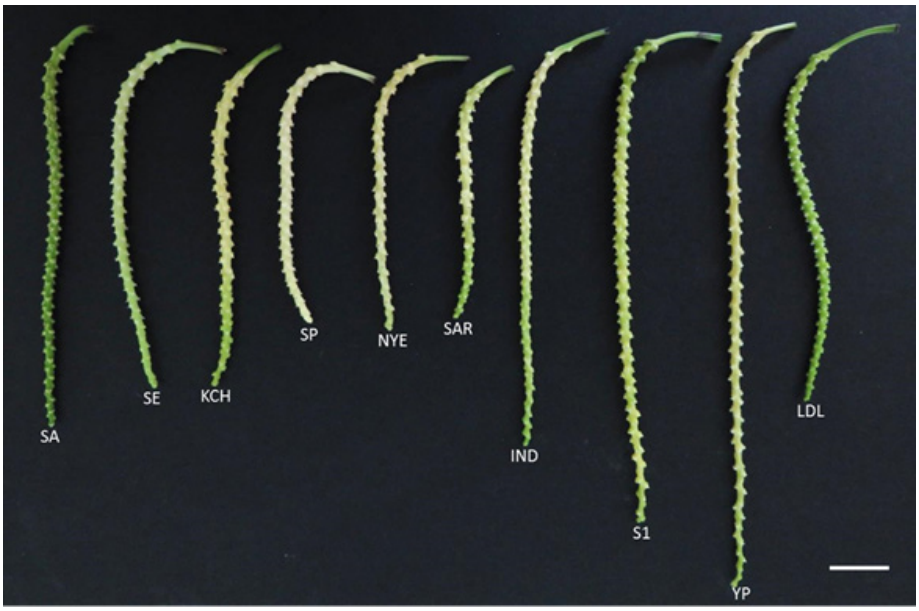

Figure 4. Inflorescence. SA - 'Semongok Aman'; SE - 'Semongok Emas'; KCH - 'Kuching'; SP - 'Semongok Perak'; NYE - 'Nyerigai'; SAR - 'Sarikei'; IND - 'India'; S1 - 'Semongok 1'; YP - 'Yong Petai', and LDL - 'Lampung Daun Lebar'. Scale bar: 2cm

Figure 5. Fruit spike. SA - 'Semongok Aman'; KCH - 'Kuching'; SE - 'Semongok Emas'; SP - 'Semongok Perak'; S1 - 'Semongok 1'; NYE - 'Nyerigai; IND - 'India'; LDL - 'Lampung Daun Lebar'; SAR - 'Sarikei', and YP - 'Yong Petai'. Scale bar: 2cm

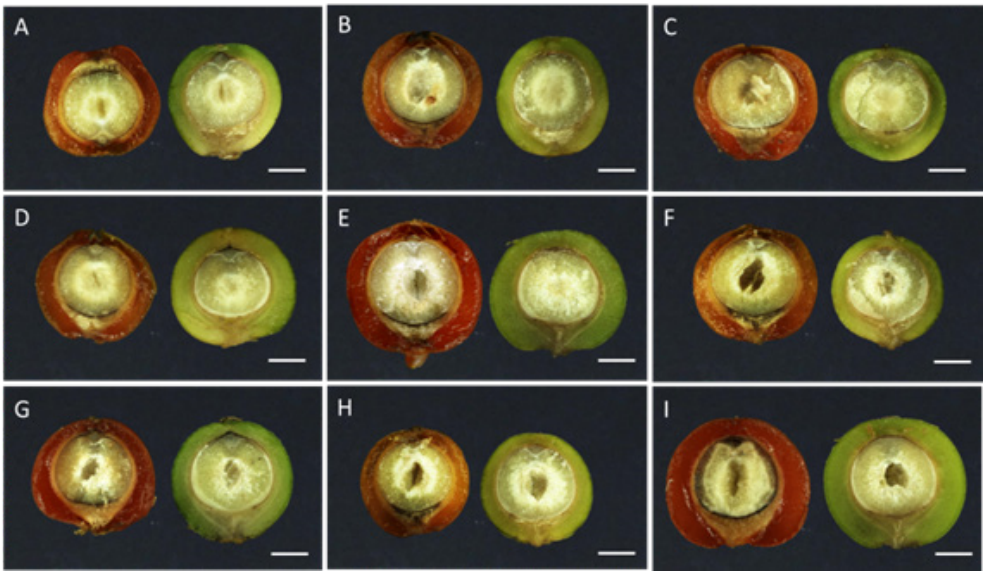

Figure 6. Cross section of ripe and mature fruit. A. 'Semongok Aman'; B. 'Kuching'; C. 'Semongok Emas'; D. 'Semongok Perak'; E. 'Semongok 1'; F. 'Nyerigai; G. 'Lampung Daun Lebar'; H. 'Sarikei', and I. 'Yong Petai'. Scale bar: 2mm

Figure 7. Shoot tips. SA - 'Semongok Aman'; KCH - 'Kuching'; SE - 'Semongok Emas'; SP - 'Semongok Perak'; S1 - 'Semongok 1'; NYE - 'Nyerigai; IND - 'India'; LDL - 'Lampung Daun Lebar'; SAR - 'Sarikei', and YP - 'Yong Petai'. Scale bar: 1cm

Qualitative trait analysis showed leaf shape, leaf apex, leaf base, branch column types (branching behaviour), and anthocyanin colouration at shoot tip were crucial for diagnosing morphological differences among cultivars. Most of the cultivars exhibited elliptical shaped leaf; however, cultivar 'India' had a lanceolate shaped leaf and the cultivar 'Semongok 1' exhibited cordate-shaped leaf. Thus, leaf shape distinctness is an important key diagnosis for the two cultivars. Leaf apex observation showed cultivar 'Semongok Aman' was substantially distinct, with a mucronate-shaped leaf apex; thus, the cultivar could be identified through this trait easily. The only cultivar 'Lampung Daun Lebar' showed ovate leaf shape and oblique leaf base at the same time, another important key diagnosis. In branch column analysis, most cultivars showed a horizontal type of branching, but the cultivar 'Nyerigai' exhibited an erect type of branching, while both cultivar 'Semongok Emas' and cultivar 'Lampung Daun Lebar' exhibited a drooping type. Another important key diagnosis is to shoot tip colouration analysis, where the arrival of anthocyanin at the shoot tip will lead to an exhibition of a purplish colour. Among the ten cultivars, the only cultivar 'Semongok 1' was anthocyanin-free at the shoot tip, thus exhibiting a whitish-green shoot tip. However, qualitative traits, like leaf colour and inflorescence colour, were less substantial for the diagnosis, because the colour intensity is influenced greatly by biotic and abiotic factors (Anita & Anna, 2012; Szakiel et al., 2011). Analyses in this study showed qualitative traits are more influential compared to quantitative traits. This is supported by Khan et al. (2015), Olakojo and Adetula (2014), and Stephan et al. (2016). However, qualitative traits alone with limited variability are insufficient for the diagnosis of certain black pepper cultivars. In this study, the ANOVA test proved the variability for quantitative traits was more substantial compare to qualitative traits. The analysis showed seed weight is among the most important quantitative trait, exhibiting seven significantly different groups among the ten cultivars. Next are traits like blade length-width ratio, fruit size in diameter, conversion rate (%) (fresh to black pepper), inflorescence thickness, number of inflorescence per branch per node, number of flower per inflorescence, per cent fruit set (%), number of node/feet of the stem, and internode length that exhibited six significant groups in the ANOVA test, respectively. The quantitative traits of blade width, blade length, pericarp thickness, seed diameter, and conversion rate (fresh to white pepper) showed five significant different groups, also an important diagnosis key for the ten black pepper cultivars. Thus, the quantitative plays a pivotal role as an additional indicator when the qualitative traits are unable to assist the identification.

The phenetic analysis was done by Chen et al. (2018) revealing that cultivars 'Semongok Aman' and 'Semongok 1' had high distinctive values for identification, thus varietal diagnostic 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 had 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) could determine the characteristic differences. Cultivar 'Lampung Daun Lebar' and the cultivar 'Yong Petai' had a similarity of 96%; however, the two showed distinctive differences on leaf width, leaf length-width ratio, spike thickness, and spike length characteristics. The study also reported cultivars 'Kuching' and 'Sarikei' showed the highest similarity index, thus were among the most difficult cultivars to diagnose morphological differences. This finding proved the importance of both qualitative analysis and quantitative analysis in varietal identification of black pepper cultivars.

CONCLUSIONS

Qualitative trait analysis has assisted the diagnosis of ten important cultivars of black pepper in Malaysia as mentioned above while the quantitative traits are crucial as an additional indicator for the diagnosis beside played the role as an indicator of the potential agronomic performance of the cultivar. This study showed cv. 'Semongok 1' exhibited two distinct qualitative traits, a cordate shaped leaf and anthocyanin free shoot tip, and was among the easiest cultivar to identify. Another cultivar with two distinct qualitative traits is cv. 'Semongok Aman', with mucronate shaped leaf apex. The identification for this cultivar can be further verified by quantitative traits, counting the per cent of fruit set. This cultivar exhibited per cent fruit set as high as 76%, averagely. Qualitative trait analysis also discovered the morphological distinctness of cultivar 'Lampung Daun Lebar'. This cultivar showed ovate leaf shape and oblique leaf base at the same time, unique among all the cultivars. The identification for this cultivar was further supported by the quantitative trait of blade length-width ratio (Lw^{-1}), where the cultivar showed the lowest ratio among all cultivars. Cultivar 'Nyerigai' exhibited a unique branching behaviour (branch column type) of an erect type, while others exhibited horizontal or drooping behaviour. The only cultivar that showed a distinctness in leaf and fruit colouration was cv. 'Semongok Emas', with the leaf colour of green group 137 series (RHS code) and fruit colour of green group 141 series (RHS). Cultivar 'India' exhibited a lanceolate shaped leaf, an important diagnosis key for this cultivar. Quantitative trait uniqueness for this cultivar was seed weight; it was the lightest seed among all. Cv. 'Kuching', cv. 'Sarikei', cv. 'Semongok Perak', and cv. 'Yong Petai' did not show qualitative trait distinctness; however, quantitative trait analysis had assisted the diagnosis. Cv. 'Sarikei' had a great distinctness in quantitative traits, including the smallest leaf area, shortest inflorescence and fruit spike, smallest fruit and seed size, and thinnest pericarp. Cv. 'Kuching' showed the highest number of inflorescence (spike) per branch per node and the greatest number of node/feet of the stem, while cv. 'Yong Petai' had the longest inflorescence and

fruit spike, but the thinnest fruit spike. Cv. 'Semongok Perak' only showed significant variability in the conversion rate (from fresh to dried berry), with the lowest rate in both conversions to black (pericarp remained) and white (pericarp removed) peppercorns. The findings of this study enable efficient identification of black pepper cultivar in Malaysia. This is prerequisite toward implementation of the varietal regulation act in the country, at the same time serve as conservation information for the crop.

ACKNOWLEDGMENTS

The authors would like to thank Mr. Kevin Muyang Anak Tawie for assistance in morphological assessment via WinFOLIA image analysis system. Appreciation also goes to Mr. Wan Ambi and Mr. Juvian Jacob, research assistants of the Malaysian Pepper Board, for their excellent technical assistance in the field. This project is fully funded by the Malaysian Pepper Board, sourced from the Economic Planning Unit (EPU) of Malaysia.

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