



Faculty of Resource Science and Technology

**Phenotypic, Taxonomy, Phytochemical and Physiological Characterization
of Black Pepper (*Piper nigrum* L.) Cultivars in Malaysia**

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Phenotypic, Taxonomy, Phytochemical and Physiological Characterization of
Black Pepper (*Piper nigrum* L.) Cultivars in Malaysia

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulation of Universiti Malaysia Sarawak. Except where due acknowledgments have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.



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DEDICATION

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ABSTRACT

To improve the global market competitiveness of Malaysian black pepper, the quality of peppercorn needs to be strengthened. The varietal regulation at farm level is the prerequisite to enhance quality of peppercorn toward strengthening the marketing strategy. This project has comprehensively documented the morphology of ten important black pepper cultivars in Malaysia, namely ‘Semongok Aman’, ‘Kuching’, ‘Semongok Emas’, ‘Semongok Perak’, ‘Semongok 1’, ‘Nyerigai’, ‘India’, ‘Lampung Daun Lebar’, ‘Sarikei’ and ‘Yong Petai’. The key diagnosis characteristics are ‘Semongok Aman’ (Mucronate shaped leaf apex; High percentage of fruit set high, *ca.* 76%), ‘Kuching’ (High number of inflorescence (spike) per branch per node, *ca.* 58.67; High number of node/feet of stem, *ca.* 4.73), ‘Semongok Emas’ (Leaf colour of Green Group 137 Series; Fruit colour of Green Group 141 Series), ‘Semongok Perak’ (Low conversion rate for production of dried black peppercorn, *ca.* 36.12%; Low conversion rate for production of dried white peppercorn, *ca.* 24.21%), ‘Semongok 1’ (Cordate shaped leaf; Anthocyanin absent at shoot tip), ‘Nyerigai’ (Erect branching behaviour at mature vine stage), ‘India’ (Lanceolate shaped leaf; Light seed weight, *ca.* 0.14 g), ‘Lampung Daun Lebar’ (Oblique shaped leaf base; Blade length-width ratio low, *ca.* 1.52), ‘Sarikei’ (Small leaf area, *ca.* 36.90 cm²; Short inflorescence, *ca.* 6.06 cm; Short fruit spike, *ca.* 8.07 cm; Small fruit size, *ca.* 5.78 mm; Small seed size, *ca.* 3.84 mm; Thin pericarp, *ca.* 1.73 mm) and ‘Yong Petai’ (Long inflorescence length, *ca.* 12.75 cm; Long fruit spike length, *ca.* 17.07 cm; Thin inflorescence, *ca.* 2.90 mm). Phenetic analysis and Principle Component Analysis enlightened that ‘Semongok Aman’ and ‘Semongok 1’ have high distinctive value for identification while ‘Kuching’ and ‘Sarikei’ have high similarity, thus identification are more complicated. In phytochemical study, analysis showed ‘Semongok Aman’ and ‘Lampung Daun Lebar’ has higher piperine

(4.20% and 4.25% respectively) and essential oil content (2.50% and 2.24% respectively). At the same time, this analysis also proved the high pleasant aroma index of peppercorn of ‘Nyerigai’ (26.11), ‘Sarikei’ (25.20) and ‘Kuching’ (18.75). In physiological analysis where the evaluation is based on gas exchange performance and leaf area index, ‘Kuching’ was proven most productive, followed by ‘Nyerigai’ and ‘India’. Besides, the efficiency of artificial pollination has been improved also via floral biology study. This study suggests pollen collection for all the ten cultivars, ideally between 0400 h and 1400 h while the stigma was proven more receptive at stage where elongation and spreading of stigmata has occurred and the emergence and wide spreading of stigmata has been completed. In general, this study has ensured efficient *in situ* identification of black pepper cultivar base on the identification key produced. Besides, the commercial potential of each cultivar particularly in pharmacological and fragrance industry has also been identified. It was hoped the study outcome could be used as industry guideline to ensure sustainable development of Malaysian black pepper industry in future.

Keywords: Black pepper cultivar; floral biology study; Malaysia; morphological analysis; phytochemical study; physiological analysis; *Piper nigrum* L.

Pencirian Fenotip, Taxonomi, Fitokimia dan Fisiologi bagi Kultivar Lada Hitam (Piper Nigrum L.) di Malaysia

ABSTRAK

Untuk meningkat persaingan pasaran sedunia lada di Malaysia, kualiti lada perlu dipertingkatkan. Pengawalan varieti lada pada peringkat kebun merupakan langkah awal dalam menjamin kualiti lada seterusnya memperkasa strategi pemasaran lada hitam Malaysia. Projek ini telah mendokumentasi morfologi bagi sepuluh jenis kultivar lada utama di Malaysia secara menyeluruh, iaitu merangkumi 'Semongok Aman', 'Kuching', 'Semongok Emas', 'Semongok Perak', 'Semongok I', 'Nyerigai', 'India', 'Lampung Daun Lebar', 'Sarikei', dan 'Yong Petai'. Kekunci diagnosis bagi ciri-ciri morfologi adalah 'Semongok Aman' (Berbentuk mukronat pada puncak daun dan asas daun; Peratusan kejadian buah yang tinggi, kira-kira 76%), 'Kuching' (Bilangan tangkai bunga yang tinggi, kira-kira 58.67; Bilangan buku ruas dalam sekaki batang yang tinggi, kira-kira 4.73), 'Semongok Emas' (Warna daun jenis Green Group 137 Series); Warna buah jenis Green Group 141 Series), 'Semongok Perak' (Peratus pertukaran lada hitam yang rendah, kira-kira 36.12%; Peratus pertukaran lada putih yang rendah, kira-kira 24.21%), 'Semongok I' (Daun berbentuk kordat; Antosianin tidak hadir pada hujung pucuk), 'Nyerigai' (Cabang sisi bersifat menegak pada peringkat dewasa pokok), 'India' (Daun berbentuk lanseolat; bijih lada ringan, kira-kira 0.14 g), 'Lampung Daun Lebar' (Asas daun berbentuk serong; Nisbah panjang-lebar daun rendah, kira-kira 1.52), 'Sarikei' (Keluasan daun kecil, kira-kira, 36.90 cm²; Tangkai bunga pendek, kira-kira 6.06 cm; Tangkai buah pendek, kira-kira 8.07 cm; Buah bersaiz kecil, kira-kira 5.78 mm; Bijih lada bersaiz kecil, kira-kira 3.84 mm; Kulit buah nipis, kira-kira 1.73 mm) dan 'Yong Petai' (Tangkai bunga panjang, kira-kira 12.75 cm; Tangkai buah panjang, kira-kira 17.07 cm;

Tangkai bunga nipis, kira-kira 2.90 mm). Analisis finetik dan Analisis Prinsip Komponen telah membuktikan 'Semongok Aman' dan 'Semongok I' mempunyai nilai perbezaan yang tinggi untuk pengecaman manakala 'Kuching' dan 'Sarikei' mempunyai persamaan yang tinggi, jadi pengecaman lebih merumitkan. Dalam kajian fitokimia, analisis menunjukkan 'Semongok Aman' dan 'Lampung Daun Lebar' mempunyai kandungan piperine (4.20% dan 4.25% masing-masing) dan kandungan minyak pati (2.50% dan 2.24% masing-masing) yang tinggi. Pada masa yang sama, analisis ini juga membuktikan index aroma wangi yang tinggi pada bijih lada yang dihasilkan oleh 'Nyerigai' (26.11), 'Sarikei' (25.20) dan 'Kuching' (18.75). Dalam analisis fisiologi yang dinilai berdasarkan prestasi pertukaran gas dan index keluasan daun, 'Kuching' telah dibuktikan paling produktif, diikuti oleh 'Nyerigai' dan 'India'. Selain itu, keberkesanan pendebungaan artifisial juga dipertingkatkan melalui kajian biologi bunga. Kajian ini mencadangkan pengumpulan polen bagi semua kultivar sebaiknya dijalankan di antara 0400 jam dan 1400 jam manakala stigma dibuktikan lebih berdaya terima pada peringkat pemanjangan dan perkembangan stigmata telah bermula dan pada peringkat kemunculan dan perkembangan stigmata telah lengkap. Secara umumnya, kajian ini telah memastikan keberkesanan pengecaman kultivar lada hitam secara in situ. Selain itu, potensi komersial bagi setiap kultivar juga dikenalpasti, khususnya dalam industri farmakologikal dan pewangi. Diharapkan hasil kajian ini boleh dijadikan sebagai panduan industri, untuk memastikan kemapanan pembangunan industri lada hitam di Malaysia pada masa depan.

Kata kunci: Kultivar lada hitam; kajian biologi bunga; analisis morfologi; kajian fitokimia; analisis fisiologi; *Piper nigrum L.*; Malaysia.

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CHAPTER 1

INTRODUCTION

1.1 Background

Piper nigrum L., commonly called black pepper is the world renowned spice from the family of *Piperaceaea*. The peppercorn harvested from the plant not only widely used for food preparation that attributed by unique aroma and pungent properties, but also its pharmacological value (Krishnapura, 2009; Menghani et al., 2010; Meghwal & Goswami, 2012; Butt et al., 2013). The alkaloid compound, piperine, is the compound that contributes to the pungency of black pepper (Krishnapura, 2009; Gorgani et al., 2017) while the aroma is attributed to the volatile oil found in the peppercorn (Nirmala Menon & Padmakumari, 2005). The pharmacological potential that has been scientifically proven include immune modulatory, anti-carcinogenic, stimulatory, hepatoprotective and anti-inflammatory by Darshan and Doreswamy (2004); antimicrobial by Yang et al. (2002); antiulcer activities by Bai and Xu (2000); biotransformative effects and enhance the bioavailability of different drugs by Atal et al. (1985) and Wu (2007); protective effect against radiation by Raman and Gaikar (2002b); Increases pancreatic lipase activity and stimulates pancreatic amylase, trypsin, and chymotrypsin by Platel and Srinivasan (2000) and reduction of blood cholesterol, triglycerides, and glucose by Mueller and Hingst (2013).

The cultivar or cultivated variety of black pepper was believed to have derived from the wild one through domestication (Ravindran, 2000) in the center of origin, the

Malabar Island of India. The plant was introduced to Malaysia as early as 1840 by Hakka Chinese (Mullen, 1960) through Babylonian-Chinese sea trade that linked Malabar Coast and South East Asian ports (Ravindran, 2000). Sarawak and Johore region were the main cultivation area at the beginning stage. However, cultivation only focused in Sarawak in later years with more than 98% of country total production was from this state (Eng & Gumbek, 2007). The distribution in peninsula state and Sabah is limited mainly due to the farmer preference to others crop such as oil palm and rubber. Recent total black pepper planting hectare is around 13,000 hectare (Ibragimov et al., 2015), mainly distributed in rural area of Sarawak. Approximately 18,000 to 20,000 pepper farm families, with 68,000 to 70,000 people involved in pepper cultivation (Wong, 2016).

The most common black pepper cultivar is cv. 'Kuching', which is widely planted throughout Sarawak (Sim, 1991). However, Paulus (2007) reported three important cultivars in Malaysia, namely 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' were introduced to Malaysian farmers (Sim, 2007). In recent year, a manual entitled 'Pepper production technology in Malaysia' released by the Malaysian Pepper Board, reported 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, 2011a). However, there is no varietal restriction or regulation in black pepper planting in the past until nowadays. Thus, the diversity of the black pepper cultivar in Malaysia black pepper farm is unrevealed.

Since 1957, black pepper germplasm has been established in Malaysia for the purpose of breeding (Sim, 1979). Botanical description and documentation on the collections has been initiated during that time, particularly on the exotic *Piper* species. A total of 47 accessions of pepper varieties and 46 accessions of other species of *Piper* are being conserved in Semongok Agricultural Research Center, Sarawak in form of living plant (Sim, 2007). In recent year, a guideline for the conduct of tests for distinctness, uniformity and stability (DUS) of black pepper variety has been developed by Sim et al. (2009), foreseeing the important of varietal regulation to the industry. This guideline was developed for the purpose of new varietal registration. However, there is nil application to date.

Malaysian pepper production used to top world production however currently ranked fifth position behind Vietnam, Indonesia, India and Brazil (IPC, 2017). Despite the diminishing of black pepper industry since 1980th, Malaysian government continued to support the industry due to the promising ROI per hectare. This crop has higher per hectare return rate compared to others crop in Malaysia. The cost benefit analysis of black pepper that has been conducted by Mohammed et al. (2017) proving benefit cost ratio of 5.7 and internal rate of return of 61% per hectare planting of black pepper, indicating high viability of this industry. In other words, the black pepper cultivation can ensure lucrative return to farmer.

1.2 Problem Statements

Pests and diseases problem are common threats for pepper cultivation in the past until now. In recent year, the high labour cost and urbanization factor had further threatened the sustainability of pepper industry. From the world top pepper producer in 1970th and 1980th (Paulus, 2007) to the fifth producer in 20th century (IPC, 2017), Malaysian black pepper industry has undergone significant diminishing. In current black pepper industry scenario, Malaysia is unable to compete with the main pepper producers such as Vietnam and Indonesia, particularly on production volume. This is mainly due to labour constraint and urbanization factor. However, Malaysian black pepper is famous for its quality, attributed to pleasant aroma and high pungency. Thus, production of quality peppercorn has become Malaysian government aspiration in black pepper industry.

Beside breeding program to produce elite black pepper variety, a mono-varietal farm concept was believed able to strengthen the quality of peppercorn. A pre-requisite to implement the new policy is to comprehensively document all the important cultivars in Malaysia, which is currently lacking. The documentation by previous researcher on cultivated black pepper in Malaysia is less comprehensive, and none of the cultivars were registered under the National Crop List of Malaysia. More specifically, the phenotypical characteristics such as morphological, physiology and also phytochemical parameters for all the important black pepper cultivars in Malaysian have not been scientifically evaluated thus determination on the vigour of black pepper cultivar is unattainable.

1.3 Objectives

This project was conducted with aims,

- To comprehensively document and analyse the morphological characteristics of all the important black pepper cultivars in Malaysia. (Refer Chapter 3 & Chapter 4)
- To evaluate yield potential of all the black pepper cultivars based on morphological yield attributing characters and physiological assessment. (Refer Chapter 4 & Chapter 6)
- To evaluate phytochemical yield and aroma quality of peppercorn from all the black pepper cultivars. (Refer Chapter 5)
- To document the reproductive biology of all the black pepper cultivars toward improving efficiency of breeding program in future. (Refer Chapter 6)

1.4 Organization of the Thesis

This dissertation comprehensively documented phenotypic characteristics of ten black pepper cultivars in Malaysia, for the purpose of identification and breeding program. The ten cultivars are namely cv. 'Semongok Aman' (SA), cv. 'Kuching' (KCH), cv. 'Semongok Emas' (SE), cv. 'Semongok Perak' (SP), cv. 'Semongok 1' (S1), cv. 'Nyerigai' (NYE), cv. 'India' (IND), cv. 'Lampung Daun Lebar' (LDL), cv. 'Sarikei' (SAR), and cv. 'Yong Petai' (YP). The overall content consists of eight chapters as outlined below:-

Chapter 1 Introduction.

Chapter 2 Literature review.

Chapter 3 Taxonomy of *Piper nigrum* L. and its Cultivars in Malaysia: This chapter illustrated botanical description for the ten black pepper cultivars. At the same time, the distributions of cultivars were also documented.

Chapter 4 Morphological Characteristics of Black Pepper Cultivars: This chapter analysed morphological characteristics of ten black pepper cultivar by using phenetic analysis and principle component analysis to assist *in situ* identification of cultivars. The key diagnoses for the identification were highlighted in this study.

Chapter 5 Phytochemical Characterization of Black Pepper Cultivars: This chapter revealed the chemical content particularly the essential oil and piperine of peppercorn. The phytochemical yields for each cultivar were compared. Besides, peppercorn aroma analyses for each cultivar were conducted also.

Chapter 6 Physiological and Floral Biology of Black Pepper Cultivars: This chapter determined the yield performance of black pepper cultivars based on gas exchange performance. The gas exchange parameter includes photosynthesis rate, stomatal

conductance and transpiration rate. Besides, the leaf area index (LAI) was also evaluated. In this chapter, anther dehiscence, pollen viability and stigma receptivity of black pepper cultivars were studied also. This study aimed to improve efficiency of the breeding program in future. The superior characteristics of black pepper cultivars that have been identified in Chapter 4, 5 and 6 will be selected as donor plant in future breeding program.

Chapter 7 General discussions, conclusions and recommendations.

CHAPTER 2

LITERATURE REVIEW

2.1 Background of Black Pepper (*Piper nigrum* L.)

2.1.1 Taxonomic History

The earliest record of description on *Piper* L. was by Rheede in 1678. Linnaeus (1753) included 17 species of *Piper* in his Species Plantarum while Roxburgh (1832) recorded 17 species from India, Miquel (1848) illustrated seven wild *Piper* in his monograph, Wight (1853) described 16 species in 'Icones Plantarum Indiae Orientalis' and De Candolle (1866) included 52 species in his monographic work. In 1886, Hooker (1886) divided the genus of *Piper* into six sections, with black pepper categorized under section Eupiper. Even though Rao (1914) was first to carry out floristic studies at Western Ghats of Southern India, where all the species closely related to pepper originated but the taxonomic key was not constructed. Gamble (1925) then documented 13 *Piper* species including *Piper nigrum* L. with taxonomic keys in his Flora of Presidency of Madras. In later year, the taxonomic and biosystematic studies on *Piper* taxa occurring in Western Ghats of South India has also been reported by Rahiman et al. (1979), Rahiman (1981), Ravindran et al. (1987, 1990, 1992a, 1997), Ravindran (1991) and Ravindran and Nirmal Babu (1994).

In Malaysia, the earliest study on *Piper* taxonomy was by Ridley and Hutchinson (1967). The preliminary identification on the *P. nigrum* collection was based on Ridley's

Floral of the Malay Peninsular (Sim, 1979). In later year, Sim (1979) has reported botanical description on *P. nigrum* that includes dioecious and monoecious classification, branching behaviour, leaf, stem, and flower spike (Sim et al., 1996). However, botanical documentation on black pepper cultivars is still lacking. There is no comprehensive reference for strategic planning of black pepper industry.

2.1.2 Taxonomic Description

Apart from the original description made by Linnaeus (1753), taxonomic description on *P. nigrum* (black pepper) has been intensively documented by Blacklock (1954), Gentry (1955), Purseglove (1974), Rahiman et al. (1979), Rahiman (1981), Ravindran et al. (1987, 1990, 1992a and 1997), Ravindran (1991) and Ravindran and Nirmal Babu (1994). In Malaysia, Sim (1979) has initiated taxonomic documentation on Black pepper for her germplasm collection. General documentation of botanical as illustrated below has been intensively reviewed by these researchers.

Black pepper is a perennial climber, with adventitious roots emerging from the nodal region of main stem which adhere to the support, living or non-living. The supports provide columnar (branching) support for the vines that may reach 1.5 m in diameter. Stem at mature stage is thick and rough, branches numerous and multiple shoots emerged from nodal region. Naturally, the plant will climb ten or more meter, but trained vine for commercial purpose is restricted to approximately 5 m or lower. The plant is propagated by cuttings obtained from half matured stem, with 4-5 nodes per cutting. Even though the vine capable of flowering and fruiting at few month stages, but thinning of flower was

encouraged at young vine stage to ensure rapid growth of shoots and branches. The plant is allowed to fruit at around one and a half years after planting and their productiveness ends more than ten years. Black pepper is a year round crop, however peak flowering and fruiting season normally occur at early of the year. The maturity of berries achieved at month seventh from initiation of flowering. The berries are collected as soon as they turn orange; they are then dried in the sun before grounded into powdered form for consumption.

Leaves is thick, glabrous and shape much variable commonly are ovate, elliptic or elliptic-lanceolate with size varies from small to large. Leaf base is rounded, acute or cordate while apex is generally acuminate. Lateral ribs in two or three pairs, prominent, and the upper most one 1-2.5 cm above the leaf base with upper surface dark green to light green while lower surface pale green. Petiole is short or long, grooved and leaf margins wavy or even.

Spike is filiform and pendulous with young ones green or whitish green, or light purple and mature ones green, pale purple or pale yellow. Flower spike length varies much. Peduncle is glabrous and bracts oblong, decurrent, sessile with free upper margin, develop into a shallow cup in female spikes, rachis and bracts glabrous. Commonly, stamens emerge in two while the anther is ditheous with single carpel and ovary is spherical with no style. Stigma is 3-5 lobed and papillate.

Fruit is drupe type, commonly known as berry is green when young and changes to red on ripening. Seed mostly spherical shaped and pungent taste. Cultivated ones are bisexual while wild forms usually dioecious. The fruit is approximately 4 to 6 mm in diameter (depending on variety) and is picked just before it ripens. The fruits are separated from the stalk, and then dried to moisture content below twelve percent. Within two to three days of drying, the pericarp turns black. These become the black pepper corns. If the pericarp is removed before drying, the product is the white pepper corn.

Inflorescence of black pepper which commonly called the flower spike is a catkin type. It is borne on the vine at the node opposite the leaf. A flower spike may have 50 to 150 green apetalous florets, with only one to three mm in diameter. The flowers are usually hermaphrodite but may be unisexual, with staminate and pistillate flowers on the same plant or on separate plants.

2.2 Commercial Black Pepper Farm in Malaysia

Most of the black pepper farms in Malaysia are operated by smallholder, with planting volume ranged from few hundreds to few thousand vines (Figure 2.1). Systematic planting system (Figure 2.2) is practiced even though mostly managed by smallholder. Black pepper planting requires technical knowledge at every growth stage, especially the maintenance of matured vine. The productivity of black pepper farm mainly depends on quality of maintenance, from initiation stage until fruit bearing vine stage. Standard procedure to establish and maintain black pepper farm has been comprehensively reported by Paulus (2011b) in manual entitled 'Pepper Production Technology in Malaysia'.

Besides, documentation on pests (Megir, 2011) and diseases (Lily, 2011) of black pepper has also been done.

Black pepper is propagated vegetatively by cuttings. Cuttings are prepared from the terminal shoot, preferably with four to five nodes per cutting. Ideally, the cuttings need to sow in sand bin to promote adventitious root growth, ensuring high survival rate. Rooted cuttings normally are readied to be transferred after 1 month period of sowing in sand medium.



Figure 2.1: Big scale black pepper farm located at Sri Aman, Sarawak, Malaysia.



Figure 2.2: Vine uniformity contributed by systematic black pepper planting system in Malaysia.

Land preparation is the most crucial step in black pepper cultivation. In Malaysia farm scenario, most of the land used for black pepper cultivation is reclaimed from lallang infested land, old rubber stand and secondary forest (Paulus, 2011b). The stump and roots must be thoroughly removed before the initiation of planting. Good drainage is another important consideration in land preparation, especially low-lying area. Field drains of 60 cm depth and 60 cm width should be dug at every 5-6 row of vines and these are joined to the peripheral drainage system (Wong, 1986). The recommended planting distance is 1.8 m x 2.4 m (Wong, 1986) or 1.83 m x 2.44 m (Aznil & Yau, 1993). Before planting, mounds of loose soil (20 cm high and 50 cm wide) at the base are prepared. The dolomite application to reduce acidic condition on the mound is also encouraged before the planting (Paulus, 2011b). At three weeks after dolomite application, field planting of black pepper cutting can be carried out. Black pepper is a climber species, thus support is necessary to promote normal growth. Traditional black pepper cultivation in Malaysia, particularly Sarawak only used dead wood pole of Belian species (*Eusideroxylon zwageri*) as support. However, cement pole or life support like Quickstick (*Gliricidia sepium*), Dedap

(*Erythrina indica*) and Simpuh (*Dillenia suffruticosa*) can be used also (Paulus, 2011b). However, life support is less favoured by the planters due to tediousness of maintaining the support trees from time to time.

In the first two years of planting, the vine considered immature. Flowering is not encouraged at immature stage to ensure rapid branch proliferation. Normally, pruning procedure is practiced to achieve this. First pruning is about 6-8 months after planting, followed by second pruning approximately at month 12-14 after planting (Paulus, 2011b). Third pruning is encouraged at second year, approximately at the stage whereby the terminal shoot has reached the peak of the support (Paulus, 2011b). The pruning practice is important to ensure high productivity of vine in future. In fertilization application, N:P:K compound fertilizer with ratio 9:9:9 or 15:15:15 is more recommended at this stage of vine (Paulus, 2011b). Animal manure with appropriate quantity can be used also. Beside adequate fertilizer, the liming practice is necessary also to avoid soil acidity problem. Pests and diseases problem is less intensive at immature stage of black pepper vine. However, root diseases such as white root and *Fusarium* (slow decline) need to be cautioned. This problem can be avoided if land preparation was done properly. Good drainage and soil sanitation are two main factors contributed to plant vigour at this stage (Paulus, 2011b).

For mature black pepper vine (2 years old and above), nutrient management plays pivotal role in ensuring the vigour and productivity of vine. The recommended fertilizer is N:P:K, with ratio 12:12:17:2 + TE or 14:14: 21:2 + TE (Paulus, 2011b). Organic fertilizer is also encouraged at appropriate quantity. Organic fertilizer can ensure good soil

properties which is important to prolong vine longevity. In ensuring high productivity of mature vine, thinning practice via manual removal of excessive leaf is recommended. Occurrence of pests and diseases incident at mature black pepper vine is very common. The common pests include green pepper bug, pepper weevil, flea beetle, tinged bug, scale insect and cigarette beetle (Megir, 2011). Beside chemical control, thinning practices is encouraged to prevent the pest infestation. Common black pepper diseases include Phytophthora foot rot, black berry disease, slow decline, velvet blight, thread blight and pink disease (Lily, 2011). Preventive measure like appropriate land selection, use of disease free planting material, selection of disease tolerance cultivar, provide good drainage system to planting plot and controlled of vector pest are important to ensure the plant vigour (Lily, 2011). Most of the root diseases are devastating to black pepper vine, especially Phytophthora foot rot and slow decline (*Fusarium* sp.) (Lily, 2011).

2.3 Black Pepper Cultivars

2.3.1 Diversity

The cultivar of black pepper was believed to derive from wild one through domestication or selection in India (Ravindran, 2000). India was the place of origin for *Piper nigrum* L., thus has the richest diversity of cultivated black pepper. More than one hundred cultivars were reported by Ravindran (2000), with thirty eight were listed as important cultivars with distinctness on leaves, inflorescence and fruit spike morphology, productivity level and topography suitability. These cultivars include cv. 'Aimpiriyan', cv. 'Arakkulam', cv. 'Munda', cv. 'Arimulaku', cv. 'Balankotta', cv. 'Cheppukulamundi', cv. 'Cheriyakamiakkadan', cv. 'Cholamundi', cv. 'Chumala', cv. 'Doddigae', cv.

‘Jeerakamundi’, cv. ‘Kalluvally’, cv. ‘Karimundi’, cv. ‘Karimunda’, cv. ‘Karivilanchi’, cv. ‘Kottanadan’, cv. ‘Kurimalai’, cv. ‘Kuriyalmundi’, cv. ‘Kuthiravally’, cv. ‘Malamundi’, cv. ‘Malligesara’, cv. ‘Mundi’, cv. ‘Narayakkodi’, cv. ‘Nedumchola’, cv. ‘Neelamundi’, cv. ‘Perambramunda’, cv. ‘Perumkodi’, cv. ‘Poonjaran munda’, cv. ‘Thulamundi’, cv. ‘Thevanmundi’, cv. ‘Thommankodi’, cv. ‘Uddaghere’, cv. ‘Uthirankotta’, cv. ‘Vadakkan’, cv. ‘Valiakaniakkadan’, cv. ‘Vattamundi’ and cv. ‘Velliyaranmunda’. The less important cultivars population is about sixty three types as reported by Ravindran et al. (2000a). However, the cultivar derived from cross pollination between (Uthirankotta X Cheriyaakaniyakadan) called cv. ‘Panniyur 1’ is the most famous cultivar in India (Pradeepkumar et al., 2003).

Ravindran et al. (2000b) also listed important cultivars for others black pepper producing countries, namely Malaysia (cv. ‘Kuching’, cv. ‘Sarikei’ and cv. ‘Miri’), Indonesia (cv. ‘Bangka’, cv. ‘Banjarmasin’, cv. ‘Belantung’, cv. ‘Beng Kayang’, cv. ‘Chunuk’, cv. ‘Chunuk Kernuga’, cv. ‘Djambi’, cv. ‘Duantebei’, cv. ‘Kerenci’, cv. ‘Kernuga’, cv. ‘Korintji’, cv. ‘LDK’, cv. ‘LDL’, cv. ‘Palulauta’, cv. ‘Petaling 1’, cv. ‘Petaling 2’, cv. ‘Merefin’, cv. ‘Natar 1’, cv. ‘Natar 2’, cv. ‘LDLN 1’ and cv. ‘LDLN 2’), Sri Lanka (cv. ‘Ceylon’), Madagascar (cv. ‘Sel. IV. 1’ and cv. ‘Sel. IV. 2’) and Thailand (cv. ‘Antique’, cv. ‘Ban Keow’, cv. ‘Prang Thi’ and cv. ‘Prang Thi Bai Vick’). Whilst, Vietnam (the world biggest black pepper producer nowadays) claimed they have five important cultivars called cv. ‘Karimunda’, cv. ‘Panniyur 1’, cv. ‘Belantoeng’, cv. ‘Vinh Linh’ and cv. ‘Tieu Trung’ (Nguyen & Bui, 2011).

A manual entitled ‘Pepper production technology in Malaysia’ released by Malaysian Pepper Board, mentioned 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, 2011a). However, Sim (2007) reported the existence of other cultivars, namely cv. ‘Lampung Daun Lebar’ and cv. ‘Lampung Daun Kecil’. There is no comprehensive documentation on black pepper cultivars in Malaysia so far, thus the diversity is ambiguous.

2.3.2 Black Pepper Germplasm in Malaysia

2.3.2.1 Accessions Record

Germplasm is the foundation for any breeding programme. Pepper germplasm collection in Malaysia that stationed at Semongok Agricultural Research Center, Sarawak has been started since 1971 and intensified ten years later by Sim (1991). In the report, she mentioned that in early 1980th, the germplasm assemblage was reported with 20 varieties of pepper, 18 unidentified species of *Piper* and 98 accessions of unidentified species of *Piper*.

However, current germplasm population has decreased more than fifty percent due to government R&D policy transition in early 2000th. Plenty of accessions were reported to have died before handling over to Malaysian Pepper Board in 2015 (Chen et al., 2018). Currently, duplication of existing accessions was given priority. As of current, the documentation of the black pepper cultivars has not been comprehensively carried out.

2.3.2.2 Genetic Relatedness of Black pepper in Malaysia

In Malaysia, evaluation of genetic relatedness among black pepper (*Piper nigrum* L.) accessions using direct amplification of minisatellite-region DNA (DAMD) has been done by Ho et al. (2005). In their study, 43 black pepper accessions (Table 2.1) collected from Agricultural Research Centre (ARC), Semongok, Sarawak were evaluated using direct amplification of minisatellite-region DNA (DAMD) analysis. UPGMA dendrogram of DAMD similarities among 43 pepper accessions has been derived from the analysis (Figure 2.3).

Table 2.1: Black pepper accessions selected for DAMD analysis.

Accessions origin	Accessions code
Sarawak	K62 (Tissue cultured), KCH, PN118, PN125, PN126, PN127, PN129, PN131, PN135, PN136, PN137, PN138, PN139, PN140
Indonesia	PN36, PN37, PN74
India	PN28, PN30, PN79, PN81, PN94, PN99, PN100, PN103, PN113, PN114, PN115, PN116, PN119, PN123, PN124, PN132, PN133, PN134
Central America	PN107, PN108, PN109, PN110, PN111, PN112, PN106, PN117

Reference: Ho et al. (2005).

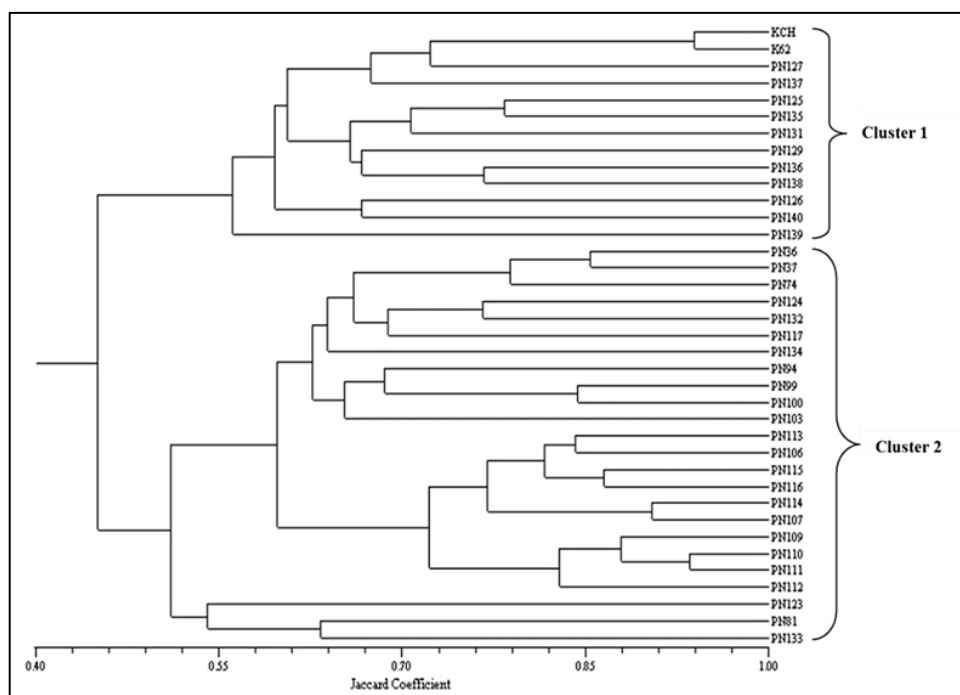


Figure 2.3: UPGMA dendrogram of DAMD similarities among 43 black pepper accessions. Sourced from Ho et al. (2005).

The paper reported that all black pepper accessions collected from Sarawak were grouped together in Cluster 1, while accessions collected from Indonesia, India and Central America were grouped together in Cluster 2 (Figure 2.3), based on the UPGMA dendrogram of DAMD. However, accession PN139 was distinctly different from the other accessions within Cluster 1. Three Indonesia black pepper accessions, namely PN36, PN37 and PN74 were grouped together and along with accessions collected from Central America, were found closer to Indian accessions than Sarawak accessions. Those accessions shared high genetic similarity to Indian black pepper accessions. This suggests that the black pepper genetic resources in Sarawak, Indonesia and Central America are most probably originated from the same ancestor. This is mainly due to India is the primary centre of diversity of pepper in the world (Pradeepkumar et al., 2001).

2.3.3 Quantitative and Qualitative Characteristics to Differentiate Morphology of Black Pepper Cultivars

Black pepper cultivars are very difficult to be distinguished when grown together at the field because most of them could have derived from similar ancestors (Pradeepkumar et al., 2001). The distinctive nature of these cultivars probably indicates domestications of cultivated black pepper from wild *Piper* species could have started long time ago (Ravindran & Nirma Babu, 1988; Ravindran, 1991).

For the morphological diagnosis of black pepper cultivars, both qualitative and quantitative traits should be taken into account. However, qualitative traits are more influential compared to quantitative traits (Ravindran, 1991; Ravindran et al., 1997a,b; Adetula, 2014; Ali Khan et al., 2015; Olakojo & Stephan et al., 2016) and have adopted principle component analysis to differentiate the characters between black pepper cultivars in India. In the analysis, eight principle components that comprised of both quantitative and qualitative characteristics, have been identified, namely component 1 (leaf size index; leaf length; leaf breath), component 2 (leaf thickness, abaxial and adaxial epidermal thickness), component 3 (leaf length-spike length ratio, spike length, peduncle length), component 4 (guard cell length and guard cell breath), component 5 (fruit size and fruit shape), component 6 (leaf shape and leaf base), component 7 (stomatal frequency and mesophyll thickness) and component 8 (Leaf shape and colour of runner shoot tip).

In Malaysia, although some morphological documentation on germplasm collection has been done by Sim (1979), but there is no scientific morphological analysis to ease varietal identification. However, a guideline for the conduct of tests for distinctness, uniformity and stability (DUS) of black pepper variety has been developed by Sim et al. (2009). This guideline was developed for the purpose of new varietal registration. This guideline has included both qualitative and quantitative characteristics for the evaluation, as listed below:-

- i. Shoot: anthocyanin coloration of orthotropic shoot tip
- ii. Shoot: internode length (of orthotropic shoot between 4th and 5th node from terminal bud)
- iii. Lateral branch: attitude (at the end of harvesting season at 1.5 m above ground)
- iv. Leaf : intensity of green colour of leaf blade
- v. Leaf: relief of upper surface
- vi. Leaf: blade shape (predominantly)
- vii. Leaf : blade length
- viii. Leaf : blade width
- ix. Leaf: blade length/width ratio
- x. Leaf: base shape (predominantly)
- xi. Leaf: petiole length
- xii. Flower spike: spike colour just after anthesis
- xiii. Fruit spike: number per lateral branch
- xiv. Fruit spike: length (most berries at Mature- green stage)
- xv. Fruit spike: length
- xvi. Fruit: percentage of fruit setting

- xvii. Fruit: diameter (mean of 100 well-developed green berries at mature-green stage)
- xviii. Fruit: weight (mean of 100 well-Developed green berries at mature-green stage)
- xix. Fruit: pericarp thickness (mean of 100 well-developed green berries at mature-green stage)
- xx. Fruit: piperine content (%)
- xxi. Oleoresin content (%)
- xxii. Volatile oil content (%)

2.4 Phytochemical of Black Pepper

2.4.1 Constituents and Compositions

The chemistry of black pepper, particularly the peppercorn or berry part is composed of 2 main components, namely, volatile (essential) oil and pungent compounds. The essential oils are responsible for their aroma while pungency attributed to piperine from oleoresin extract. Andri et al. (2010) reported Malaysian peppercorn constituted by Piperine with 5.8 wt % and essential oil with 1.7 v/w %. However, Tainter and Grenis (1993) reported the piperine content of peppercorns lies within the range of 3-8 g/100g, whereas the content of the minor alkaloids piperidine and piperettine have been estimated as 0.2-0.3 g/100g and 0.2-1.6 g/100g, respectively.

The most recent report on phytochemical constituent analysis of peppercorn is by Mohammad et al. (2016). The team mentioned a total of 55 bioactive compounds were identified in the methanolic extract of peppercorn. Gas chromatography-mass spectrometry (GC-MS) analysis has revealed the existence of the Propanedioic acid, dimethyl ester,

Bicyclo[3.1.1]heptane,6,6-dimethyl-2-methylene-,(1S), 3-Carene, Cyclohexene, 1-methyl-5-(1-methylethenyl)-,(R), 1,6-Octadien-3-ol,3,7-dimethyl, 2-Methyl-1-ethylpyrrolidine, 2-Isopropenyl-5-methylhex-4-enal, L- α -Terpineol, (R)-lavandulyl acetate, Pyrrolizin-1,7-dione-6-carboxylic acid, methyl(ester), 7-epi-cis-sesquisabinene hydrate, Phenol, 2-methoxy-4-(1-propenyl)-,(Z), Eugenol, Alfa. Copaene, Naphthalene,1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methyl), Epiglobulol, Caryophyllene, 1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl-,Z,Z,Z, α -ylangene, β -copaene, Cedran-diol,8S,13, Isocalamendiol, Cinnami acid , 4-hydroxy -3-methoxy-,{5-hydroxy-2-hydroxymethyl, (-)-Spathulenol, 1-Heptatriacotanol, Desacetylanquidine, 5-Isopropyl-2,8-dimethyl-9-oxatricyclo[4.4.0.0.(2,8)]decan-7-one, Estra-1,3,5(10)-trien-17 β -ol, Trans-1,2-Diamino-cyclohexane-N,N,N',N'-tetraacetic acid, Phytol, Piperidine,1-(1-oxo-3-phenyl-2-propenyl), Eicosanoic acid, 2-(acetyloxy-1-[(acetyloxy)methyl]ethyl ester, 2,5,5,8a-Tetramethyl-6,7,8,8a-tetrahydro-5H-chromen-8-ol, Z-5-methyl-6-heneicosen-11-one, 2H-1,2-Benzoxazine- 3-carbonitrile, 2-cyclohexyloctahydro-4a,8a-d, Indoxazin-4-one, 4,5,6,7-tetrahydro-3-undecyl, 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3 β ,5Z,7E), 3-Oxo-10(14)-epoxyguai-11(13)-en-6,12-olide, 7-[2-(Ethoxycarbonyl)-3 α ,5 β -dimethoxycyclopentyl -1]-heptanoic acid, 2H-Benzo[f]oxireno[2,3-E]benzofuran-8,(9H)-one,9-[[1,3-benzodio, Nalorphine, 2-Cyclohexen -3-ol-1-one, 2-[1-iminotetradecyl]-, Piperine, Fenretinide, 11-Dehydrocorticosterone,5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one,1,1a,1b,4,4a,7a,7b,17a-Ethyl-3 β -methoxy-17a-aza-D-homoandrost-5-ene-17-one, Bufo-20,22-dienolide m,14,15-epoxy-3,11-dihydroxy-, (3 β ,5 β ,11 α ,15,9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol, Retinal ,9-cis-, 6- β -Naltrexol, Piperine, Ursodeoxycholic acid, 5 α -Cholan-24-oic acid, 12 α -hydroxy-3,7-dioxo-,methyl ester and Stigmasterol. However, the composition percentage was not mentioned in the report.

According to Buckle (1985) and Lawrence (1985), the volatile fraction of pepper oil is dominated by monoterpene hydrocarbons (30-70%) such as R- and α -pinene, limonene, sabinene, myrcene, R-phellandrene, and α -3-carene. Sesquiterpenes such as R- and α -caryophyllene and α -farnesene make up 25-45% of the total essential oil content, whereas oxygen-containing sesquiterpenes such as caryophyllene oxide occur in amounts of 4-14%. In comparison by country, Jirovetz et al. (2002) reported major African phytochemical constituents were germacrone D (11.01%) and limonene (10.26%) while Nirmala Menon and Padmakumari (2005) identified sabinene (3.9-18.8%) and β -pinene (3.9-10.9%) for Indian black pepper. In Malaysia, Rezvanian et al. (2016) reported major black pepper oils constituents on local cultivars (specifically cv. 'Kuching'), such as Z, β -farnesene (10.57-40.61%), α -terpinene (10.01-22.23%), Z, β -ocimene (8.05- 13.97%). β -pinene (4.59-8.71%), α -pinene (2.27-5.33%), α -cubebene (1.14-4.61%), α -phellandrene (1.96-3.35%), 2-acetyl naphthalene (0.92-2.39%), cubenol (0.64-2.35%) and γ -gurjunene (0.84-2.31%). The volatile fraction of pepper oil is dominated by monoterpene hydrocarbons, sesquiterpenes, and oxygen-containing sesquiterpenes (Richard et al., 1971; Lawrence, 1985; Buckle et al., 1985; Rmili et al., 2014) with monoterpenes were the primary constituents in the oils that comprised about 57% of the total oil content. However, the results were inconsistent and only cv. 'Kuching' was evaluated in the study. The composition variation of essential oil can vary considerably relating to the cultivar, harvest year, and ripening stage (Richard et al., 1971; Lawrence, 1985).

2.4.2 Importance: Pungency, Aromatic and Medicinal Values

Piperine which derived from oleoresin is considered the principal pungent of black pepper (Parthasarathy et al., 2008) as it constitutes around 98% of the total alkaloids (Hirasa & Takemasa, 1998). The pungent compounds composted 5-9% of the alkaloids piperine and piperettine (Tewtrakul, 2000).

The other major component of peppercorn is volatile (essential) oil, with 1-2.5% of total composition (Tewtrakul, 2000). Essential oil of black pepper is normally extracted by steam distillation of dried peppercorn. It does not contain pungent compounds and only represents aromatic and odorous constituents. Essential oil consists mainly of terpenes, sesquiterpenes, and their derivatives that are responsible for the characteristic aroma, and imparts the identifying flavor and odor most closely associated with the plant itself. Black pepper essential oil is a mixture consisting of 90% hydrocarbons and 10% oxygenated terpenes and aromatic compounds (Ferreira et al., 1999). The hydrocarbon fraction is composed of monoterpenes (70-80%) and sesquiterpenes (20-30%), which appear to possess the desirable attributes of pepper flavor. Though oxygenated terpenes are relatively minor constituents, they contribute to the characteristic odor of pepper oil (Pino et al., 1990). Major aromatic compounds of pepper constituted by α - and β - pinene, limonene, myrcene, linalool, α -phellandrene, sabinene, β -caryophyllene, germacrene- D (Jagella & Grosch, 1999; Jirovetz et al., 2002; Nirmala Menon & Padmakumari, 2005; Wang et al., 2009). In later year, the aroma types that attributed to specific chemical compounds have been reported by Meghwal and Goswami (2012), such as α -terpineol (Floral), Acetophenone (Irritant, sharp), Hexonal (Green apple), Nerol (Fresh, Floral, Herbal),

Nerolidol (Mild spicy, Rooty), 1, 8-cineol (Camphory), Dihydrocarveol (Warm, Woody), Citral (Citrusy), α -pinene (Terperic, Oxidised) and Piperolnol (Sweet, Floral). Black pepper oil is highly valued in the fragrance industry and is used in high-grade toiletry products and the perfume industry as well as the flavour industries attributed to the unique aroma (Nair, 2011).

Besides the pungent and aromatic value, black pepper also rich in pharmacological value. The therapeutic properties of piperine are abundant. In ancient time, black pepper was used as a natural medicinal for alleviation of pain, chills, rheumatism, influenza, muscular pains, chills, and fevers. Parthasarathy et al. (2008) claimed consumption of pepper based tea help relieving migraine headaches, strep throat, poor digestion, and even coma. It was also used for enhancing the circulation of blood, increasing the flow of saliva, and stimulating appetite (Pruthi, 1993). In modern medicinal applications, Gorgani et al. (2017) has very comprehensive record on therapeutic properties of piperine according to findings by several of researchers, as listed at Table 2.2. However, piperine was proven slightly soluble in water (40 mg/L at 18 °C) (Vasavirama & Upender, 2014). The pharmaceutical activities of piperine are limited due to its low water solubility and poor dissolution because high concentrations of piperine can be toxic for the central nervous and reproductive systems (Veerareddy et al., 2004; Pachauri et al., 2015). Besides piperine, essential oils from black pepper have also been used as antibacterial, antifungal, antioxidant and insecticidal agents (Charles & Simon, 1990; Bakkali et al., 2008).

Table 2.2: Piperine therapeutic properties for modern medical applications.

No.	Types of therapeutic properties	References
1.	Immunomodulatory, anticarcinogenic, stimulatory, hepatoprotective, antiinflammatory	Darshan and Doreswamy (2004)
2.	Antimicrobial	Yang et al. (2002)
3.	Antiulcer activities	Bai and Xu (2000)
4.	Biotransformative effects; enhance the bioavailability of different drugs	Atal et al. (1985); Wu (2007)
5.	Protective effect against radiation	Raman and Gaikar (2002b)
6.	Increases pancreatic lipase activity and stimulates pancreatic amylase, trypsin, and chymotrypsin	Platel and Srinivasan (2000)
7.	Reduction of blood cholesterol, triglycerides, and glucose	Mueller and Hingst (2013)

2.5 Physiology of Black Pepper Vine

2.5.1 Gas Exchange Parameters

Photosynthetic rate, stomatal conductance and transpiration rate are main physiological characters that linked to plant growth performance. Photosynthetic performance has been proven positively correlated to yield and growth performance in

plant, likes grain crop (Long et al., 2006b), rubber (Conforto et al., 2015), cassave (De Tafur et al., 1997; El Sharkawky et al., 1993) and also in mung bean (Ignacimuthu & Babu, 1989). In black pepper, the positive correlation particularly on yield has been proven by Mathai (1983) and Krishnamurthy and Chempakam (2009). Beside net photosynthesis rate, the leaf stomatal conductance (Ishihara & Saito, 1987; Hirasawa et al., 1988; Kusumi et al., 2012; Golam Sarwar et al., 2013; Sabagh et al., 2017) and transpiration rate (De Wit, 1958; Smeal et al., 1991; Ben-Gal & Shani, 2002; Madrid, 2013) were among the physiological characters that related to plant productivity. Varietal difference is mainly contributed to variation in physiological performance of crop (Thorne, 1963; Dornhoff & Shibles, 1970; Hirasawa et al., 2010).

In recent years, the evaluation of gas exchange was done using a Portable Photosynthesis System (LI-COR LI-6400 Infrared Gas Analyzer, Lincoln, Nebraska, USA). The photosynthetic rate in black pepper varies from 1 to 4 μ moles in different varieties while stomatal conductance is in the range of 0.04 to 0.15 mol H₂O m⁻²s⁻¹ (Krishnamurthy & Chempakam, 2009) as reported by Indian researcher. The researcher further highlighted that the ideal physiological characters for black pepper ideotype is photosynthetic rate with minimum of 3.0-3.5 μ moles (Krishnamurthy & Chempakam, 2009) as observed in Indian cultivar such as cv. 'Panniyur 1', cv. 'OPKM', cv. 'HP 780', Acc. 1041 and Acc. 4129 while ideal stomatal conductance ranged 0.06-0.10 mol H₂O m⁻²s⁻¹ as observed in cv. 'Panniyur 1', cv. 'OPKM', Acc. 1041 and Acc. 4129. To ensure high productivity, carbon fixation >50 % of the fixed carbon should reach berries (Mathai, 1983).

In Malaysia, gas exchange measurement on black pepper via Portable Photosynthesis System was initiated by Sulok et al. (2014). The finding was reported in a manuscript entitled ‘Effect of Leguminous Cover Crop (*Calopogonium mucunoides*. Desv.) on Leaf N, Chlorophyll Content and Gas Exchange Rate of black Pepper (*Piper nigrum* L.). The paper mentioned that average photosynthesis rate of $10.47 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$, leaf stomatal conductance of $0.09 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$ and transpiration rate of $1.55 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ was observed in field grown vine of cv. ‘Kuching’. In 2018, gas exchange assessment via Portable Photosynthesis System on black pepper vine was conducted on various of kenaf-based composite posts. The pole, namely Belian, kenaf extrusion, kenaf pultrusion, kenafkrete and fibreglass were evaluated on suitability as alternative supports for black pepper in this project. Assessment showed photosynthesis rate ranged 10.02 to $12.58 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ while stomatal conductance ranged 0.24 to 0.39 $\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$ on black pepper vine (Khew et al., 2018).

2.5.2 Leaf Area Index (LAI) of Black pepper

Leaf area index (LAI) is derived by integrating the leaf area density over the canopy height and it corresponds to the one-sided leaf area per unit horizontal ground surface area (Watson, 1947). However, in later year Chen and Black (1992) proposed to define LAI as half the total developed area of leaves per unit ground horizontal surface area, on the basis of radiative transfer considerations. This definition is in accordance to work of Lang (1991) also, whereby this definition not only valid for flat broad leaves but also for needles and non-flat leaves. LAI assessment by using Plant Canopy Analyser, LAI-2000 (LI-COR Inc., Lincoln, NE, USA) is common nowadays. This indirect

measurement method is non-destructive and adopting principle of transmission of radiation through the canopy (Anderson, 1971; Ross, 1981) and based on a statistical approach to foliar element distribution and arrangement in the canopy (Jones, 1992).

The leaf area index (LAI) is an important trait that is related to canopy photosynthetic rate (Vaesen et al., 2001) or more specifically crop growth (Patil et al., 2018). The leafiness of the crop was reflected and leafiness in one way reflects the photosynthetic capability of the crop (Patil et al., 2018). The linear correlation between LAI and crop growth is generally supported by De Jong and Ryugo (1998); Friedrich and Fischer (2000); Fischer (2011); Guo and Sun, (2001); Hirose et al. (1997); Lakso and Flore (2003); Monteith (1977) and Senyigit et al. (2013). In black pepper, positive correlation between vine production and LAI has been reported by Varughese and Ghawas (1993) and Yap (2012). Yap (2012) in his paper entitled ‘Impact of Different Fertilization Methods on the Soil, Yield and Growth Performance of Black Pepper (*P. nigrum* L.)’ mentioned the LAI index was used as growth performance indicator in his black pepper nutrient management study. The LAI recorded in the study are 5.03 (chemical fertilizer), 3.81 (organic fertilizer), 5.62 (chemical and organic fertilizer) and 2.01 (control or zero fertilizer).

Besides, leaf area density is significantly correlated with radiation interception and light environment in the fruit zone (Dokoozlian & Kliever, 1995) that linked to vine productivity. Mathai (1983) reported that the upper part of the canopy with a relatively higher leaf area during the spike development period promotes the growth and

development of productive laterals and sustains relatively large number of spikes. For higher productivity in black pepper, exposure of higher leaf area to light, accumulation of high dry matter in the fruiting branches before spike initiation in June, higher carbon fixation capacity and higher translocation of photosynthates from leaves to the developing berries are very important. This is proven in high productivity cultivars like cv. 'Panniyur 1' and cv. 'Karimunda' in India, whereby more than 50% of the radioactive uptake occurred in the adjacent spikes and the berries of these cultivars also mobilized more photosynthates (Mathai, 1983). This suggest that, the black pepper vine need sufficient lateral branches with optimum leaf area to absorb maximum light, to ensure better yields. Krishnamurthy et al. (2000) further emphasized that 75% leaf area reduction of subtending leaves may significantly reduce berry set percentage.

2.6 Floral Biology of Black Pepper

2.6.1 Flower Morphology and Development

Sim (1979) and Chen et al. (2009) have comprehensively been reported the morphology of flower and its development in Malaysia. Black pepper flowers are minute, sessile and borne in axil of ovate fleshy bracts, while perianth is absent. In bisexual flower, two stamens with two conspicuous anthers emerge from the sides of the ovary. The flowers are usually hermaphrodite with staminate and pistillate flowers on the same plant or on separate plants. Sim (1979) has described six stages of flower development in black pepper as illustrated at Table 2.3.

Table 2.3: Stages of black pepper flower development.

Stages	Descriptions
Stage 1: First appearance of stigmata.	Three or four succulent and opaque stigmata rays protruding from the tip of a bract. If view with naked eye, they appear like small dots at the tip of the bract.
Stage 2: Elongation and spreading of stigmata.	Three to four stigmata are visible. They elongate after 2 days of emergence and the top parts are spread out. They appear like curved finger-like structure emerging from the bract.
Stage 3: Complete emergence and wide spreading of stigmata.	Four stigmata are well spread. They are whitish crystal in colour and appear like star when viewed with naked eyes. The stigmata curve towards the ovary. The ovary is light green in colour.
Stage 4: Withering of stigmata.	Browning stigmata surface visible. The ovary is green colour, slightly darker compared to stage 3.
Stage 5: Emergence of anthers.	Stigmata become black. Anthers appear on one side of the ovary which has protruded from the sustaining bract. The ovary is green in colour.
Stage 6: Anthesis.	Stigmata are black in colour. Stamen appears on both sides of the ovary. Anthers dehiscence occurred. Ovary has risen from the sustaining bracts and is green in colour.

Reference: Sim (1979).

2.6.2 Inflorescence Morphology and Development

Inflorescence of black pepper is catkin type with 50 to 150 rather inconspicuous yellowish green apetalous florets, sized only 1-3 mm in diameter (Ravindran et al., 2000c). Sim (1979) has described nine stages of inflorescence development (Table 2.4) as illustrated below:

Table 2.4: Stages of black pepper inflorescence development.

Stages	Descriptions
Stage 1: Emergence of flower spike from the sheath.	Day 0: The spike is yellowish green in colour and pointed horizontally.
Stage 2: First appearance of stigmata.	Day 16: The spike is green in colour and pointed downward.
Stage 3: Half of the flower spike covered with visible stigmata.	Day 19
Stage 4: First flower with stigmata well spread.	Day 20
Stage 5: Half of the spike with well spread stigmata.	Day 22
Stage 6: Appearance of the first pair of anthers.	Day 27: The colour of spike turned from green to pale green.
Stage 7: Anthesis of the first pair of anthers.	Day 29: The spike is yellow in colour.
Stage 8: First appearance of the second pair of anthers.	Day 30: The spike remains yellow in colour and berry formation initiated.
Stage 9: Anthesis of the second pair of anthers.	Day 32

Reference: Sim, 1979.

2.6.3 Fruit Set and Development

The fully developed fruits only come with successful pollination, fertilization and development while the underdeveloped fruit occurred when fruit development retarded midway (Martin & Gregory, 1962). The berry maturity period ranged 6-8 months, varied by variety and climate factor (Ravindran et al., 2000e). Fruit ripening of black pepper is uneven, either among fruit spike of vine or among berries of same fruit spike. Sim et al.

(1996) has characterized black pepper fruit development into twelve stages by evaluation on the size, colour and firmness of the fruit. The description as outlined at Table 2.5.

Table 2.5: Stages of black pepper fruit development.

Stages	Descriptions
Stage 1: Withering of stigma.	After anthesis, the stigmas curved towards the ovary, turned brown and eventually blacken.
Stage 2: Emergence of fruit.	Within the first week after anthesis, the ovary enlarged and rose from the sustaining bracts. Young fruits emerged as small green pinheads.
Stage 3: Fruit half covered in the subtending bracts.	The young fruit is still half covered by the subtending bracts. This stage initiated at one to two weeks after anthesis and lasted within a week.
Stage 4: Early watery stage.	The fruit had emerged to about two to three times the length of the bract. It was fragile and juicy. It could be pressed open very easily. The exocarp, mesocarp and perisperm were light green in colour. This stage started from the second or third week and lasted till the sixth week after anthesis.
Stage 5: Late watery stage.	The fruit grew in size, with uniform close distance, near touching each other. It was still fragile and could readily be pressed open. The juice was watery and cloudy. The exocarp and mesocarp were green in colour. This stage started from the sixth and lasted till the tenth week after anthesis.
Stage 6: Milky stage.	The fruit continued to grow in size. Exocarp and mesocarp were remaining light green. The perisperm cells were filled with more starch. The fruit could easily be pressed open and the juice was white or a little bit milky in colour. This starch filling stage lasted for about ten weeks starting from the tenth week after anthesis.

Table 2.5 continued

Stage 7: Soft dough stage.	The fruit sized almost optimum, had become firm and could not be pressed open easily. The exocarp and mesocarp remained green. Increase of starch in the perisperm could be seen from the longitudinal section of the fruit, as well as from the chemical analysis. This stage started from the 20 th to 21 st week after anthesis and lasted for two to four weeks.
Stage 8: Early hard dough stage.	The size of fruit continues increased and the colour of the exocarp and mesocarp was green. The fruit become firm and pretty difficult to press open. The area of the perisperm continues filled with starch. This stage started at 22 to 24 weeks after anthesis and lasted for two to four weeks.
Stage 9: Late hard dough or mature green stage.	Full sized fruit achieved and texture becomes very hard. The exocarp is still green in colour whereas the mesocarp showed tint of yellowish green. The endocarp layer still joined the mesocarp firmly and could not be separated neatly. This stage started at the 25 th week after anthesis and lasted for about two weeks.
Stage 10: Breaker stage.	Colour change could be seen on the exocarp. The fruit approaching ripen stage. The colour of mesocarp had also changed to greenish yellow. The endocarp layer could be separated pretty neatly from the mesocarp. This stage started at about 26 weeks after anthesis and lasted for two to four weeks.
Stage 11: Early ripe stage.	The colour of the fruit either greenish yellow, yellow or greenish orange, lack uniformity. The mesocarp has soft texture and the endocarp could be separated neatly from the mesocarp. This stage started at 27 th to 28 th week after anthesis and lasted for two to three weeks.
Stage 12: Ripe stage.	The fruit could be yellowish orange, bright orange or reddish orange in colour. The mesocarp had become very soft, easily rotten. This stage occurred at about 28 to 29 weeks after anthesis and lasted for two to three weeks.

Reference: Sim et al., 1996.

2.6.4 Pollination Biology of Black Pepper

2.6.4.1 Anther Dehiscence, Stigma Receptivity and Pollen Viability

De Waard and Zevan (1969) first mentioned the dehiscence of black pepper flower usually takes place between 12.00 p.m and 2.00 p.m during day time when relative humidity of 60 percent is attained and at temperature of 32°C. However, anther dehiscence is influenced to a very great extent by temperature and relative humidity (Martin & Gregory, 1962). Whilst, on stigmata of black pepper, the full emergence stigma may be receptive for 10 days, with peak receptivity at 3 to 5 days (Purseglove, 1974) or 3-9 days (KAU, 1978). However, Sim (1979) reported 16 days for emergence of full stigma from inflorescence and 22 days for appearance of receptive stigma specifically on cv. 'Kuching', for her observation in Malaysia. However, in later year, Chen (2011) revealed no significant difference at any stages of stigma receptivity, including the early emergence stage. This is supported by Helen and Lauren (2002) that reported stigmatic age is uncorrelated with receptivity and Wetzstein and Sparks (1989) that mentioned the stigma normally remains receptive at any stage before receiving pollen.

According to Ravindran et al. (2000c), pollen size of black pepper is minute, diameter along the equatorial axis ranges from 9.5-13.0 µm and along the polar axis between 7.0-11.0 µm. This is in agreement with Iljas (1960) and Martin and Gregory (1962), who reported pollen grains with mean diameter approximately 10 µm, regardless of cultivars. The pollen was proven released in gelatinous masses to pollinate receptive stigmas of other flowers (Ravindran et al., 2000c). However, information on pollen viability of black pepper has not comprehensively been reported so far. There is a need to

investigate the viability on different pollen stages and life span of pollen viability for all the selected cultivar to improve efficiency of conventional breeding.

2.6.4.2 Pollination Ecology

Pollination ecology of black pepper is complicated due to sensitive to climatic factor. The emergence of flowers within inflorescence is uneven (Gentry, 1955), where the flowering begins at the base of the inflorescence and continues to the tip over a seven to eight day period. The hermaphrodite flower is reported protogynous, with two to three stamens appearing at the base of the ovary only after the stigma matured (McGregor, 1976). The protogynous nature of black pepper flowers has separated emergence of male and female flower by 1-14 days (Ravindarn et al., 2000c), encouraging cross pollination of flower.

Cross-fertilization was the main pollination mechanism of black pepper (McGregor, 1976). McGregor (1976) explained self-pollination of the floret is prevented effectively by the protogynous nature of *P. nigrum*, but he apparently referred to transfer of pollen between flowers on a plant rather than between plants. However, Martin and Gregory (1962) and Sasikumar et al. (1992) have contradicted view in the mode of pollination of black pepper. They suggest self-pollination between flowers on a plant was undoubtedly the rule. But their statement was not scientifically proven.

In pollination agent, rain drop was proven the main pollinating agent of *P. nigrum* by Anadan (1924) and Menon (1949). De Waard and Zevan (1969) stated that although the pollen was in gelatinous masses, a light rain would break up these masses, and then the pollen grains would be dispersed and finally caught in the papillae of the stigma. They concluded that water is a medium for pollen distribution. This is supported by McGregor (1976) also. He reported that rain drops or water help in scattering pollen grains in different directions, either wash down the pollens to lower spikes or carry them to neighbouring vines. At the same time, McGregor (1976) also attributed the transfer of pollen to wind and insect such as ants and bees. However, Martin and Gregory (1962) stated that no insect pollinator visited the spikes during anthesis also ensured successful fruit set, in their observation. This is supported by Anadan (1924). He mentioned that arrival of bees to inflorescence failed to promote fruit set if the inflorescence was protected from rain. Free (1970) has different view on the pollination mechanism of black pepper. He stated that pollination was the result of geitonogamy (gravity) that was aided by rain or wind which is in accordance to observation by Anandan (1924).

Gentry (1955), was the first to report about the possible occurrence of apomixes in black pepper. He found fruit set occurred on male sterile cv. 'Uthiranotta' without exposure to pollen. Apomixes was common in wild *Piper* species (Ravindran et al., 2000d). Examples are the *P. longum* (Ravindran et al., 2000b) and *P. chaba* (Ravindran et al., 2000b) that were growing in the germplasm conservatory of the Indian Institute of Spices Research, IISR. However, in the case of cultivars, which are mostly bisexual, apomixes are difficult to be verified. In a study by Sasikumar et al. (1992), emasculation and bagging done on cv. 'Karimunda' resulted in 7.0 percent spike achieving successful

fruit setting with 8.2 percent for each spike, indicating the occurrence of apomixes to a small extend. The team further elaborated that apomixes may be the mechanism of fruit setting in predominantly female cultivars such as cv. 'Karuvilanchy', cv. 'Uthirancotta' and cv. 'Cholamundi'. However, in Malaysia, Chen (2013) concludes that apomixes do not occurred in black pepper. In his study, the inflorescence prevented from all the possible pollinator resulted to zero fruit set, indicating apomixes is not possible.

CHAPTER 3

TAXONOMY OF *PIPER NIGRUM* L. AND ITS CULTIVARS IN MALAYSIA

3.1 Introduction

Rheede (1678) was the first to record botanical description on *Piper*. Linnaeus (1753) later included 17 species of *Piper* in his *Species Plantarum*. Subsequently, the taxonomy of *Piper* was studied by Roxburgh (1832), Miquel (1848), Wight (1853), De Candolle (1869) and Rao (1914) in later years. However, Rahiman et al. (1979), Rahiman (1981), Ravindran et al. (1987), Ravindran et al. (1990), Ravindran (1991), Ravindran et al. (1992a), Ravindran & Nirmal Babu (1994) and Ravindran et al. (1997) have more comprehensive study, up to taxonomic key level that enable identification of species.

In Malaysia, the earliest study on *Piper* taxonomy was by Ridley and Hutchinson (1967). The preliminary identification on the collection was based on Ridley's *Floral of the Malay Peninsular* (Sim, 1979). In later year, Sim (1979) in her morphological study on *Piper nigrum* L. has included dioecious and monoecious classification, branching behaviour, leaf, stem, and flower spike. Sim et al. (1996) also studied fruit development of three selected cultivars, cv. 'Kuching', cv. 'Semongok emas' and cv. 'Hybrid 10' while Chen (2011) reported on the floral biology study of *P. nigrum*, and the apomixis study, with regard to floral morphology. In 2018, a phenetic analysis on important cultivars in Malaysia has been reported by Chen et al. (2018). The paper enlightened morphological distinctness of each cultivar based on ANOVA test and Principle Component Analysis.

Besides, a guideline for the conduct of tests for distinctness, uniformity and stability on black pepper (*Piper nigrum* L.) has been developed by Sim et al. in 2009 but no application to date.

The cultivar of black pepper was believed to have derived from wild one through domestication or selection (Ravindran, 2000) in India. Ravindran (2000) in a book entitled 'Black pepper (*Piper nigrum* L.)' that was published by Harwood Academic Publishers has described 38 cultivars of black pepper in India, with description focused on leave shape, spike length, percent fruit set, topography suitability and yield performance. The team also listed important cultivars for others countries, like Malaysia (cv. 'Kuching', cv. 'Sarikei' and cv. 'Miri'), Indonesia (cv. 'Bangka', cv. 'Banjarmasin', cv. 'Belantung', cv. 'Beng Kayang', cv. 'Chunuk', cv. 'Chunuk Kernuga', cv. 'Djambi', cv. 'Duantebei', cv. 'Kerenci', cv. 'Kernuga', cv. 'Korintji', cv. 'LDK', cv. 'LDL', cv. 'Palulauta', cv. 'Petaling 1', cv. 'Petaling 2', cv. 'Merefin', cv. 'Natar 1', cv. 'Natar 2', cv. 'LDLN 1' and cv. 'LDLN 2'), Sri Lanka (cv. 'Ceylon'), Madagascar (cv. 'Sel. IV. 1' and cv. 'Sel. IV. 2') and Thailand (cv. 'Antique', cv. 'Ban Keow', cv. 'Prang Thi' and cv. 'Prang Thi Bai Vick'). However, these cultivars only differentiated by yield performance evaluation. The world biggest black pepper producer nowadays, i.e. Vietnam claimed it has five important cultivars called cv. 'Karimunda', cv. 'Panniyur 1', cv. 'Belantoeng', cv. 'Vinh Linh' and cv. 'Tieu Trung' (Nguyen & Bui, 2011). A manual entitled 'Pepper production technology in Malaysia' that released by Malaysian Pepper Board in 2011, mentioned 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, 2011a). However, Sim (2007) reported the

existence of other cultivars, namely cv. 'Lampung Daun Lebar' and cv. 'Lampung Daun Kecil'.

This study not only reviewed botanical description of *Piper nigrum* L. in Malaysia but also conducted comprehensive observation on important cultivars. The morphological description, diversity and distribution of the cultivars were documented.

3.2 Materials and Methods

Extensive fieldwork (Table 2.6) was undertaken in cooperation with extension staff of Malaysian Pepper Board, to cover all the possible black pepper cultivation area within Malaysia to come out with a representative botanical description of black pepper cultivar in Malaysia.

Malaysian Pepper Board has developed an APP called E-Pekebun (*Sistem Maklumat Pekebun Lada*) that enables documentation on all the pepper planting area throughout Malaysia from time to time. In this study, the APP has assisted identification on farm location for pepper cultivars distribution and taxonomy study. All the farm that have been recorded in this system were accessed for verification on the cultivars. In each farm, two extension officers were instructed to tag the entire pepper vine of the farm and photos for parts like leaf, shoot tip, inflorescences and fruits were taken simultaneously for each vine. The cultivar identification was preliminary done via observation on photos provided by the officers. After examination and confirmation, the branches (with leaf, inflorescence

or fruit spike) of identified pepper vine were then collected for preparation of specimen. Only one specimen per farm was prepared. This procedure was repeated until all the targeted farms completed. The details of the fieldwork coverage and outcome as illustrated at Table 2.6.

For further verification, pot experiment was established based on Completely Randomized Design (CRD) (Figure 3.1) that consisted of a total of 50 potted vine, 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 pots were arranged 1 m x 1 m (between vine x between row). The data collection was initiated at 2 years old vine. In this study, description by previous black pepper botanist particularly Ravindran et al. (1997) and Sim (1979) were comprehensively reviewed. Meanwhile, a botanical drawing and photography documentation were produced as evidence to the description. Vouchers (dried specimens) for the ten cultivars have also been prepared and deposited at herbarium that located at Forest Department Sarawak and the duplicates at Malaysia Pepper Board, Kuching.

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 3.1: Completely Randomized Design (CRD) pot arrangement for the ten treatments. 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.

Table 2.6: Fieldwork area coverage and observations.

Fieldtrip schedule	Locations	Cultivars observed	Collection number	Collector
February 2018	State: Sarawak	cv. ‘Semongok Aman’	CYS-01-1, CYS-01-2, CYS-01-3, CYS-01-4, CYS-01-5, CYS-01-6, CYS-01-7, CYS-01-8, CYS-01-9, CYS-01-10, CYS-01-11, CYS-01-12, CYS-01-13, CYS-01-14	Chen.Y.S
– June 2018	Kuching Division- Bau, Sematan, Padawan, Mambong, Tarat, Matang; Samarahan Division- Muara Tuang, Simunjan and Batang Sadong; Serian Division- Tarat, Tebakang, Kedup, Bukit Semuja, Tedebu and Gedong; Sri Aman Division- Balai Ringin, Lachau, Lubok Antu, Bukit Begunan and Batang Lupar; Betong Division- Saratok, Layar, Saribas and Bukit Saban; Bintulu Division- Niah and Subis; Miri Division- Marudi and Sibuti; Sibuan Division- Dalat, Selangau, Kanowit and Lanang; Sarikei Division- Julau and Repok; Limbang Division- Lawas, Batu Danau and Bukit Kota; Bintulu Division- Tatau; Mukah Division- Dalat; Limbang Division- Lawas and Ba’Kelalan; Kapit Division- Song, Bukit Garam and Belaga; Miri Division- Marudi	cv. ‘Kuching’	CYS-02-1, CYS-02-2, CYS-02-3, CYS-02-4, CYS-02-5, CYS-02-6, CYS-02-7, CYS-02-8, CYS-02-9, CYS-02-10, CYS-02-11, CYS-02-12, CYS-02-13, CYS-02-14, CYS-02-15, CYS-02-16, CYS-02-17, CYS-02-18, CYS-02-19, CYS-02-20, CYS-02-21, CYS-02-22, CYS-02-23, CYS-02-24, CYS-02-25	Chen.Y.S
		cv. ‘Semongok Emas’	CYS-03-1, CYS-03-2, CYS-03-3, CYS-03-4, CYS-03-5, CYS-03-6, CYS-03-7,	Chen.Y.S
		cv. ‘Semongok Perak’	CYS-04-1, CYS-04-2, CYS-04-3, CYS-04-4, CYS-04-5	Chen.Y.S
		cv. ‘Semongok 1’	CYS-05-1, CYS-05-2, CYS-05-3, CYS-05-4, CYS-05-5	Chen.Y.S
		cv. ‘Nyerigai’	CYS-06-1, CYS-06-2, CYS-06-3,	Chen.Y.S

Table 2.6 continued

				CYS-06-4, CYS-06-5	
		cv. 'India'		CYS-07-1, CYS-07-2, CYS-07-3, CYS-07-4, CYS-07-5, CYS-07-6, CYS-07-7	Chen.Y.S
		cv. 'Lampung Daun Lebar'		CYS-08-1, CYS-08-2, CYS-08-3, CYS-08-4, CYS-08-5	Chen.Y.S
		cv. 'Sarikei'		CYS-09-1, CYS-09-2, CYS-09-3, CYS-09-4, CYS-09-5	Chen.Y.S
		cv. 'Yong Petai'		CYS-10-1, CYS-10-2, CYS-10-3, CYS-10-4, CYS-10-5, CYS-10-6	Chen.Y.S
July 2018	State: Johor Kukup, Pontian, Kulai, Kluang, Batu Pahat; Tebrau; Parit Sulong; Kota Tinggi; Yong Peng, Bakri and Lok Heng.	cv. 'Kuching'		CYS-02-15, CYS-02-16, CYS-02- 17, CYS-02-18, CYS-02-19	Chen.Y.S
August 2018	State: Sabah Inanam; Pensiangan; Kimanis; Ranau; Keranaan.	cv. 'Semongok Aman'		CYS-01-15, CYS-01-16	Chen.Y.S
		cv. 'Kuching'		CYS-02-20, CYS-02-21, CYS-02- 22, CYS-02-23, CYS-02-24	Chen.Y.S
September 2018	State: Terengganu Ajil; Marang and Dungun	cv. 'Semongok Aman'		CYS-01-17, CYS-01-18	Chen.Y.S
		cv. 'Kuching'		CYS-02-25, CYS-02-26, CYS-02- 27	Chen.Y.S
		cv. 'Semongok Emas'		CYS-03-16, CYS-03-17	Chen.Y.S

Table 2.6 continued

November 2018	State: Kelantan Jeli and Pasir Puteh	cv. 'Kuching'	CYS-02-28, CYS-02-29	Chen.Y.S
	State: Kedah Kulim, Belantok, Baling, Jerai and Sik	cv. 'Kuching'	CYS-02-30, CYS-02-31, CYS-02-32, CYS-02-33, CYS-02-34	Chen.Y.S
	State: Pulau Pinang Bukit Mertajam.	cv. 'Kuching'	CYS-02-35	Chen.Y.S
	State: Negeri Sembilan Mambau; Seremban and Jelebu.	cv. 'Kuching'	CYS-02-36, CYS-02-37, CYS-02-38	Chen.Y.S
	State: Pahang Lipis and Rompin.	cv. 'Kuching'	CYS-02-39, CYS-02-40	Chen.Y.S
	State: Selangor Subang Jaya.	cv. 'Kuching'	CYS-02-41	Chen.Y.S
	State: Perak Gopeng; Canning; Batu Gajah; Kampar; Sungai Siput and Tambun.	cv. 'Kuching'	CYS-02-42, CYS-02-43, CYS-02-44, CYS-02-45, CYS-02-46	Chen.Y.S
	State: Kelantan Jeli and Pasir Puteh	cv. 'Kuching'	CYS-02-47, CYS-02-48	Chen.Y.S

3.3 General Botanical Descriptions of *Piper nigrum* L.

Habit - Woody perennial climber bearing essential oil, with epiphytic ivy-like root emerge from the nodal region of main stem; root adhere to death or living support pole; shoot growing vertically to develop vigorous vine; branching laterally where flowering and fruiting occur.

Stem- Nodal stem with internode ranged from 8-13 cm when mature; old stem rough with filiform cracking bark, young stem with thick cuticle dull dark green; nodes commonly multilacunar, single to multiple branching from node; shoot tip purplish green (anthocyanin present) or whitish green (anthocyanin absent).

Leaves- Simple, alternate, petiolate, entire, shape commonly lanceolate-ovate, lanceolate or ovate; size much variable, small to large; base rounded, acute, ovate or oblique; tip commonly obtuse, acute or rounded; leaf margin wavy or even; upper surface dark green to light green, lower surface dull green.

Inflorescence- Catkin type, acropetal, length much variable ranged from 6 cm to 13 cm; green, whitish green or light yellow.

Flower- Minute, bracteates, naked, bisexual or unisexual, hypogynous, protogynous.

Perianth- Absent

Androecium- Stamens 2; anthers dithecal.

Gynoecium- Carpels 1; ovary spherical; orthotropous ovule, 1 in each locule; style absent; stigma 2-5 lobed, papillate.

Fruits- Drupe; light green when young, dark green at mature, yellow and eventually red on ripening.

Seeds- Shape spherical, some slightly pointed at hilum or remains of style region, seed coat with 10-12 whitish thread-like stripes from hilum to remains of style; small, diameter 3-5 mm; embryo minute, near to hilum.

The botanical drawing and photography documentation of black pepper is as illustrated at Figure 3.2 and Figure 3.3.

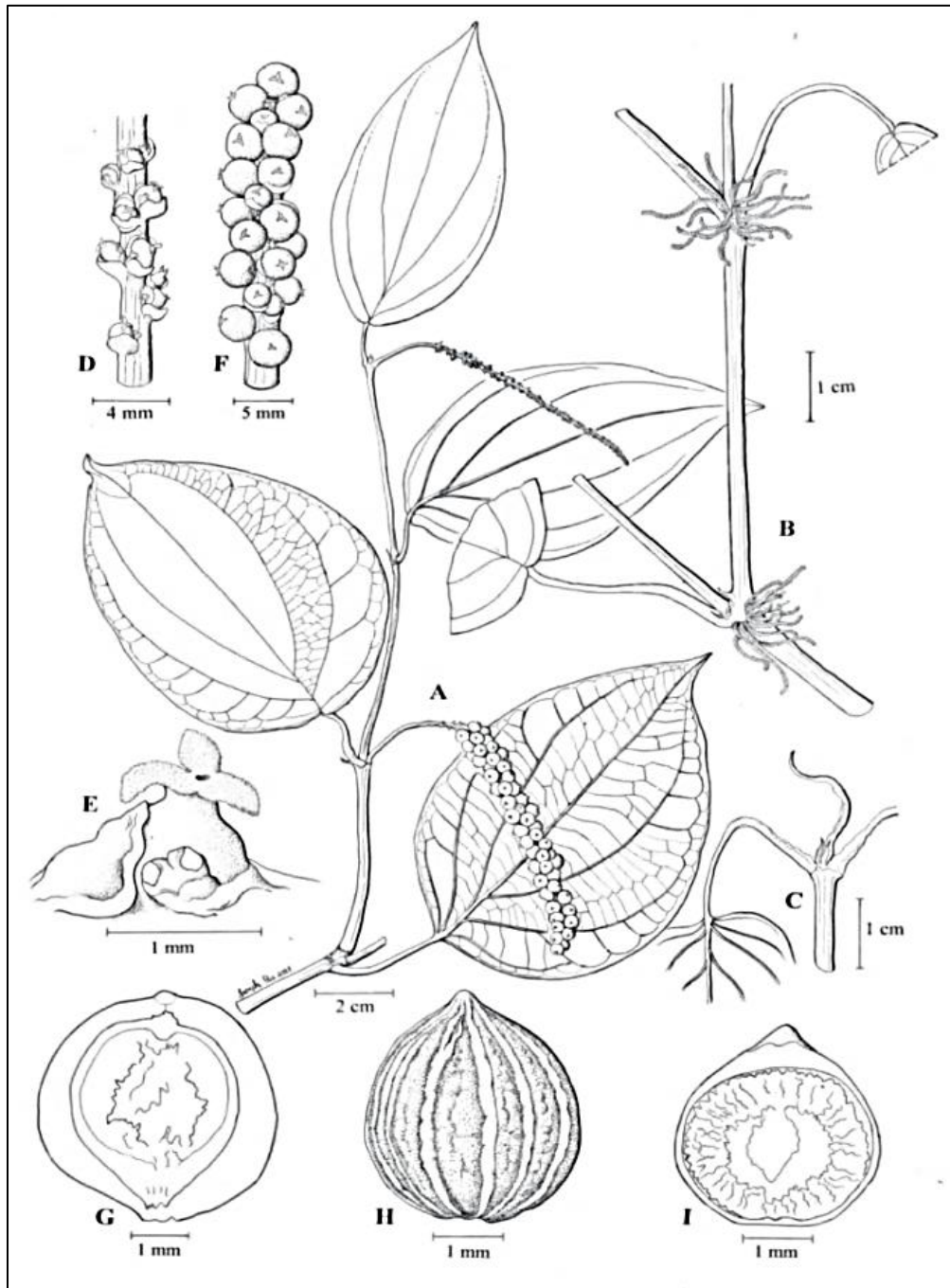


Figure 3.2: Botanical drawing of cultivated black pepper. A. Lateral branch. B. Terminal stem node. C. Shoot tip. D. Inflorescence/early stage of fruit development. E. Single flower. F. Mature stage of fruit spike. G. Cross section of fresh peppercorn. H. Outer surface of peppercorn (pericarp removed). I. Cross section of pepper seed.



Figure 3.3: Photography documentation of important morphological characteristics of cultivated black pepper. A. Mature vine. B. Ivy-like root emerge from node of stem and cling on support pole. C. Old stem with branches. D. Single branch. E. Leaf lower surface. F. Leaf upper surface. G. Shoot tips. H. Ripen fruits. I. Mature fruits. J. Inflorescence-Catkin. K. Bisexual flower. L. Unisexual flower. M. Single flower with 3 lobbed stigma and two pairs of anthers (2 stamens). N. Close up of inflorescence- Minute and bracteates. O. Dissected fruit- Drupe type. P. Spherical seed. *St- stamen; Stg- stigma; Br- bracteates.

3.4 Cultivars of Black Pepper in Malaysia

3.4.1 Diversity

A manual entitled ‘Pepper Production Technology in Malaysia’ that released by Malaysian Pepper Board in 2011 has mentioned 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, 2011a). However, Sim (2007) reported the existence of other cultivars, namely cv. ‘Lampung Daun Lebar’ and cv. ‘Lampung Daun Kecil’ also while Ravindran (2000) reported important cultivars in Malaysia constituted by cv. ‘Kuching’, cv. ‘Sarikei’ and cv. ‘Miri’. Thus, the diversity of black pepper in Malaysia is undefined. However, extensive field work that has been conducted managed to verify ten cultivar of black pepper based on morphological uniqueness. The designations of cultivars were confirmed through germplasm of *Piper* that located at Agricultural Research Center, Semongok Sarawak. The verified cultivars includes 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’. Botanical descriptions for these cultivars as described at sub-chapter 3.4.1.1 to 3.4.1.10. In this project, comprehensive qualitative and quantitative analysis for reliable varietal identification or diagnostic purpose have also been carried out, as in Chapter 4.0 Morphological characteristics of black pepper cultivars.

3.4.1.1 Cultivar ‘Semongok Aman’

Ridley, The floral of the Malay peninsular, Vol.III. Apetale. L. Reeve and Co. Ltd. London (1924); Asokan Nair & Ravindran, J. Econ. Taxon. Bot. 10(1): 167 (1988).

Description: Dioecious and glabrous climber. Adventitious root emerged at nodal region from 360°. Plagiotropic branch erect when young and become drooping as plant ages. Leaf is glabrous and elliptical shaped. Leaf apex is mucronate shaped while base is acute shaped. Leaf colour fresh green to dark green and blade surface is wavy. Shoot tip is purplish green. Protogynous present with 2-3 days interval and distance between flowers is close, around 0.1 mm. Flower bisexual with staminate and pistillate part uniformly emerge. Inflorescence length is 7-8 cm long and thickness 3-4 mm while inflorescence colour is fresh green. Berry is dark green at mature stage. Fruit set percentage is high, *ca.*70% while fruit spike length is 9-11 cm. Seed colour is greyish light brown with size, *ca.*4.80 mm.

Distribution: This cultivar diversely distributed in state of Sarawak (Figure 3.6). Some also found in Sabah and Terengganu (Figure 3.6).

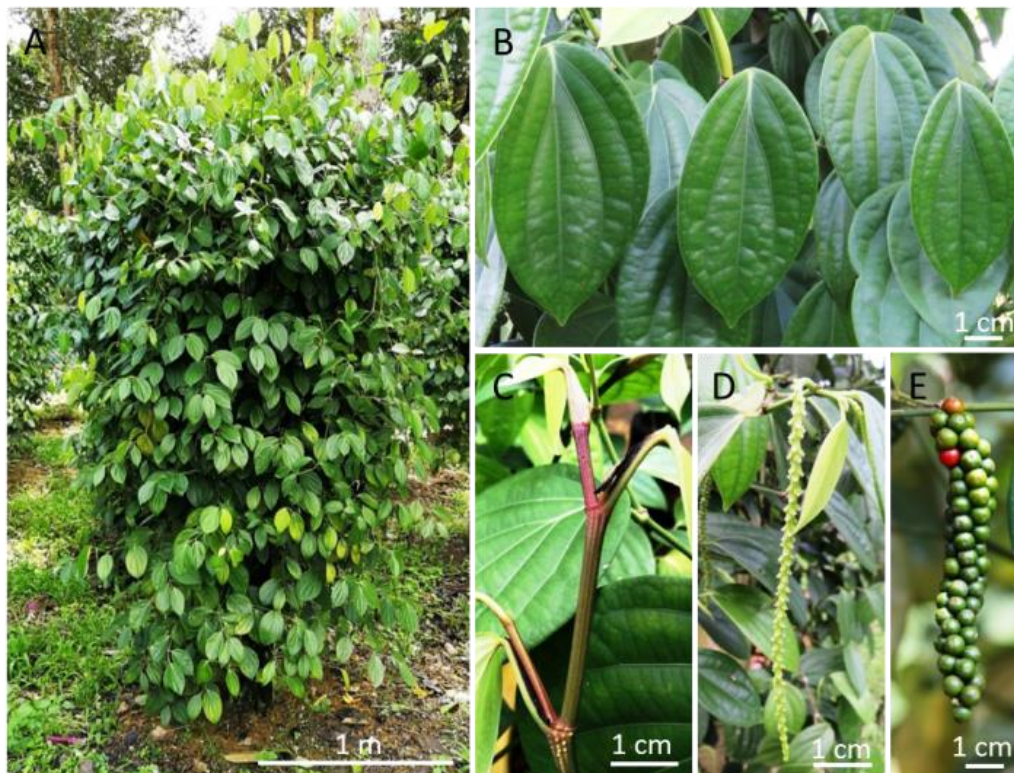


Figure 3.4: Photography documentation of cv. 'Semongok Aman'. A. Mature vine. B. Leaves. C. Shoot tip. D. Inflorescence. E. Fruit spike.



Figure 3.5: Dried specimen of cultivar cv. 'Semongok Aman' (Voucher No. CYS-01).



Figure 3.6: Distribution of cv. 'Semongok Aman'. **State of Sarawak:** Kuching Division- Bau, Sematan, Padawan, Mambong, Tarat, Matang; Samarahan Division- Muara Tuang, Simunjan and Batang Sadong; Serian Division- Tarat, Tebakang, Kedup, Bukit Semuja, Tedebu and Gedong; Sri Aman Division- Balai Ringin, Lachau, Lubok Antu, Bukit Begunan and Batang Lupar; Betong Division- Saratok, Laya, Saribas and Bukit Saban; Bintulu Division- Niah and Subis; Miri Division- Marudi and Sibuti; Sibu Division- Dalat, Selangau, Kanowit and Lanang; Sarikei Division- Julau and Repok; Limbang Division- Lawas, Batu Danau and Bukit Kota; Bintulu Division- Tatau; Mukah Division- Dalat; Limbang Division- Lawas and Ba'Kelalan; Kapit Division- Song, Bukit Goram and Belaga; Miri Division- Marudi. **State of Sabah:** Inanam; Pensiangan; Kimanis; Tuaran; Kota Marudu; Tawau; Kota Belud; Panampang; Tenom. **State of Terengganu:** Ajil and Marang. ● Black dot represents the general mapping for black pepper planting location.

3.4.1.2 Cultivar ‘Kuching’

Ridley, The floral of the Malay peninsular, Vol.III. Apetale. L. Reeve and Co. Ltd. London (1924); Asokan Nair & Ravindran, J. Econ. Taxon. Bot. 10(1): 167 (1988).

Description: Dioecious and glabrous climber. Adventitious root emerged at nodal region, from region attached to support. Plagiotropic branch is horizontal when young and become slightly drooping as plant ages. Leaf is small, glabrous and thin while leaf shape is ovate, with acute apex and rounded base. Leaf colour is fresh green to yellowish green while blade surface is flat. Shoot tip is purplish green. Protogynous present with inconsistent interval ranged 3-5 days. Distance between flowers is close, *ca.*0.2 mm. Flower is unisexual or bisexual. Inflorescence length is 6-7 cm long with thickness 3-4 mm, while colour is whitish green when young and yellowish green when mature. Berry is fresh green at mature stage. Fruit set percentage is moderate *ca.*60% with fruit spike length, 9-10 cm. Seed colour is whitish brown with size *ca.*4.44 mm.

Distribution: This cultivar diversely distributed in all black pepper planting area (Figure 3.9).

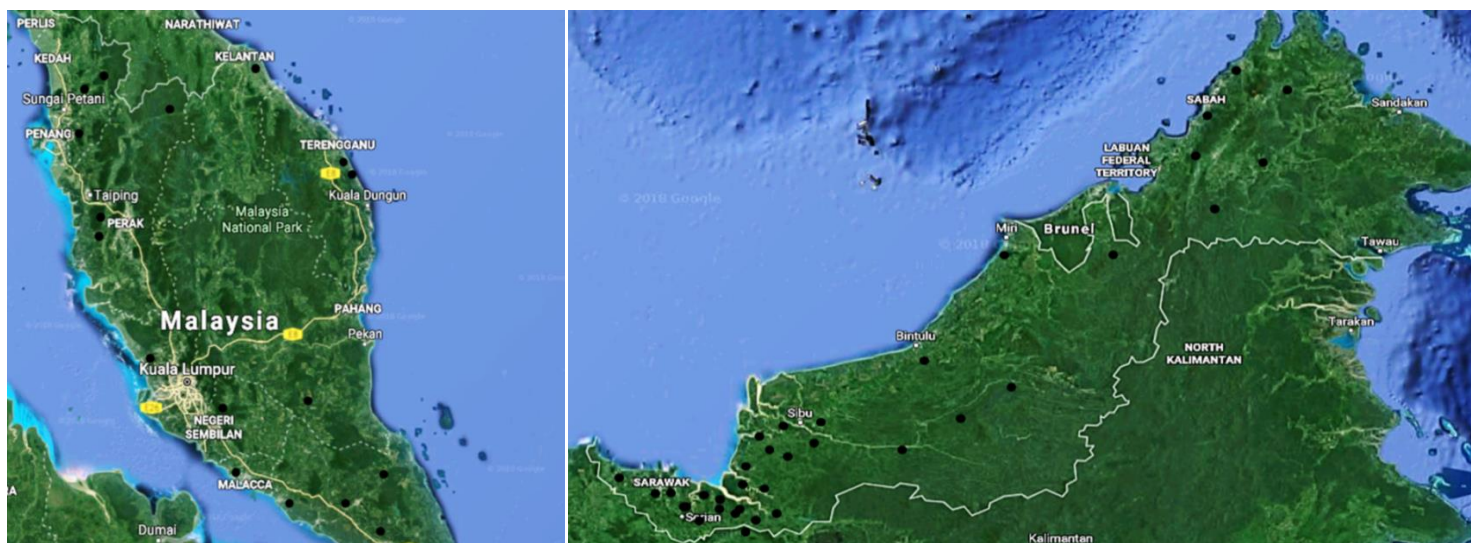


Figure 3.9: Distribution of cv. 'Kuching'. **State of Sarawak:** Kuching Division- Bau, Lundu, Sematan, Tanjung Datu, Padawan, Mambong, Tarat, Matang, Batu Kawa and Bako; Samarahan Division- Muara Tuang, Asajaya, Simunjan and Batang Sadong; Serian Division- Tarat, Tebakang, Kedup, Bukit Semuja, Tedebu and Gedong; Sri Aman Division- Balai Ringin, Lachau, Lubok Antu, Bukit Begunan and Batang Lupar; Betong Division- Saratok, Layar, Saribas and Bukit Saban; Bintulu Division- Niah, Subis and Samalaju; Miri Division- Marudi and Sibuti; Sibu Division- Dalat, Selangau, Kanowit, Lanang, Bawang Assan and Nangka; Sarikei Division- Julau, Pakan and Tanjong Manis; Limbang Division- Lawas, Batu Danau and Bukit Kota, Batu Danau, Bukit Kota and Ba'Kelalan; Bintulu Division- Niah, Tatau, Subis and Samalaju; Mukah Division- Dalat and Balingian; Kapit Division- Song, Bukit Goram, Hulu Rajang and Belaga; Miri Division- Marudi and Sibuti. **State of Sabah:** Kota Kinabalu Division- Inanam; Keningau Division- Pensiangan; Papar Division- Kimanis; Ranau Division- Keranaan. **State of Negeri Sembilan:** Mambau; Seremban and Jelebu. **State of Pahang:** Lipis and Rompin. **State of Selangor:** Subang Jaya. **State of Johor:** Kukup, Pontian, Kulai, Kluang, Batu Pahat; Tebrau; Parit Sulong; Kota Tinggi; Yong Peng, Bakri and Lok Heng. **State of Perak:** Gopeng; Canning; Batu Gajah; Kampar; Sungai Siput and Tambun. **State of Terengganu:** Ajil, Dungun and Marang. **State of Kelantan:** Jeli and Pasir Puteh. **State of Kedah:** Kulim, Belantok, Baling, Jerai and Sik. **State of Pulau Pinang:** Bukit Mertajam. ● Black dot represents the general mapping for black pepper planting location.

3.4.1.3 Cultivar ‘Semongok Emas’

Ridley, The floral of the Malay peninsular, Vol.III. Apetale. L. Reeve and Co. Ltd. London (1924); Asokan Nair & Ravindran, J. Econ. Taxon. Bot. 10(1): 167 (1988).

Description: Dioecious and glabrous climber. Adventitious root emerged at nodal region, from region attached to support. Plagiotropic branch horizontal when young and become slightly drooping as plant ages. Leaf is glabrous and elliptical shaped while apex is acute type and base is acute type. Leaf colour is greyish green to yellowish green with flat surface and wavy leaf margin. Shoot tip is light purplish green. Protogynous present with inconsistent interval ranged 3-5 days. Distance between flowers is close, *ca.*0.2 mm. Inflorescence length is 7-9 cm long while thickness is 3-3.5 mm. Inflorescence colour is whitish green when young and yellowish green when mature. Berry is yellowish green at mature stage. Fruit set percentage is moderate, *ca.*69%. Fruit spike length is 10-11 cm. Seed colour is whitish brown with size *ca.*4.60 mm.

Distribution: This cultivar diversely distributed in state of Sarawak and Terengganu (Figure 3.12).



Figure 3.10: Photography documentation of cv. 'Semongok Emas'. A. Mature vine. B. Leaves. C. Shoot tip. D. Inflorescence. E. Fruit spike.

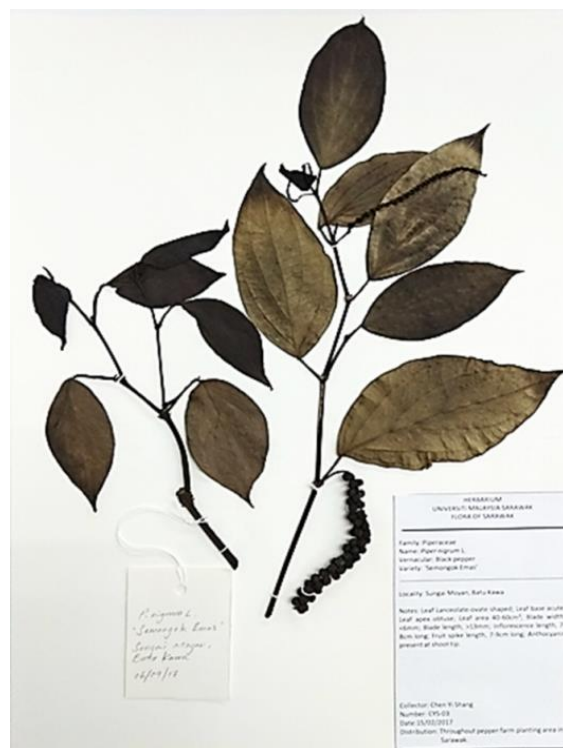


Figure 3.11: Dried specimen of cultivar cv. 'Semongok Emas' (Voucher No. CYS-03).



Figure 3.12: Distribution of cv. 'Semongok Emas'. **State of Sarawak:** Kuching Division- Bau, Sematan, Padawan, Mambong, Tarat, Matang; Samarahan Division- Muara Tuang, Simunjan and Batang Sadong; Serian Division- Tarat, Tebakang, Kedup, Bukit Semuja, Tedebu and Gedong; Sri Aman Division- Balai Ringin, Lachau, Lubok Antu, Bukit Begunan and Batang Lupar; Betong Division- Saratok, Layar, Saribas and Bukit Saban; Bintulu Division- Niah and Subis; Miri Division- Marudi and Sibuti; Sibu Division- Dalat, Selangau, Kanowit and Lanang; Sarikei Division- Julau and Repok; Limbang Division- Lawas, Batu Danau and Bukit Kota; Bintulu Division- Tatau; Mukah Division-Dalat; Limbang Division- Lawas and Ba'Kelalan; Kapit Division- Song, Bukit Goram and Belaga; Miri Division- Marudi. **State of Terengganu:** Ajil. ● Black dot represents the general mapping for black pepper planting location.

3.4.1.4 Cultivar ‘Semongok Perak’

Ridley, The floral of the Malay peninsular, Vol.III. Apetale. L. Reeve and Co. Ltd. London (1924); Asokan Nair & Ravindran, J. Econ. Taxon. Bot. 10(1): 167 (1988).

Description: Dioecious and glabrous climber. Adventitious root emerged at nodal region, from region attached to support. Seed colour whitish brown. Stem texture crusty. Plagiotropic branch erect when young and become horizontal as plant ages. Leaf is glabrous and thick while leaf shape is elliptical with acute apex and oblique base. Leaf colour is greyish dark green with flat surface and blade vein vivid. Shoot tips purplish green. Protogynous present with inconsistent interval ranged 3-5 days. Distance between flowers is close, *ca.*0.2 mm. Inflorescence length is 6.5-7.5 cm long with thickness 3.5-4 mm. Inflorescence colour is whitish green when young and yellowish green when mature. Berry is dark green at mature stage. Fruit set percentage is moderate, *ca.*62% while fruit spike length is 9-10 cm. Seed colour is whitish brown with size *ca.*4.60 mm.

Distribution: The distribution of this cultivar limited to state of Sarawak (Figure 3.15).



Figure 3.13: Photography documentation of cv. 'Semongok Perak'. A. Mature vine. B. Leaves. C. Shoot tip. D. Inflorescence. E. Fruit spike.

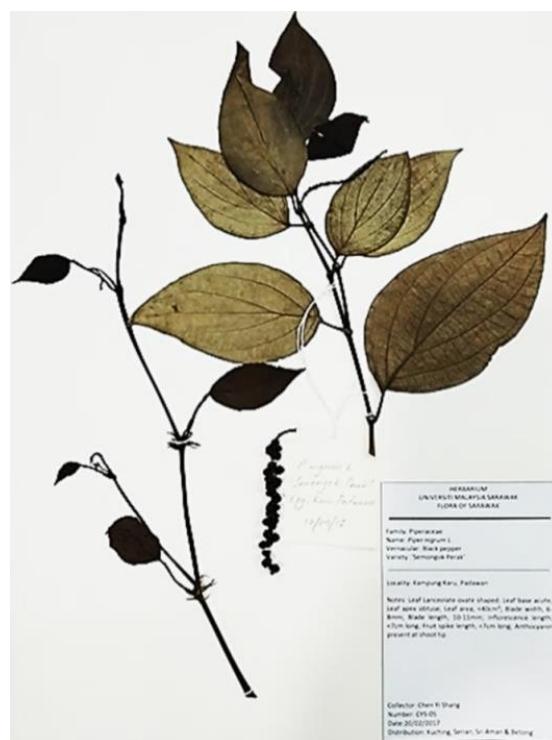


Figure 3.14: Dried specimen of cultivar cv. 'Semongok Perak' (Voucher No. CYS-04).

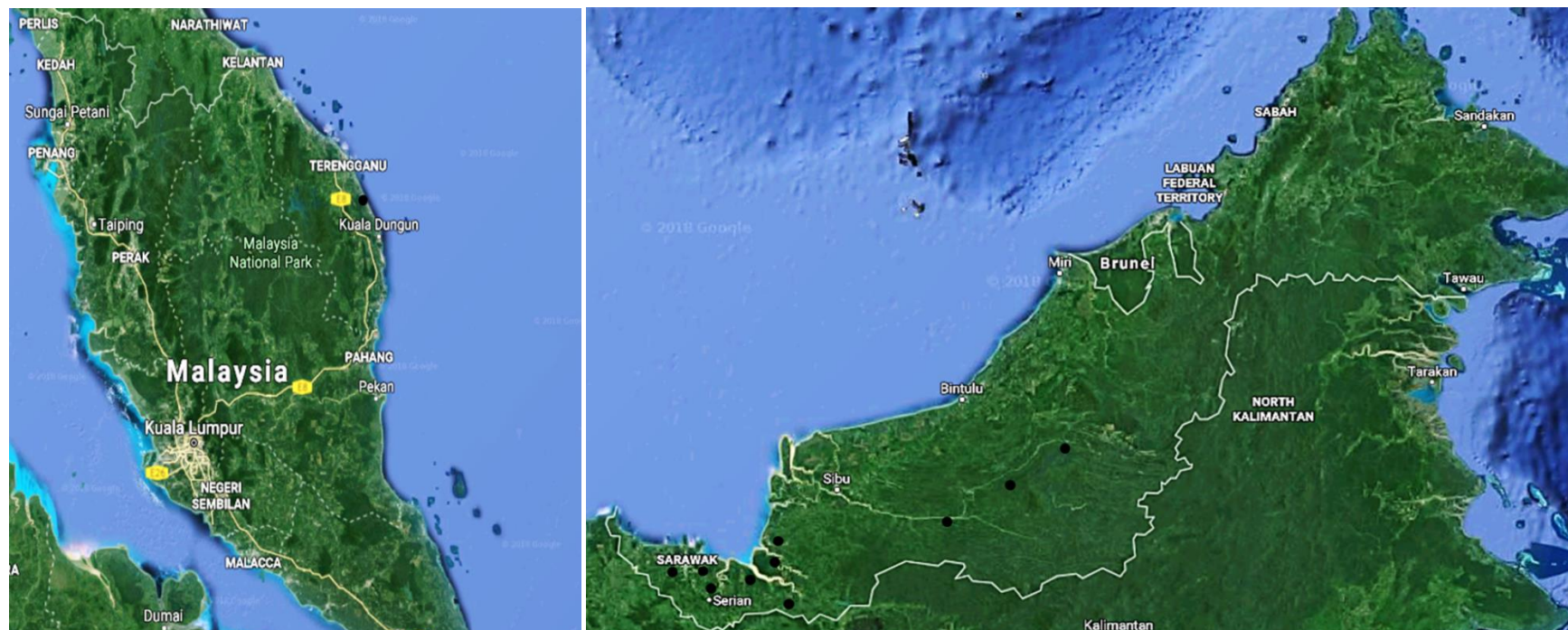


Figure 3.15: Distribution of cv. 'Semongok Perak'. **State of Sarawak:** Kuching Division- Padawan, Mambong; Serian Division- Kedup and Tebedu; Sri Aman Division- Lachau and Lubok Antu; Sarikei Division- Julau; Betong Division- Saratok, Layar and Bukit Saban; Kapit Division- Song. State of Terengganu: Ajil. ● Black dot represents the general mapping for black pepper planting location.

3.4.1.5 Cultivar ‘Semongok 1’

Ridley, The floral of the Malay peninsular, Vol.III. Apetale. L. Reeve and Co. Ltd. London (1924); Asokan Nair & Ravindran, J. Econ. Taxon. Bot. 10(1): 167 (1988).

Description: Dioecious and glabrous climber. Adventitious root rare and emerged at nodal region, from region attached to support. Seed colour whitish brown. Plagiotropic branch erect when young and become horizontal as plant ages. Leaf is glabrous with big sized blade, with width *ca.* 12 cm and length *ca.* 17 cm. Leaf shape is cordate with acute apex and cordate base. Leaf colour is fresh light green with blade surface slightly wavy. Shoot tip is whitish green. Protogynous present with inconsistent interval ranged 3-6 days. Distance between flowers is close, *ca.* 0.6 mm. Inflorescence length is 12-13 cm long with thickness 4-4.5 mm and colour is whitish green when young and yellowish green when mature. Berry is fresh dark green at mature stage. Fruit set percentage is moderate *ca.* 65% while fruit spike length is 16-17 cm. Seed colour is whitish brown with size *ca.* 4.82 mm.

Distribution: The distribution of this cultivar limited to state of Sarawak (Figure 3.18).

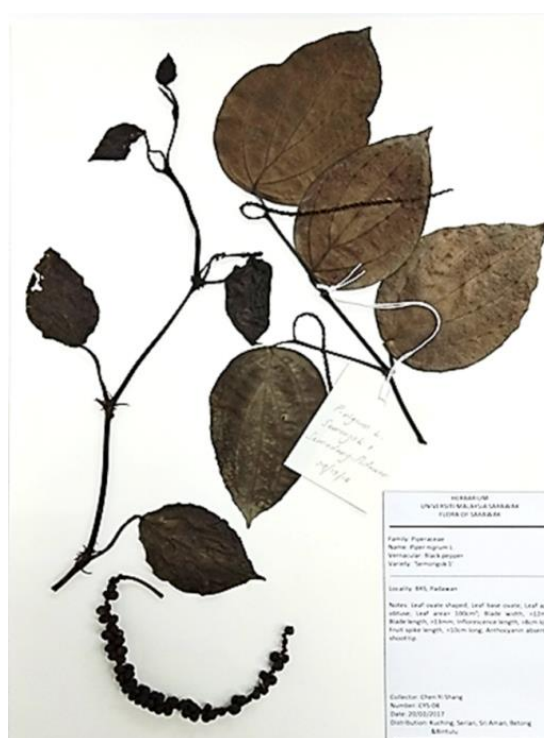




Figure 3.18: Distribution of cv. ‘Semongok 1’. **State of Sarawak:** Kuching Division- Bau, Padawan, Mambong; Samarahan Division- Simunjan; Serian Division- Tarat, Tebakang, Kedup, Tedebu and Gedong; Sri Aman Division- Balai Ringin, Lachau, Lubok Antu; Betong Division- Layar and Bukit Saban; Sibu Division- Selangau, Kanowit and Lanang; Sarikei Division- Julau; Bintulu Division- Tatau; Kapit Division- Song and Belaga. ● Black dot represents the general mapping for black pepper planting location.

3.4.1.6 Cultivar ‘Nyerigai’

Ridley, The floral of the Malay peninsular, Vol.III. Apetale. L. Reeve and Co. Ltd. London (1924); Asokan Nair & Ravindran, J. Econ. Taxon. Bot. 10(1): 167 (1988).

Description: Dioecious and glabrous climber. Adventitious root emerged at nodal region, from region attached to support. Plagiotropic branch erect when young and remain erect as plant ages. Leaf is glabrous and thin, with elliptical leaf shape. Leaf apex is acute while base is oblique. Leaf colour is greyish dark green with flat blade surface. Shoot tip is purplish green. Protogynous present with inconsistent interval ranged 3-6 days. Distance between flowers is close, *ca.* 0.2 mm. Inflorescence length is 7-8.5 cm long, with thickness 3.5-4 mm and colour is whitish green when young and pale green when mature. Berry is dark green at mature stage. Fruit set percentage is moderate, *ca.* 66% while fruit spike length is 10-11 cm. Seed is small with diameter *ca.* 4.44 mm and colour is whitish brown. Highly similar to cv. ‘Kuching’ but distinct by branching morphology.

Distribution: The distribution of this cultivar limited to state of Sarawak (Figure 3.21).



Figure 3.19: Photography documentation of cv. 'Nyerigai'. A. Mature vine. B. Inflorescence. C. Fruit spike. D. Shoot tip. E. Branches. F. Leaves.



Figure 3.20: Dried specimen of cultivar cv. 'Nyerigai' (Voucher No. CYS-06)



Figure 3.21: Distribution of cv. 'Nyerigai'. **State of Sarawak:** Kuching Division- Padawan; Serian Division- Tebakang, Kedup and Gedong; Samarahan Division- Simunjan and Batang Sadong; Sri Aman Division- Balai Ringin, Lachau, Lubok Antu, Bukit Begunan and Batang Lupar; Betong Division- Saratok, Layar, Saribas and Bukit Saban. ● Black dot represents the general mapping for black pepper planting location.

3.4.1.7 Cultivar ‘India’

Ridley, The floral of the Malay peninsular, Vol.III. Apetale. L. Reeve and Co. Ltd. London (1924); Asokan Nair & Ravindran, J. Econ. Taxon. Bot. 10(1): 167 (1988).

Description: Dioecious and glabrous climber. Adventitious root emerged at nodal region, from region attached to support. Plagiotropic branch horizontal when young and become drooping as plant ages. Leaf density is high with relatively smaller leaves size compare to others variety. Leaf is glabrous and thin with lanceolate shaped leaf, acuminate apex and rounded base. Leaf colour is dark green to greyish green while blade surface is flat. Shoot tip is purplish green. Protogynous present with inconsistent interval ranged 3-6 days. Distance between flowers is close, *ca.*0.1 mm. Inflorescence length is 7.5-8.5 cm long with thickness 2.5-3 mm and colour is pale green when young and yellowish green when mature. Berry is dark green at mature stage. Fruit set percentage is moderate, *ca.*66% with fruit spike length is 10-11 cm. Seed is small with diameter *ca.* 3.90 mm and whitish brown in colour.

Distribution: The distribution of this cultivar limited to state of Sarawak (Figure 3.24).



Figure 3.22: Photography documentation of cv. 'India'. A. Mature vine. B. Inflorescence. C. Fruit spike. D. Leaves. E. Shoot tip.



Figure 3.23: Dried specimen of cultivar cv. 'India' (Voucher No. CYS-07)



Figure 3.24: Distribution of cv. 'India'. **State of Sarawak:** Kuching Division- Bau, Padawan, Mambong, Tarat; Samarahan Division- Simunjan and Batang Sadong; Serian Division- Tarat, Tebakang, Kedup, Bukit Semuja, Tedebu and Gedong; Sri Aman Division- Balai Ringin, Lachau, Lubok Antu, Bukit Begunan and Batang Lupar; Betong Division- Saratok, Layar, Saribas and Bukit Saban; Sibu Division- Selangau, Kanowit and Lanang; Sarikei Division- Julau and Repok; Kapit Division- Song. ● Black dot represents the general mapping for black pepper planting location.

3.4.1.8 Cultivar ‘Lampung Daun Lebar’

Ridley, The floral of the Malay peninsular, Vol.III. Apetale. L. Reeve and Co. Ltd. London (1924); Asokan Nair & Ravindran, J. Econ. Taxon. Bot. 10(1): 167 (1988).

Description: Dioecious and glabrous climber. Adventitious root emerged at nodal region, from region attached to support. Delicate plagiotropic branch drooping when young and become more drooping as plant ages. High leaf density and leaf is glabrous. Leaf shape is ovate with ocute apex and oblique base. Leaf colour is greyish dark green and blade surface slightly wavy. Shoot tip is purplish green. Protogynous present with inconsistent interval ranged 3-5 days. Distance between flowers is close, *ca.*0.2 mm. Flowers is bisexual and stable. Inflorescence length is 7-8.5 cm long with thickness 3.5-4 mm and colour is whitish green when young and fresh green when mature. Distance between flowers is close, *ca.*0.2 mm. Berry is dark green at mature stage. Fruit set percentage is moderate, *ca.*55%. Fruit spike length is 10-11 cm and peduncle attachment is strong. Seed is small with diameter *ca.* 4.32 mm and whitish brown in colour.

Distribution: The distribution of this cultivar limited to state of Sarawak (Figure 3.27).

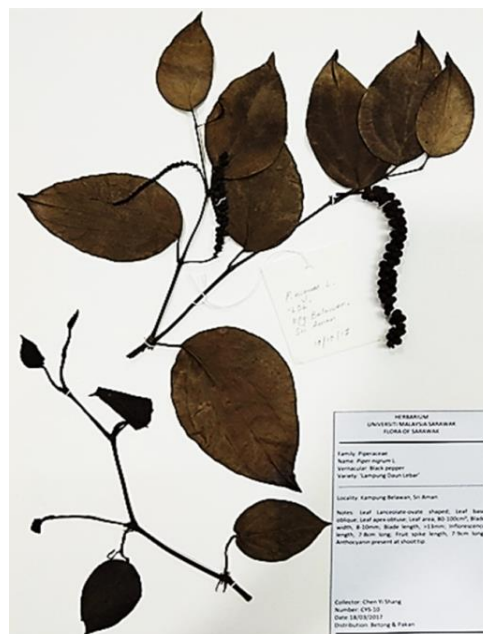
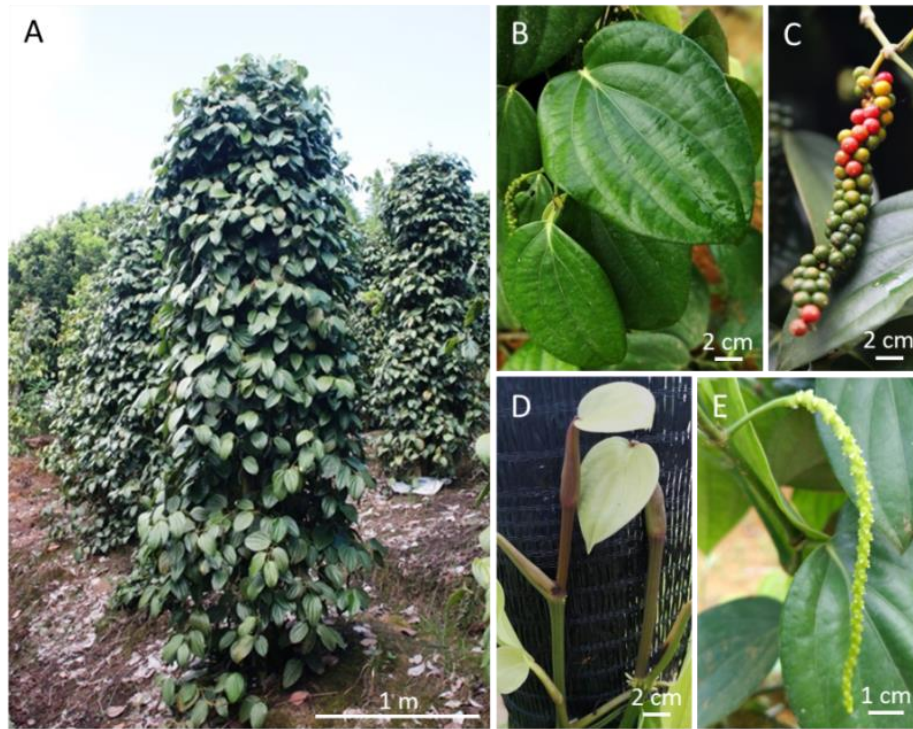




Figure 3.27: Distribution of cv. 'Lampung Daun Lebar'. **State of Sarawak:** Kuching Division- Padawan; Sri Aman Division- Layar.
 ● Black dot represents the general mapping for black pepper planting location.

3.4.1.9 Cultivar ‘Sarikei’

Ridley, The floral of the Malay peninsular, Vol.III. Apetale. L. Reeve and Co. Ltd. London (1924); Asokan Nair & Ravindran, J. Econ. Taxon. Bot. 10(1): 167 (1988).

Description: Dioecious and glabrous climber. Adventitious root emerged at nodal region, from region attached to support. Plagiotropic branch horizontal when young and become slightly drooping as plant ages. Leaf is small, glabrous and thin. Leaf shape is elliptical, with acute apex and acute base. Leaf colour is fresh green to yellowish green and blade surface is flat. Protogynous present with inconsistent interval ranged 3-7 days. Distance between flowers is close *ca.*0.2 mm. Distance between flowers is close, *ca.*0.1mm. Flower is unisexual or bisexual. Inflorescence length is 6-7 cm long while thickness is 3-4 mm. Inflorescence colour is whitish green when young and yellowish green when mature. Berry is fresh green at mature stage. Fruit set percentage is moderate, *ca.*65%. Fruit spike length is 8-9 cm. Seed is small with diameter *ca.*3.84 mm and whitish brown in colour. This cultivar is highly similar to cv. ‘Kuching’.

Distribution: The distribution of this cultivar limited to state of Sarawak (Figure 3.30).

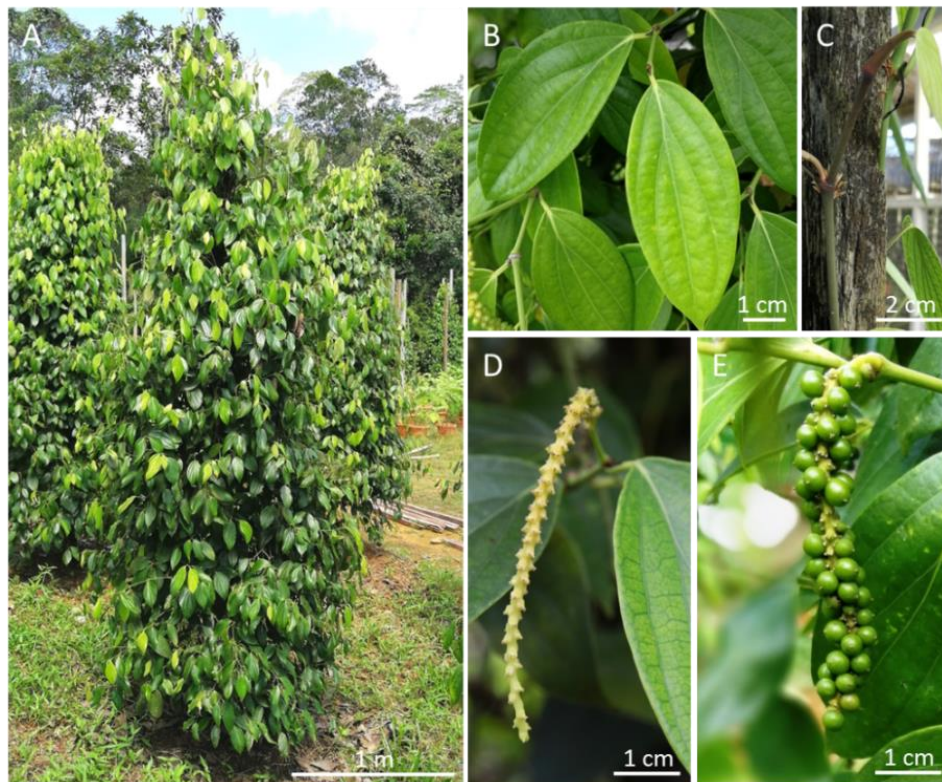


Figure 3.28: Photography documentation of cv. 'Sarikei'. A. Mature vine. B. Leaves. C. Shoot tip. D. Inflorescence. E. Fruit spike.

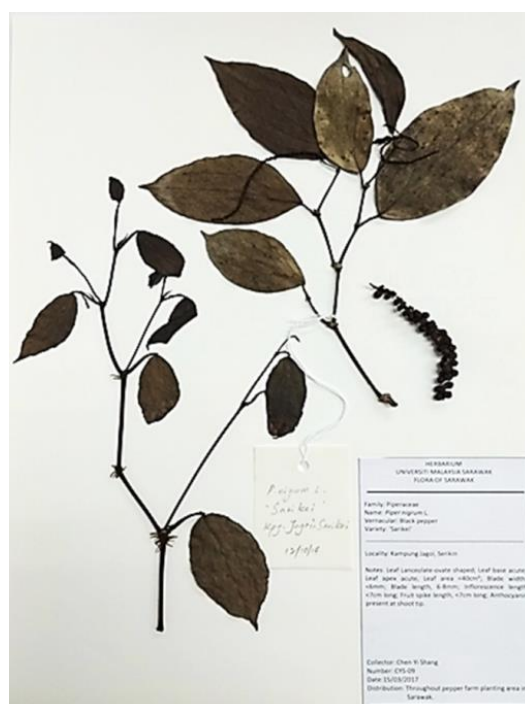


Figure 3.29: Dried specimen of cultivar cv. 'Sarikei' (Voucher No. CYS-09)



Figure 3.30: Distribution of cv. 'Sarikei'. **State of Sarawak:** Kuching Division- Bau; Sarikei Division- Julau and Repok.
 ● Black dot represents the general mapping for black pepper planting location.

3.4.1.10 Cultivar ‘Yong Petai’

Ridley, The floral of the Malay peninsular, Vol.III. Apetale. L. Reeve and Co. Ltd. London (1924); Asokan Nair & Ravindran, J. Econ. Taxon. Bot. 10(1): 167 (1988).

Description: Dioecious and glabrous climber. Adventitious root emerged at nodal region, from region attached to support. Plagiotropic branch horizontal when young and become drooping as plant ages. Leaf is glabrous and thin with elliptical shape while apex is acute and base is acute in shape. Leaf colour is fresh green to yellowish green with flat blade surface. Shoot tip is purplish green. Protogynous present with inconsistent interval ranged 3-7 days. Distance between flower is close *ca.*0.6 mm. Inflorescence is delicate with weak attachment of peduncle. Inflorescence length is 12-13 cm long while thickness is 3-4 mm. Inflorescence colour is whitish green when young and pale green when mature. Berry is dark green at mature stage. Fruit set percentage is poor, *ca.*56% with fruit spike length 10-11 cm. Seed size is big with diameter *ca.* 4.45 mm and whitish brown in colour.

Distribution: The distribution of this cultivar limited to state of Sarawak and Terengganu (Figure 3.33).



Figure 3.31: Photography documentation of cv. 'Yong Petai'. A. Mature vine. B. Inflorescence. C. Fruit spike. D. Shoot tip. E. Leaves.

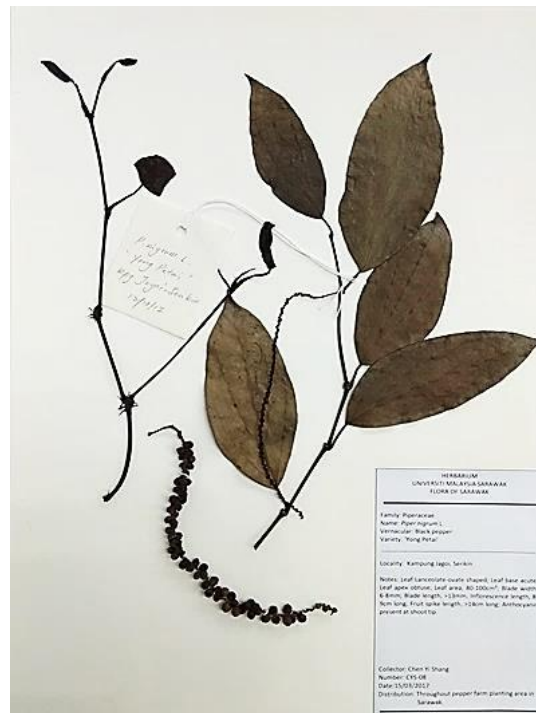


Figure 3.32: Dried specimen of cultivars cv. 'Yong Petai' (Voucher No. CYS-10).



Figure 3.33: Distribution of cv. 'Yong Petai'. **State of Sarawak:** Kuching Division- Bau, Padawan, Mambong; Serian Division- Tarat, Tebakang, Kedup, Bukit Semuja, Tedebu and Gedong; Sri Aman Division- Balai Ringin, Lachau, Lubok Antu, Bukit Begunan and Batang Lupar; Betong Division- Saratok, Layar, Saribas and Bukit Saban; Sibu Division- Selangau, Kanowit and Lanang; Sarikei Division- Julau, Pakan and Tanjong Manis. **State of Terengganu:** Ajil. ● Black dot represents the general mapping for black pepper planting location.

3.5 Conclusions

Black pepper is vegetatively propagated for commercial farm purpose. Cultivars of black pepper believed to have been derived from the wild one through domestication. Thus, morphology of black pepper cultivars exhibited high similarity regardless of distribution distance. In Malaysia, observation that has been conducted in this project has identified ten common cultivars of black pepper. These cultivars include 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'. Despite high similarity, the cultivars identification is possible through comprehensive assessment on leaf, inflorescent, flower, shoot, stem, berry and seed of each cultivar. The qualitative and quantitative analysis has also been conducted as analysed and discussed at Chapter 4, to ascertain the distinctness for cultivar identification. Cultivar 'Kuching' is the most common cultivar in Malaysia. This cultivar coverage is approximately 50% of the total planting area. Cultivars like 'Semongok Emas' and 'Semongok Aman' are also considered popular, attributed to about 20% of total planting area. Whilst, 'Semongok Perak', cv. 'Semongok 1', cv. 'Nyerigai', cv. 'India', cv. 'Lampung Daun Lebar', cv. 'Sarikei', and cv. 'Yong Petai' are among the less popular cultivars with limited distribution. These cultivars were most probably only propagated when the farmer were short of planting materials. Beside the pest and disease resistant consideration, the yield potential factor could be the main reason for the cultivar selection by farmer. These will further discussed in Chapter 4 Morphological Analysis and Chapter 6 Physiological Analysis, of this project. The black pepper distribution in Malaysia was believed to be influenced by climate, particularly the rainfall. The planting also involved very much through the state agricultural policy and encouragement to the farmer. The soil suitability

factor does not restrict the distribution. The state in West Coast of Peninsular Malaysia like Kelantan and Terengganu and also Sabah have good distribution potential by judging on climate suitability factor.

CHAPTER 4

MORPHOLOGICAL CHARACTERISTICS OF BLACK PEPPER CULTIVARS

4.1 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 cultivation of black pepper (*Piper nigrum* L.) is believed to have derived from a wild plant through domestication or selection (Ravindran et al., 2000a). It was introduced to Malaysia as early as 1856 (Dalton, 1912); however, cultivation was only focused in Sarawak (Sim et al., 1996). 95% of pepper planting areas are in the state of Sarawak. It was mainly distributed in rural area in Division of Kuching, Serian, Sri Aman, Betong, Sarikei and Sibu while 5% from Peninsular Malaysia that consist of Johor, Pahang, Negeri Sembilan, Selangor Terengganu, Kelantan, Perak, Pulau Pinang and Kedah (Sim et al., 1996). Distribution in Sabah still very limited up to date.

The earliest study on the taxonomy of black pepper cultivars in Malaysia was by Sim (1979). She mentioned that the preliminary identification on the collection was based on Ridley’s Floral of the Malay Peninsula. Sim’s morphological descriptions include dioecious and monoecious classification, branching behaviour, leaf, stem, and inflorescence. In 1996, Sim et al. also studied fruit development of only three selected cultivars, cv. ‘Kuching’, cv. ‘Semongok emas’, and cv. ‘Hybrid 10’, while Chen (2011) and Chen (2013) reported on the stigma receptivity and apomixis study of *P. nigrum*,

respectively, regarding floral morphology. Recently, the Black Pepper Test Guideline for Plant Variety Protection Act implementation has been developed (Sim et al., 2009). This guideline listed the entire important characteristic for the diagnosis of black pepper variety. In India, a book entitled ‘Black pepper (*Piper nigrum* L.)’ described 38 cultivars of black pepper, with descriptions focused on leaf shape, spike length, percent fruit set, topography suitability, and yield performance (Ravindran et al., 2000a).

Before this, the diversity of the black pepper cultivar in Malaysia is unidentified because varietal control is not practiced. The most common black pepper cultivars were cv. ‘Kuching’ and cv. ‘Sarikei’, both widely planted throughout Sarawak (Sim, 1991), while Paulus (2007) reported three important cultivars in his publication: 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’ were introduced to Malaysian farmers (Sim, 2007). 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, 2011a). 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, 2007). However, there is no updated report on current accession diversity.

The Malaysian government strategized a new policy to ensure the sustainability of the industry through strengthening the quality of peppercorn. A mono-varietal farm concept was believed able to strengthen the quality of peppercorn (Chen et al., 2018). This can be achieved through varietal control, and a pre-requisite to this policy is comprehensive documentation on all important cultivars in Malaysia. The existing documentation on cultivated black pepper in Malaysia is less comprehensive, and none of the cultivars were registered under the National Crop List of Malaysia (Chen et al., 2018). Cultivar identification could be very difficult for an inexperienced farmer. This project aimed 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. At the same time, the morphology correlations among cultivars were analysed. The 10 cultivars as mentioned at chapter 3 were selected for this investigation.

4.2 Materials and Methods

4.2.1 Analysis of Qualitative and Quantitative Trait Variability

4.2.1.1 Material Preparation

The black pepper farm distribution information was sourced from the Department of Crop, Extension, and Farmer's Development, Malaysian Pepper Board. Photography data particularly on leaf, inflorescence, fruit spike, and shoot tip were comprehensively generated for the preliminary cultivars 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.

Field grown vine was established in Jan 2015 at three locations, namely Kampung Jagoi, Serikin; Kampung Karu, Padawan and Kampung Belawan, Sri Aman; and one experimental plot under controlled environment at the Agriculture Research Center (ARC) Semongok, Department of Agricultural Sarawak. The planting procedure followed the standard practice as described by Paulus et al. (2011b) in 'Pepper production technology in Malaysia'. The planting material used is pepper cutting of 5 nodes, planted with spacing of 1.8 m x 2.0 m (between vine x between row). Observation has been conducted starting from 1 year old vine (January 2016) until 2 years old vine (December 2017).

4.2.1.2 Experimental Design

The field experiment at three locations i.e. Kampung Jagoi, Serikin; Kampung Karu, Padawan and Kampung Belawan, Sri Aman was laid out in a Randomized Complete Block Design (RCBD) (Figure 4.1) 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 location containing ten treatments, consist of 50 vines. Whilst, pot experiment was based on Completely Randomized Design (CRD) (Figure 4.2) that consist of a total of 50 potted vine, 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. Vine growing morphology or vigour was assessed on field-grown vines at the three field experimental plots at Kampung Jagoi, Serikin; Kampung Karu, Padawan and Kampung Belawan, Sri Aman while leaf, inflorescence, fruit, and seed morphology studies were based on samples collected from potted plants grown under controlled environment at Semongok Agricultural Research Center, Sarawak. Data collection was carried out from January 2016 to December 2017. Microscopy assessment and data analysis were performed at the Malaysian Pepper Board, Kuching.

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 4.1: 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 4.2: Completely Randomized Design (CRD) pot arrangement for experimental plot under 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.

4.2.1.3 Data Analysis

A total of 26 morphological characteristics, consisting of both qualitative and quantitative traits, have been assessed in this study, as listed at Table 4.1. Measurement of morphological characteristics as variables is performed in this analysis. Quantitative characteristics used were all analysed via ANOVA by using SPSS software.

Table 4.1: Morphological characteristic used for diagnosis of cultivar distinctness.

Morphological characters	Measurement methods
1. Leaf characters Leaf shape; Leaf apex and Leaf base Leaf area (cm ²); blade width (w) cm; blade length (L) cm and blade length-width ratio (Lw ⁻¹) Leaf colour (fully expanded leaf)	Description based on Benson (1979). Measured by WinFOLIA image system. RHS colour codes used.
2. Inflorescence characters Inflorescence length at stigma withering stage (cm) and Inflorescence thickness at stigma withering stage (mm) Inflorescence colour Number of flower per inflorescence Number of inflorescence (spike) per branch per node	Measured by Vernier calliper. RHS colour codes used. Counted via stereomicroscope. Counted manually.
3. Fruit characters Fruit spike length (cm) and fruit size in diameter (mm) Fruit weight (single fresh berry) (g) Fruit colour (hard dough stage) Percent fruit set (%) Conversion rate % (fresh to black pepper) Conversion rate % (fresh to white pepper) Pericarp thickness (mm)	Measured by Vernier calliper. Measured by analytical balance. RHS colour codes used. Counted manually. Percent = (Number of developed fruit)/ (Number of developed fruit + number of under developed fruit) x 100%. Measured by analytical balance. (Drying specification: Oven dry at 40°C; moisture content ≤12%) Measured by analytical balance. (Drying specification: Oven dry at 40°C; moisture content ≤12%) 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)

Table 4.1 continued

Seed weight (g)	Measured by analytical balance.
5. Vigour	
Branch column	By observation.
Internode length (cm)	Measurement by 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- Union for the Protection of New Varieties of Plants; RHS- Royal Horticultural Society.

4.2.2 Phenetic Analysis

4.2.2.1 Material Preparation and Experimental Design

Ten cultivated varieties were selected in this study as the Operational Taxonomic Unit (OTU) for the phenetic analysis. The ten OTUs were cultivar ‘Semongok Aman’ (SA), cultivar ‘Kuching’ (KCH), cultivar ‘Semongok Emas’ (SE), cultivar ‘Semongok Perak’ (SP), cultivar ‘Semongok 1’ (S1), cultivar ‘Nyerigai’ (NYE), cultivar ‘India’ (IND), cultivar ‘Lampung Daun Lebar’ (LDL), cultivar ‘Sarikei’ (SAR), and cultivar ‘Yong Petai’ (YP). A total of 27 characteristics covering important parts of the plant were targeted for the assessment, including Leaf shape; Leaf apex; Leaf base; Leaf area (cm²); Blade width (w) (cm); Blade length (cm); Blade length-width ratio (Lw-1); Leaf colour (fully expanded leaf); Inflorescence length at stigma withering stage (cm); Inflorescence thickness at stigma withering stage (mm); Inflorescence colour; Number of flower per inflorescence;

Number of inflorescence (spike) per branch per node; Fruit spike length (cm); Fruit size in diameter (mm); Fruit weight (single fresh berry) (g); Fruit colour (hard dough stage); Percent fruit set (%); Conversion rate % (fresh to black pepper); Conversion rate % (fresh to white pepper); Pericarp thickness (mm); Seed diameter (mm); Seed weight (g); Branch column types; Internode length (cm); Number of node /foot of stem and Anthocyanin: Absent or present. At least 15 readings for qualitative characteristics and 15 readings for quantitative characteristics were collected for the analysis. All of the data used in this phenetic analysis obtained from the experimental plot as explained in section 4.2.1.2 Experimental Design.

4.2.2.2 Data Analysis

Measurement of morphological characteristics as variables is performed in this analysis. Both data collected from field grown vine and planthouse vine were transformed into data metric and analysed together. Characteristics used for phenetic analysis all tested significantly different between at least two cultivars under the ANOVA test, using SPSS and further analyse by the Duncan test on 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 (David, 1975). Phenogram was presented based on average linkage (between groups) using Squared Euclidean Distance Method. Whilst, the PCA helped extract the valuable information from a multivariate data table and express this information as new variables (Jolliffe, 2002).

4.3 Results and Discussion

4.3.1 Analysis of Qualitative and Quantitative Trait Variability

4.3.1.1 Variability Analysis

Comprehensive assessment consisting of both qualitative and quantitative traits has been carried out as shown at Table 4.2. This analysis aimed to determine key diagnostic morphology for each of the cultivars. ANOVA test has been performed on the quantitative characteristics (Table 4.2). The mean, range, and standard deviation for these characteristics were analysed (Figure 4.8-4.26) also.

Table 4.2: Qualitative and quantitative traits used to diagnose the differences among black pepper cultivars.

No.	Morphological characteristic	Cultivars									
		SA	KCH	SE	SP	S1	NYE	IND	LDL	SAR	YP
	Leaf (Refer Figure 4.3)										
1.	Leaf shape	E	O	E	E	C	E	L	O	E	E
2.	Leaf apex	M	A	A	A	A	A	Ac	A	A	A
3.	Leaf base	A	R	A	Ob	C	Ob	R	Ob	A	A
4.	Leaf area (cm ²)	45.40 ^{abc}	37.70 ^{ab}	46.60 ^{bc}	62.80 ^d	132.60 ^f	53.60 ^c	50.90 ^c	81.40 ^e	36.90 ^a	66.50 ^d
5.	Blade width (w) (cm)	6.36 ^b	5.37 ^a	5.67 ^a	7.47 ^c	11.87 ^e	6.49 ^b	5.75 ^a	8.85 ^d	5.26 ^a	6.62 ^b
6.	Blade length (L) (cm)	10.70 ^a	10.83 ^a	13.31 ^c	13.20 ^c	16.67 ^e	12.07 ^b	13.63 ^c	13.50 ^c	10.93 ^a	14.75 ^d
7.	Blade length- width ratio (Lw ⁻¹)	1.70 ^b	2.02 ^d	2.35 ^{ef}	1.77 ^{bc}	1.41 ^a	1.86 ^c	2.39 ^f	1.52 ^a	2.10 ^d	2.24 ^e
8.	Leaf colour (fully expanded leaf)	Green group 139 series	Green group 139 series	Green group 137 series	Green group NN137	Green group 139 series	Green group 139 series	Green group 139 series	Green group NN137	Green group 139 series	Green group 139 series

Table 4.2 continued

Inflorescence (Refer Figure 4.4)											
9.	Inflorescence length (cm)	7.84 ^d	7.03 ^{bc}	7.95 ^d	6.93 ^b	12.40 ^e	7.06 ^{bc}	7.80 ^d	7.68 ^{cd}	6.06 ^a	12.75 ^e
10.	Inflorescence thickness (mm)	3.50 ^d	3.56 ^d	3.47 ^d	3.73 ^e	3.85 ^f	3.10 ^c	3.00 ^b	3.75 ^e	3.54 ^d	2.90 ^a
11.	Inflorescence colour	Green group 144 series	Green group N144	Green group N144	Green group 145 series	Green group N144	Green group N144	Green group N144	Green group 144 series	Green group N144	Green group 145 series
12.	Number of flower per inflorescence (average)	88.30 ^{de}	67.57 ^{ab}	86.33 ^{de}	72.70 ^{abc}	127.90 ^g	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)	12.47 ^a	58.67 ^f	19.57 ^{abc}	22.20 ^{bc}	17.60 ^{ab}	35.83 ^e	27.37 ^{cd}	21.47 ^{bc}	34.73 ^{de}	16.20 ^{ab}

Table 4.2 continued

Fruit (Refer Figure 4.5 and 4.6)											
14.	Fruit spike length (cm)	10.38 ^c	9.37 ^b	10.62 ^c	9.27 ^b	16.48 ^d	9.39 ^b	10.38 ^c	10.22 ^c	8.07 ^a	17.07 ^d
15.	Fruit size in diameter (mm)	6.68 ^e	6.75 ^e	6.76 ^e	6.86 ^e	7.27 ^f	6.48 ^d	6.02 ^b	6.30 ^c	5.78 ^a	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	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 141 series	Green group NN137 series	Green group NN137 series	Green group NN137 series	Green group NN137 series	Green group 139 series	Green group NN137 series	Green group NN137 series
18.	Percent fruit set (%)	70.68 ^f	61.10 ^{bc}	68.75 ^{ef}	61.51 ^{bc}	64.24 ^{cde}	66.93 ^{def}	65.76 ^{cdef}	55.10 ^a	64.28 ^{cde}	56.26 ^{ab}
19.	Conversion rate (%) (fresh to black pepper)	37.35 ^{ab}	41.68 ^{cd}	42.24 ^{cd}	36.12 ^a	42.35 ^d	41.06 ^{bcd}	40.51 ^{bcd}	38.31 ^{abc}	39.55 ^{abcd}	36.25 ^a

Table 4.2 continued

20.	Conversion rate (%) (fresh to white pepper)	30.37 ^{de}	31.08 ^e	31.68 ^e	24.21 ^a	27.70 ^{bc}	31.89 ^e	28.16 ^{cd}	29.62 ^{cde}	29.69 ^{cde}	25.70 ^{ab}
21.	Pericarp thickness (mm)	2.00 ^{bc}	2.20 ^c	2.16 ^{bc}	2.22 ^{cd}	2.46 ^{de}	2.25 ^{cd}	1.94 ^{ab}	2.06 ^{bc}	1.73 ^a	2.69 ^e
Seed (Refer Figure 4.6)											
22.	Seed diameter (mm)	4.80 ^e	4.44 ^c	4.60 ^d	4.60 ^d	4.82 ^e	4.44 ^c	3.90 ^a	4.32 ^b	3.84 ^a	4.45 ^c
23.	Seed weight (x 10 ⁻²) (g)	6.11 ^g	5.13 ^e	5.40 ^f	4.85 ^d	5.46 ^f	4.91 ^d	4.07 ^a	4.30 ^b	4.56 ^c	4.98 ^{de}
Vigour											
24.	Branch column types	H	H	D	H	H	E	H	D	H	H
25.	Internode length (cm)	11.42 ^e	8.33 ^a	11.40 ^e	10.10 ^{cd}	8.73 ^{ab}	9.77 ^{bc}	9.57 ^{bc}	11.23 ^{de}	9.83 ^{bc}	12.7 ^f
26.	Number of node /feet of stem (average)	3.67 ^{ab}	4.73 ^f	3.33 ^a	4.13 ^{cdf}	4.17 ^{cdf}	3.83 ^{bc}	4.30 ^{de}	4.43 ^{ef}	3.97 ^{bcd}	3.33 ^a

Table 4.2 continued

Shoot tips

(Refer Figure 4.7)

27. Anthocyanin:

Absent or present	Present	Present	Present	Present	Absent	Present	Present	Present	Present	Present
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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'; E- Elliptical; O- Ovate; C- Cordate; L- Lanceolate; M- Mucronate; A- Acute; Ac- Acuminate; Ob- Oblique; R- Rounded; H- Horizontal; D- Drooping; E- Erect. Means followed by the different superscript letter within the same row are significantly different at $P \leq 0.05$.

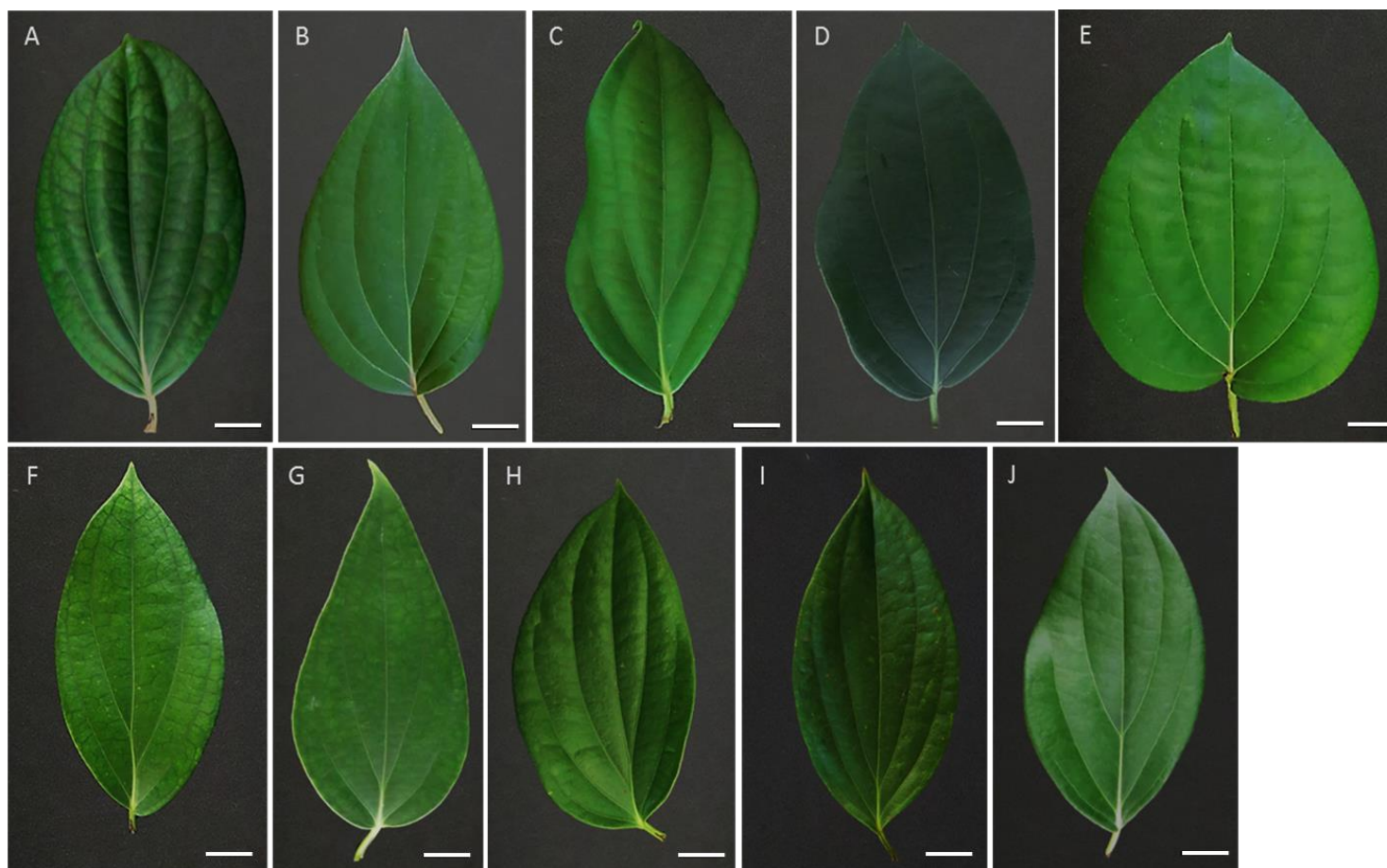


Figure 4.3: Cultivar designation and leaf shape. A. cv. 'Semongok Aman'- Elliptical. B. cv. 'Kuching'- Ovate. C. cv. 'Semongok Emas'- Elliptical. D. cv. 'Semongok Perak'- Elliptical. E. cv. 'Semongok 1'- Cordate. F. cv. 'Nyerigai'- Elliptical. G. cv. 'India'- Lanceolate. H. cv. 'Lampung Daun Lebar'- Ovate. I. cv. 'Sarikei'- Elliptical. J. cv. 'Yong Petai'- Elliptical. Scale bar: 1 cm.

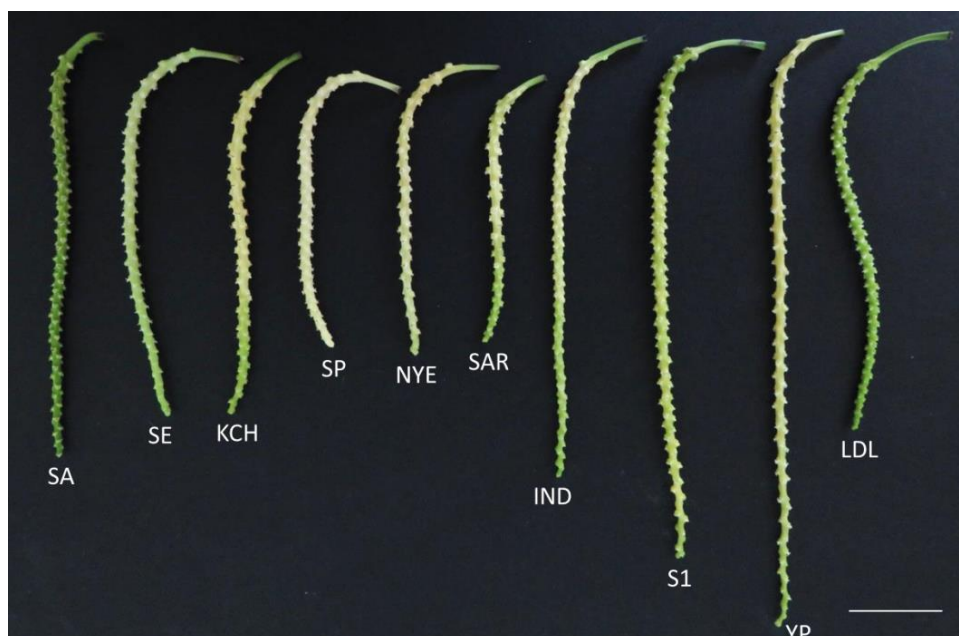


Figure 4.4: Inflorescence. SA - 'Semongok Aman', SE - 'Semongok Emas', KCH - 'Kuching', SP - 'Semongok Perak', NYE - 'Nyerigai', SAR - 'Sarikei', IND - 'India', S1 - 'Semongok 1', YP - 'Yong Petai' and LDL - 'Lampung Daun Lebar'. Scale bar: 2 cm.



Figure 4.5: 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: 2 cm.

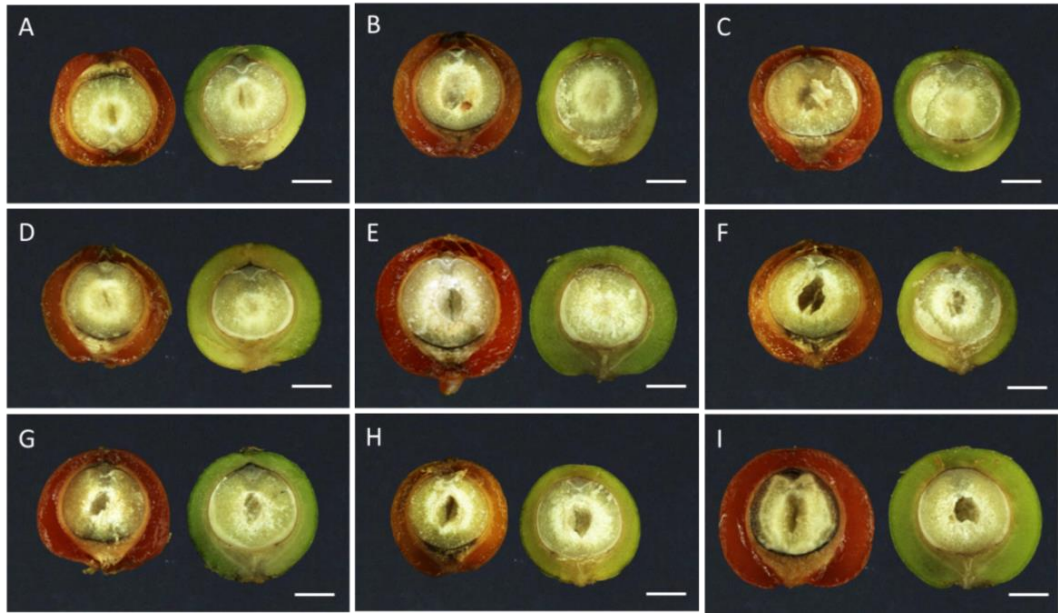


Figure 4.6: Cross section of ripe and mature fruit. A. ‘Semongok Aman’, B. ‘Kuching’, C. ‘Semongok Emas’, D. ‘Semongok Perak’, E. ‘Semongok 1’, F. ‘Nyerigai, IND - ‘India’, G. ‘Lampung Daun Lebar’, H. ‘Sarikei’ and I. ‘Yong Petai’. Scale bar: 2 mm.



Figure 4.7: 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: 1 cm.

The Whiskers plot analysis proved that the characteristic of leaf length-width ratio (Figure 4.8) and number of flowers per inflorescence (Figure 4.9) have seven significant difference groups, among the characteristics with highest distinctness values. Characteristics like leaf width (cm) (Figure 4.10), leaf area (cm²) (Figure 4.11), inflorescence/ spike thickness (mm) (Figure 4.12), number of inflorescence or spike per branch per node (Figure 4.13), percent of fruit set (Figure 4.14), fruit weight (single berry) (g) (Figure 4.15), internode length (cm) (Figure 4.16) and number of nodes per foot of stem (Figure 4.17) have six significant groups among ten cultivars.

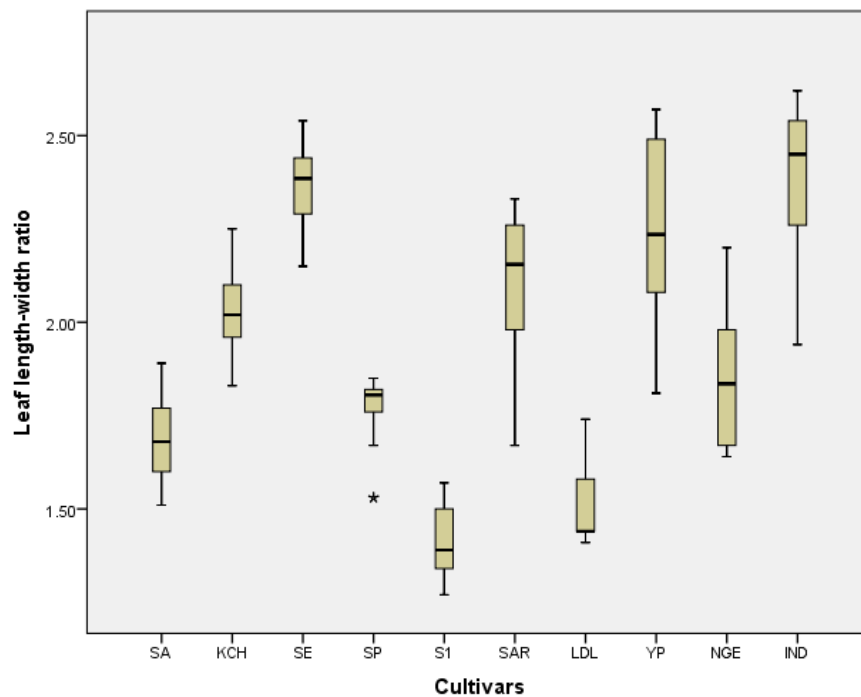


Figure 4.8: Leaf length-width ratio. Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error.

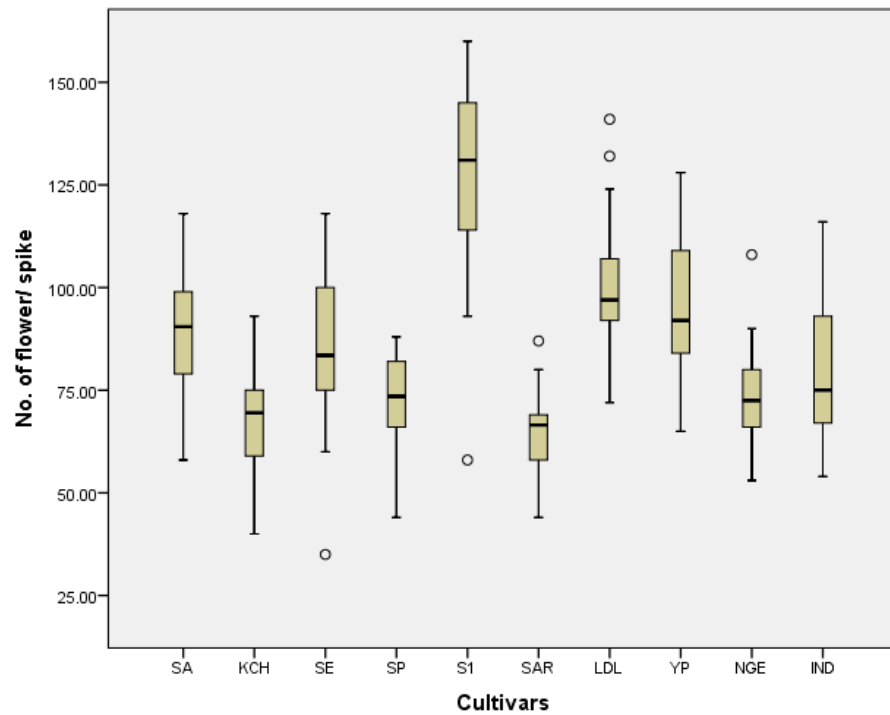


Figure 4.9: Number of flower per inflorescence. Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error. Outliers represented by open circles.

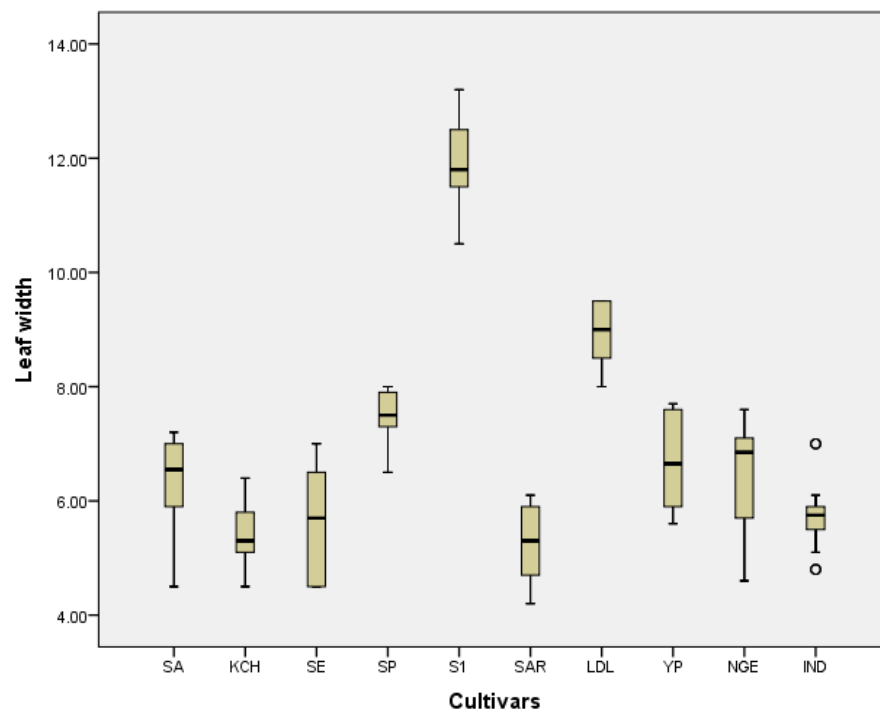


Figure 4.10: Leaf width (cm). Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error. Outliers represented by open circles.

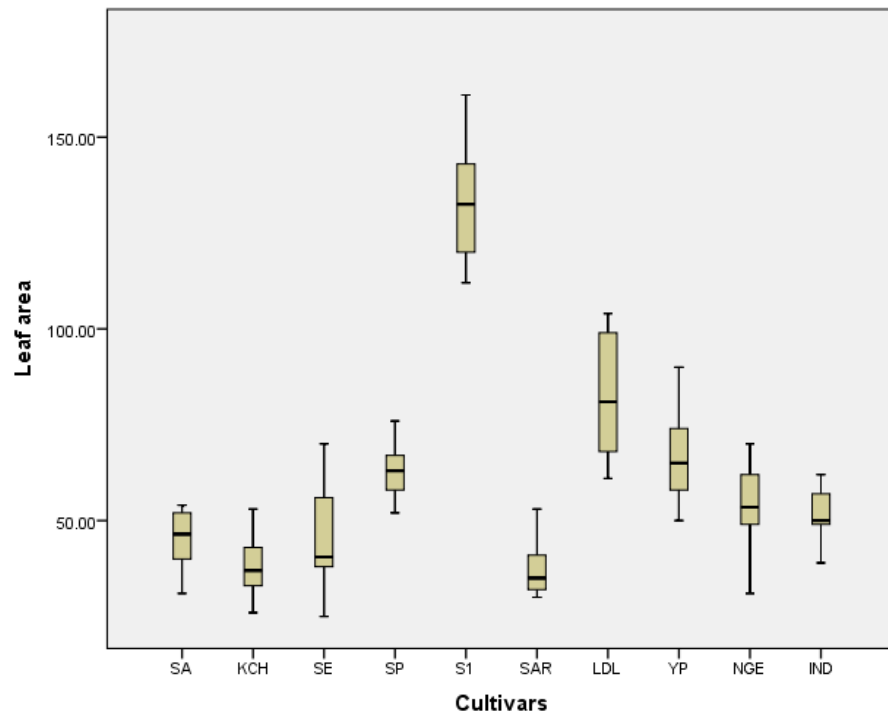


Figure 4.11: Leaf area (cm²). Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error.

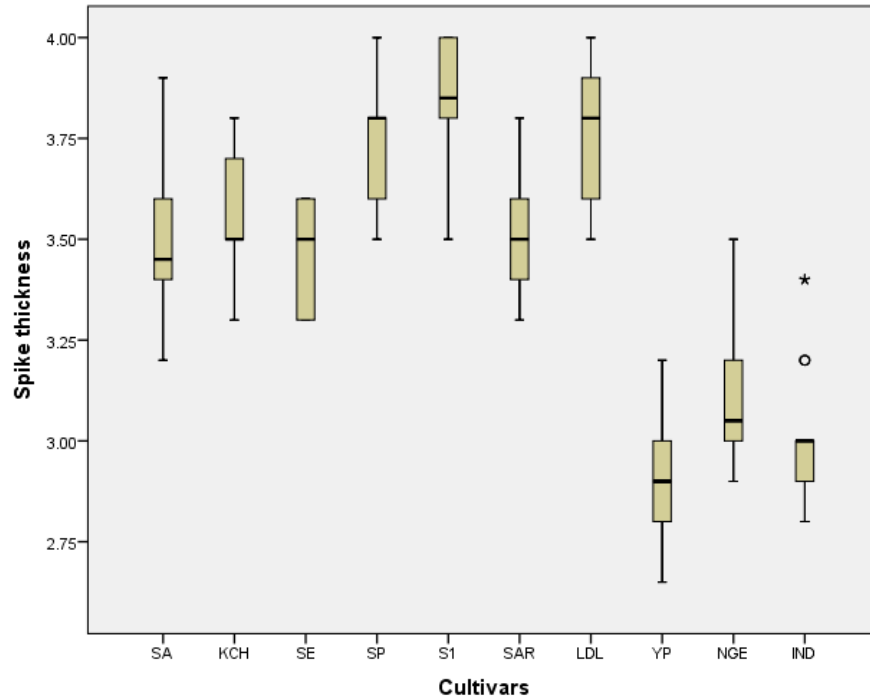


Figure 4.12: Spike or inflorescence thicknesses (mm). Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error. Outliers represented by open circles and asterisks.

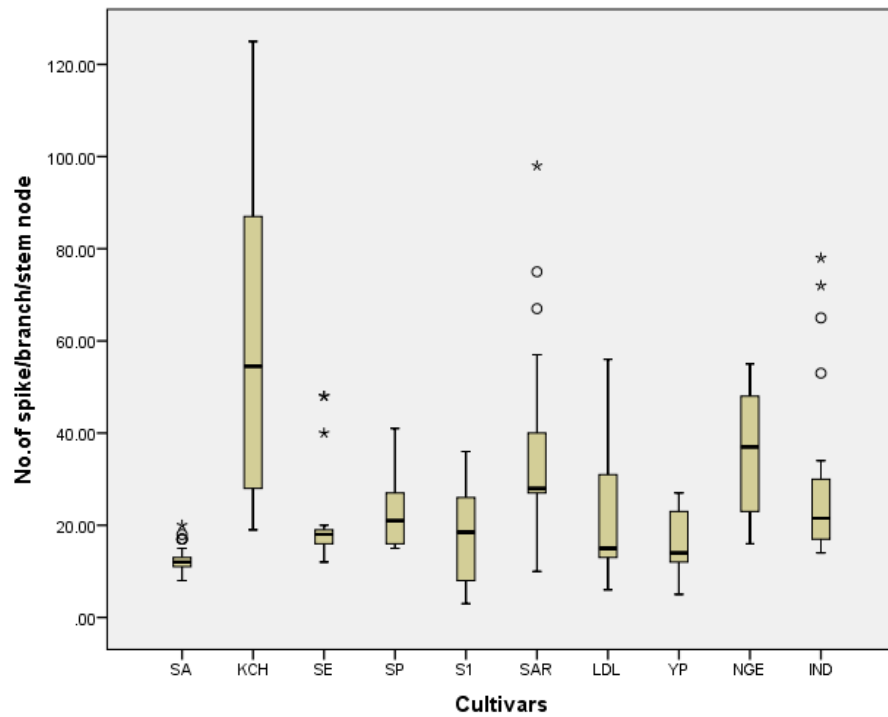


Figure 4.13: Number of inflorescence (spike) per branch per node. Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error. Outliers represented by open circles and asterisks.

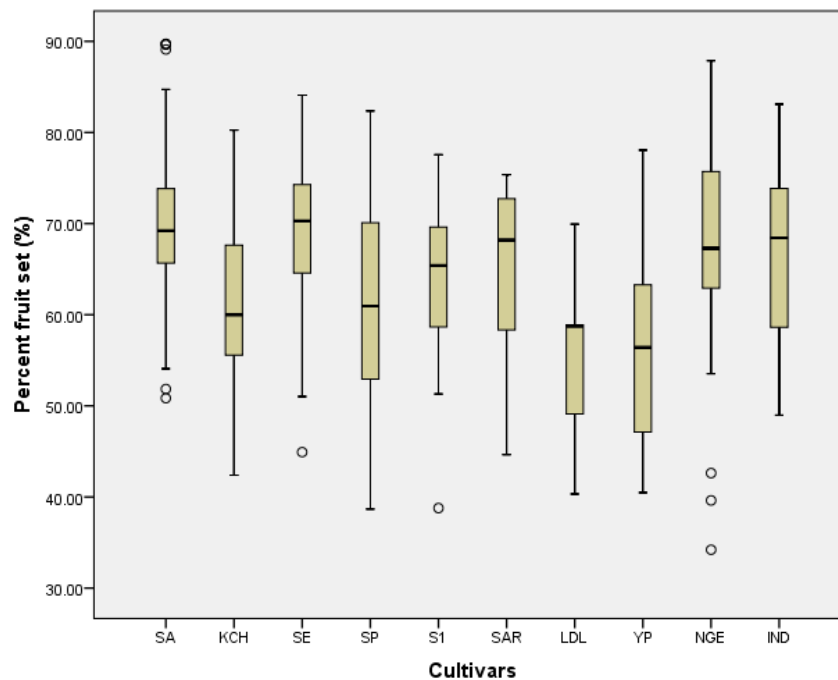


Figure 4.14: Percent fruit set (%). Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error. Outliers represented by open circles.

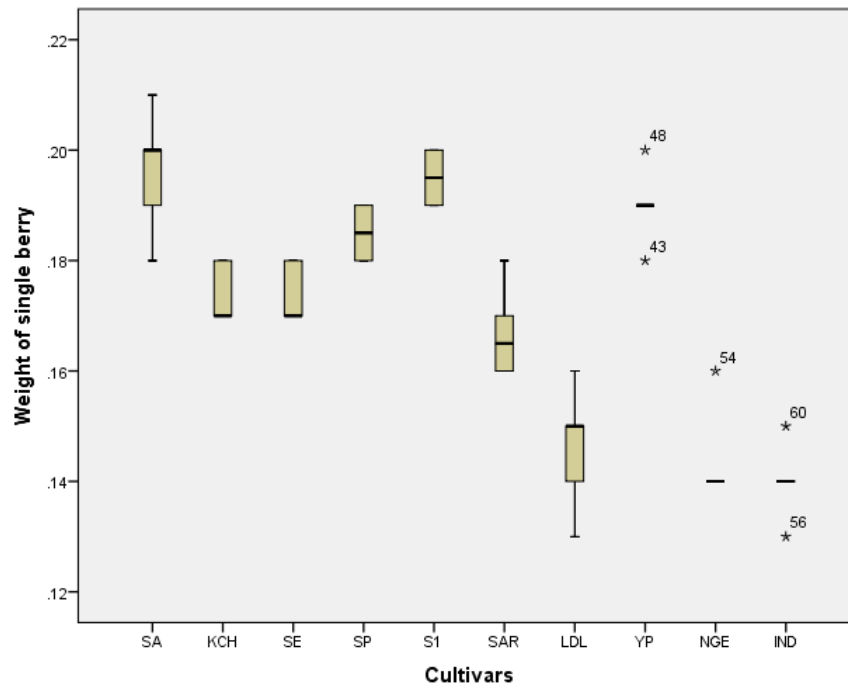


Figure 4.15: Fruit weight (single berry) (g). Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error. Outliers represented by asterisks.

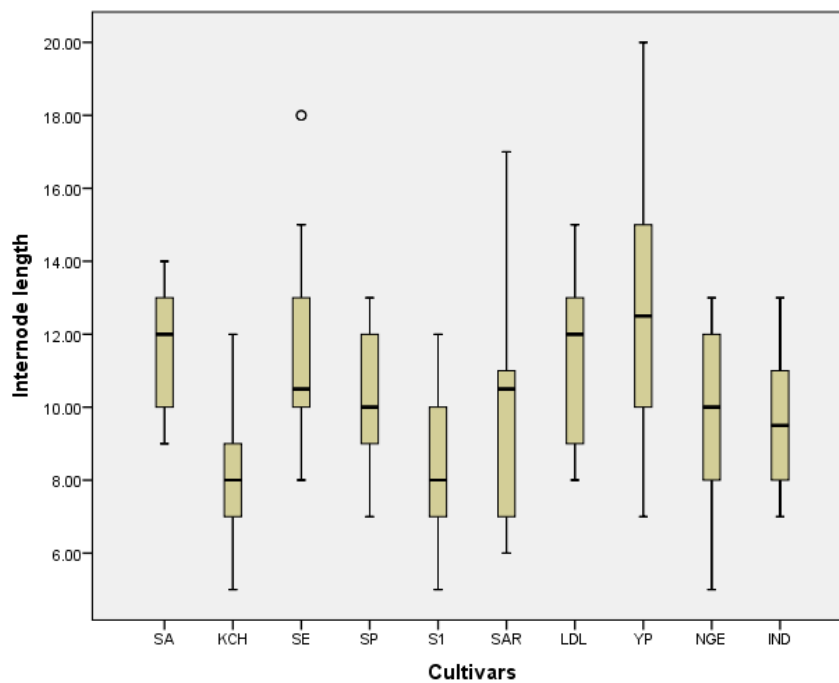


Figure 4.16: Internode lengths (cm). Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error. Outliers represented by open circles.

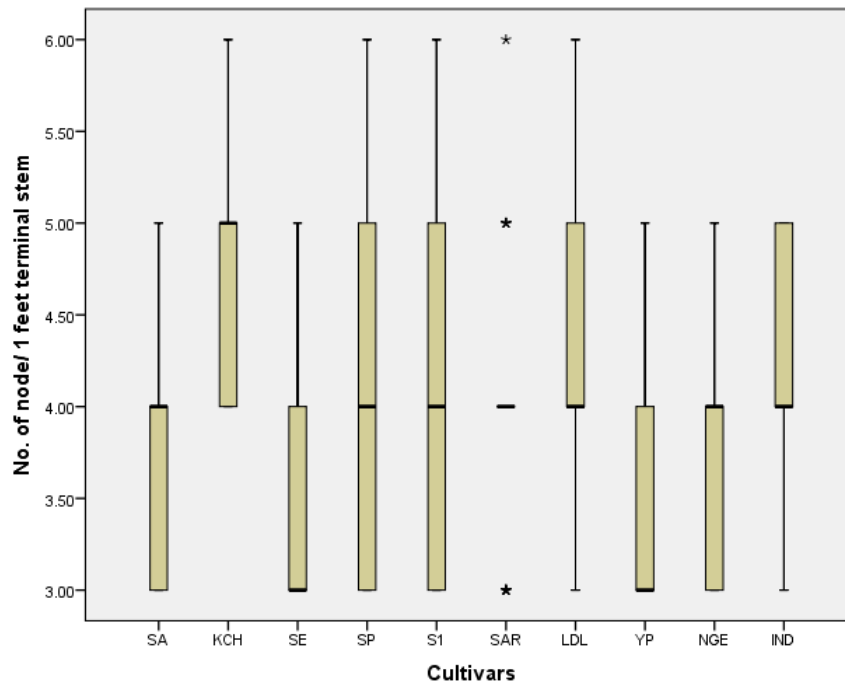


Figure 4.17: Number of nodes per foot of stem. Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error. Outliers represented by open circles.

Meanwhile, characteristics like leaf length (cm) (Figure 4.18), inflorescence length (cm) (Figure 4.19), conversion rate from fresh to dried white pepper (Figure 4.20), pericarp thickness (mm) (Figure 4.21), and seed diameter (mm) (Figure 4.22) have five significant groups. The analysis also showed that characteristics like single berry size in diameter (mm) (Figure 4.23), conversion rate from fresh to dried black pepper (Figure 4.24), fruit spike length (cm) (Figure 4.25) and seed weight (single berry) (g) (Figure 4.26) have only four significant groups. However, assessment on characteristics like number of branches per node, hilum-micropyle distance, petiole length (mm), blade thickness (mm), colour of immature fruit, colour of ripened fruit, seed shape, and venation pattern do not show a significant difference, thus are not included in the analysis.

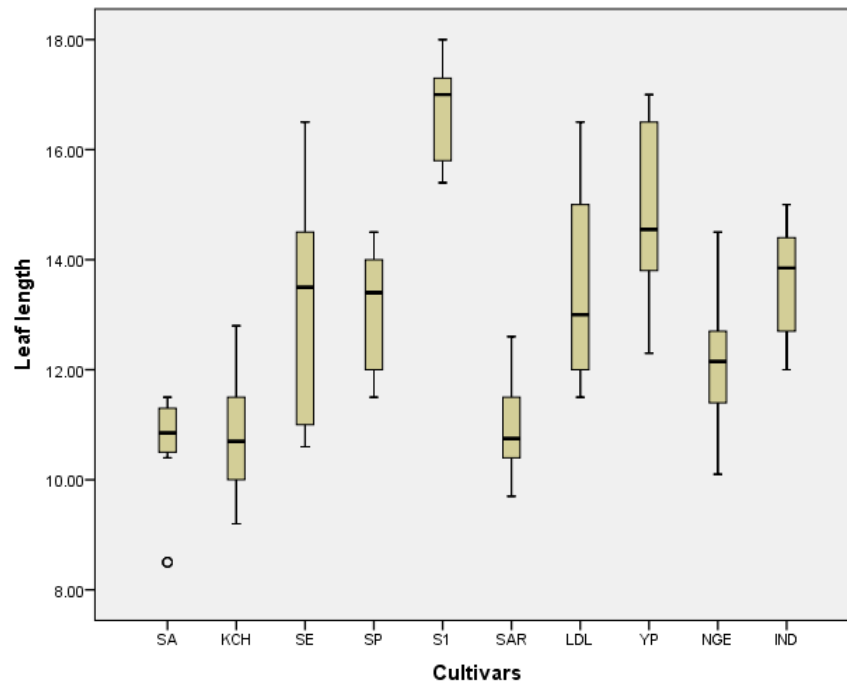


Figure 4.18: Leaf length (cm). Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error. Outliers represented by open circles.

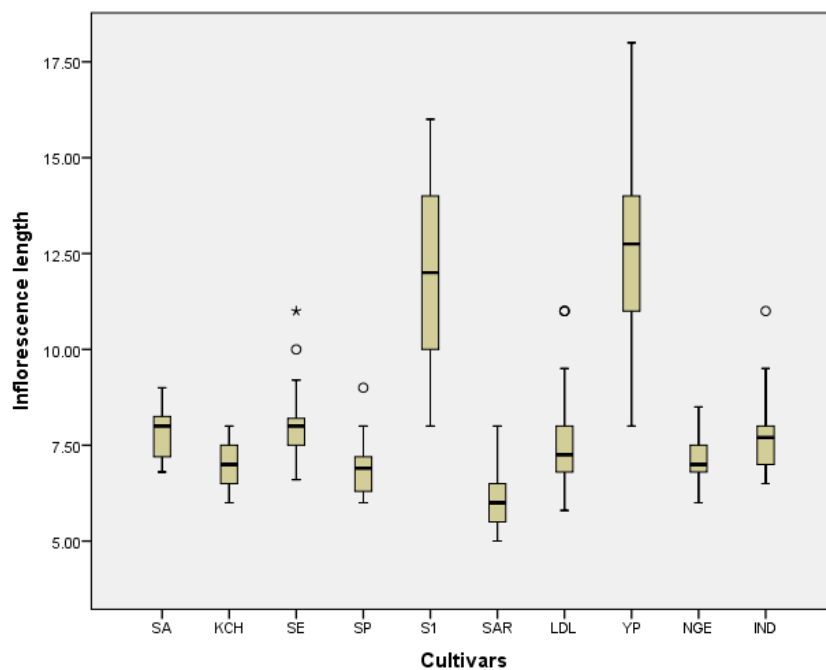


Figure 4.19: Inflorescence lengths (cm). Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error. Outliers represented by open circles and asterisks.

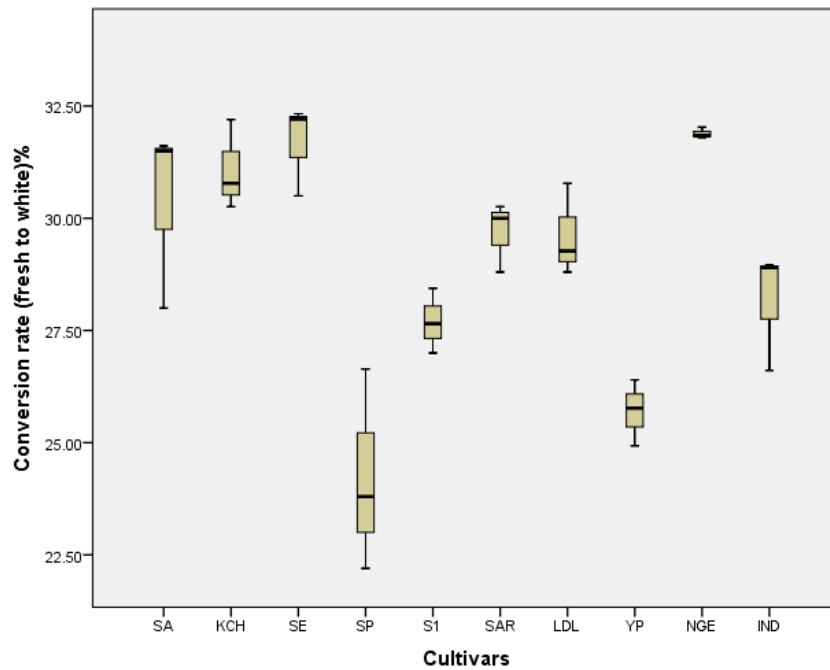


Figure 4.20: Fresh to dried (white pepper conversion rate %). Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error.

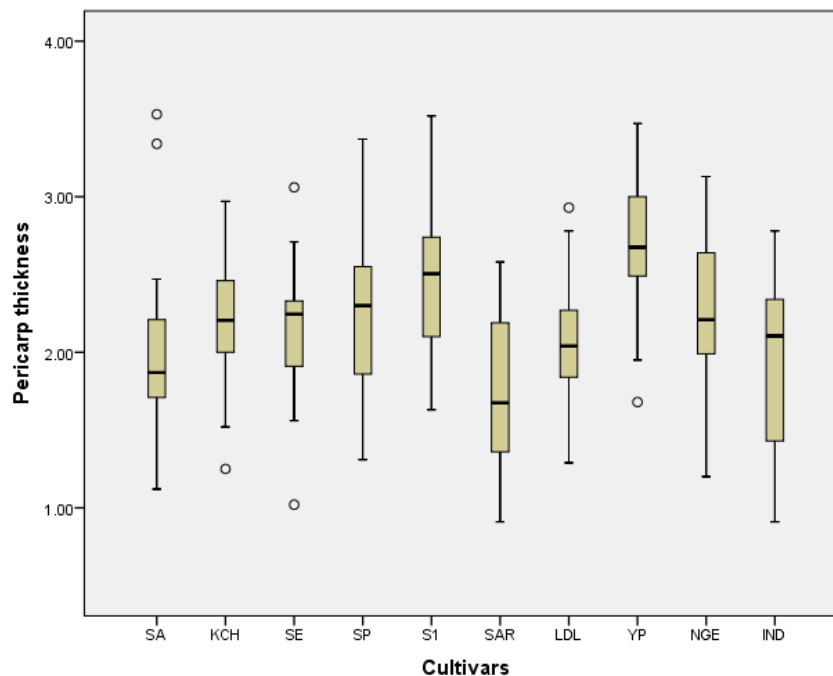


Figure 4.21: Pericarp thicknesses (mm). Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error. Outliers represented by open circles.

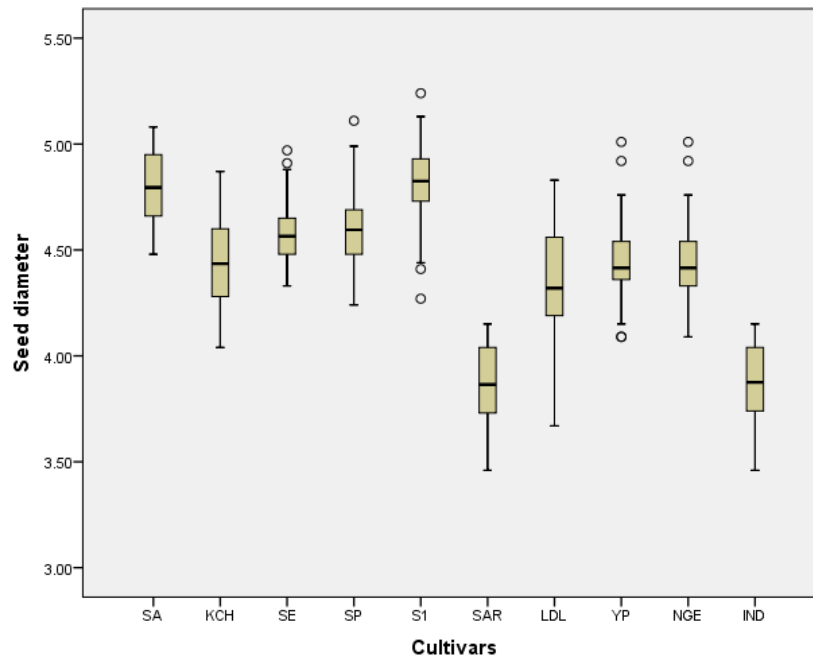


Figure 4.22: Seed diameter (mm). Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error. Outliers represented by open circles.

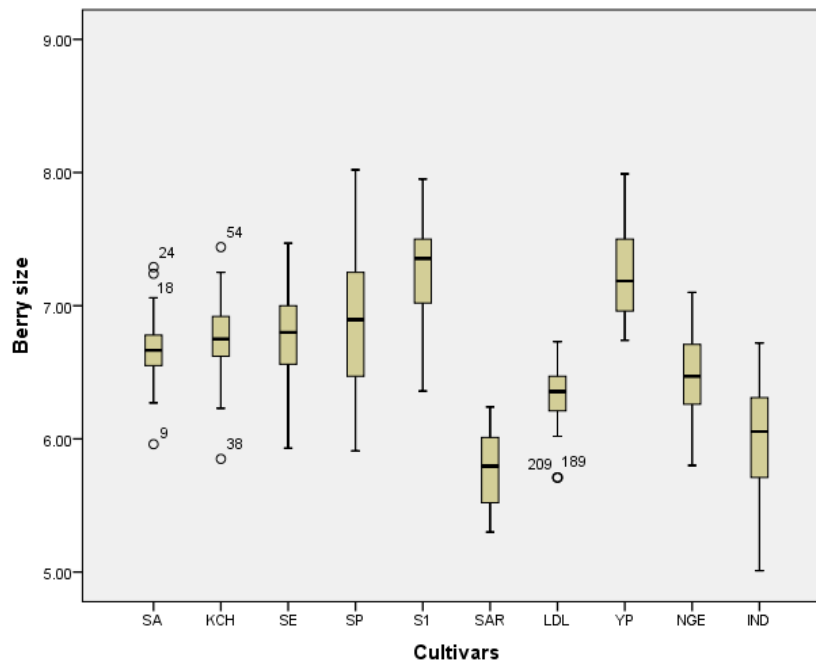


Figure 4.23: Single fresh berry sizes in diameter (mm). Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error. Outliers represented by open circles.

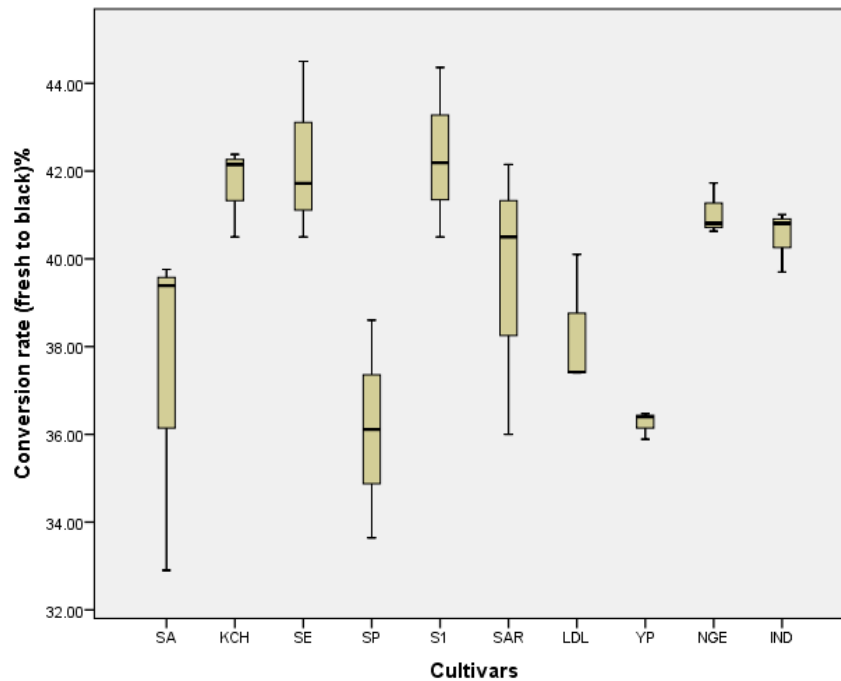


Figure 4.24: Fresh to dried (black pepper conversion rate %). Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error. Outliers represented by open circles.

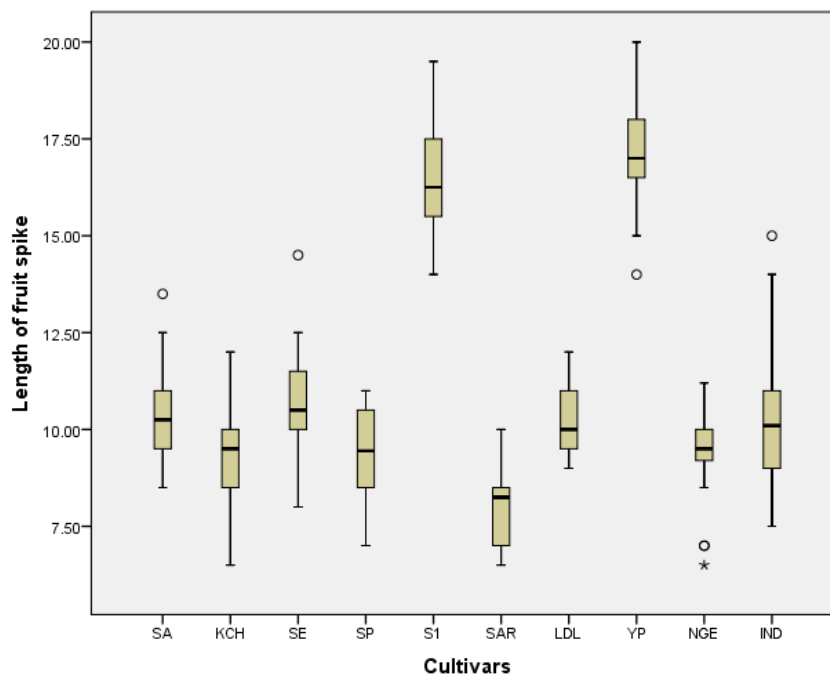


Figure 4.25: Fruit spike length (cm). Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error. Outliers represented by open circles and asterisks.

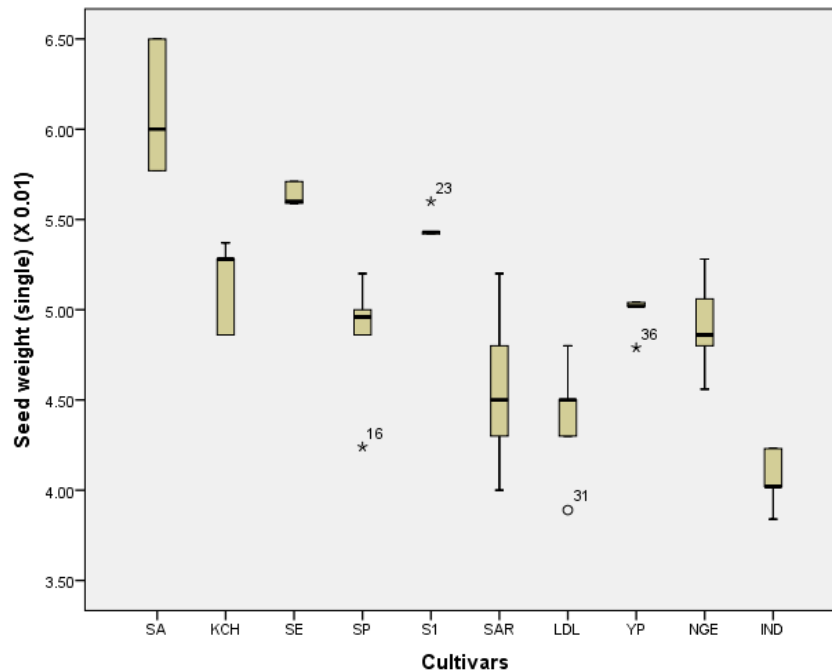


Figure 4.26: Seed weight (10^{-2}) (g). Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error. Outliers represented by open circles and asterisks.

Qualitative trait analysis showed leaf shape, leaf apex, leaf base, branch column types (branching behaviour), and anthocyanin colouration at shoot tip are crucial for diagnosing morphological differences among cultivars. Most of the cultivars exhibited lanceolate-ovate shaped leaf; however, cultivar ‘India’ has a lanceolate shaped leaf and cultivar ‘Semongok 1’ exhibited an ovate-shaped leaf. Thus, leaf shape distinctness is an important key diagnosis for the two cultivars. Leaf apex and leaf base observation showed cultivar ‘Semongok Aman’ was substantially distinct, with a rounded leaf apex and base; thus, the cultivar could be identified through the two traits easily. Only cultivar ‘Lampung Daun Lebar’ showed oblique shape in leaf base analysis, another important key diagnosis. In branch column analysis, most cultivars showed a horizontal type of branching, but 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 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, 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 colour intensity is influenced greatly by biotic and abiotic factors (Szakiel et al., 2011; Anita & Anna, 2012).

Qualitative traits are more influential (Olakojo & Adetula, 2014; Ali Khan et al., 2015; Stephan et al., 2016) compared to quantitative traits. However, qualitative traits alone with limited variability are insufficient for the diagnosis of ten black pepper cultivars. In this study, the ANOVA test proved the variability for quantitative traits (Table 4.2) was more substantial compare to qualitative traits. Analysis showed seed weight is among the most important quantitative trait, exhibiting seven significantly difference 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, percent fruit set (%), number of node/feet of 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.

4.3.1.2 Dichotomous Key for Cultivar Diagnosis

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 $<80 \text{ cm}^2$ (fully developed leaf from matured and vigorous vine); Number of flower per inflorescence less than 90----- 2
- 1b Leaf area $>80 \text{ cm}^2$ (fully developed leaf from matured and vigorous vine); Number of flower 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 $5.0-5.2 (x 10^{-2}) \text{ g}$ ----- ‘Kuching’
- 3b Pericarp thickness 1.6-1.8 mm thick; Seed weight $<4.8 (x 10^{-2}) \text{ g}$ ----- ‘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 ocute; 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’

4.3.1.3 Yield Potential Based on Morphological Analysis

The analysis of qualitative and quantitative trait variability not only eases identification of cultivars but also assist in making reliable assumption on yield potential for each cultivar. Several studies have been conducted on yield attributing factor for black pepper, including Mathai (1983), Ibrahim et al. (1985 and 1987), Sujatha and Namboodiri (1995) and Sim and Rosmah (2011). This chapter mainly discussed on yield attributing factor that could be evaluated through morphological analysis. Generally, the calculation of yield potential include three components of yield attributing factor, namely the vine factor that constituted by branching and inflorescence per branch; inflorescence factor that include number of flower per inflorescence and percent fruit set and fruit factor that comprised of berry weight and conversion rate (fresh to dried). The yield potential analysis based on morphological characteristics is in accordance to studies done by Mathai (1983) and Paulus (2011a) on vine factor particularly the bushiness of branching and inflorescence intensity per branch while Ibrahim et al. (1985 and 1987) and Sujatha and Namboodiri (1995) supported the inflorescence factor, especially the percent fruit set within spike. The fruit factor that constituted by fruit weight and conversion rate (fresh to dried berry) was take into account for the yield potential evaluation because the morphological analysis showed significant different among cultivars on these factors (Table 4.2). The formula that based on morphological attributing yield parameters is as below:-

$$\begin{aligned}
\text{Yield Potential Index} &= (\text{Vine yield attributing characters}) \times (\text{Inflorescence yield attributing characters}) \times (\text{Fruit yield attributing characters}) \\
&= (\text{Number of inflorescence/spike per branch per node} \times \text{Number of branch per feet of stem}) \times (\text{Number of flower per inflorescence} \times \text{Percent fruit set}) \times (\text{Fruit weight of single fresh berry} \times \text{Fresh to dry conversion rates } \%)
\end{aligned}$$

*Assumption: 1 feet length at middle region of mature vine exhibited uniform and stable growth characteristics.

*Formula is created based on morphological study by Mathai (1983); Ibrahim et al. (1985 and 1987); Sujatha and Namboodiri (1995); Sim and Rosmah, (2011) and outcome of this project.

Yield potential index (Table 4.3) has been evaluated for the ten cultivars based on formula as outlined above. Cv. ‘Kuching’ was found to have the highest yield potential compared to others cultivars. The high potential index is mainly contributed by high number of inflorescent per branch. Cv. ‘Semongok 1’ and cv. ‘Nyerigai’ occupied second and third position, attributed to high number of flower per inflorescence and high conversion rate for cv. ‘Semongok 1’ while high number of inflorescent per branch and high number of flower per inflorescence for cv. ‘Nyerigai’. Among all the cultivars, cv. ‘Yong Petai’ has the lowest yield potential index. This cultivar has long inflorescence and fruit spike, however does not showed high yield potential index mainly due to poor performance on vine attributing parameters (branching and inflorescence per branch) and low percent fruit set. Whilst, cv. ‘Sarikei’, cv. ‘India’, cv. ‘Lampung Daun Lebar’, cv. ‘Semongok Perak’, cv. ‘Semongok Emas’ and cv. ‘Semongok Aman’ ranked forth to ninth position respectively in the evaluation.

This evaluation is merely an assumption study to rank the yield potential among all cultivars based on morphological characteristics illustrated by black pepper cultivars. The actual yield of black pepper could be varied by soil fertility factor (Mathew et al., 1995), cultivation system (Nybe & Sujatha, 2008), maintenance quality (Pillai, 1977; Kurien & Nair, 1988; Mathai & Sastry, 1988; Azmil & Yau, 1993; Gunaratne & Heenkenda, 2004) and climate factor (Krishnamurthy et al., 2010). However, the physiological evaluation as reported in this thesis in Chapter 6 Physiological and floral biology of black pepper cultivars, aimed to further analyse the yield potential for the ten cultivars. The comparison between the two analyses will reveal more comprehensive conclusion on the yield potential.

Table 4.3: Yield potential evaluation for the ten cultivars based on morphological yield attributing characters.

Yield Attributing Characters	Cultivars									
	SA	KCH	SE	SP	S1	NYE	IND	LDL	SAR	YP
A) Vine yield characters										
i. Number of inflorescence (spike) per branch per node	12.47	58.67	19.57	22.20	17.60	35.83	27.37	21.47	34.73	16.20
ii. Number of branch per feet of stem (1 node develop 1 branch)	3.67	4.73	3.33	4.13	4.17	3.83	4.30	4.43	3.97	3.33
B) Inflorescence yield characters										
i. Number of flower per inflorescence	88.30	67.57	86.33	72.70	127.90	73.77	80.40	100.53	65.10	93.57
ii. Percent fruit set (%)	70.68	61.10	68.75	61.51	64.24	66.93	65.76	55.10	64.28	56.26

Table 4.3 continued

C) Fruit yield characters											
i.	Fruit weight of single fresh berry	0.20	0.17	0.17	0.19	0.20	0.14	0.14	0.15	0.17	0.19
ii.	Conversion rate (%) (fresh to black pepper)	37.35	41.68	42.24	36.12	42.35	41.06	40.51	38.31	39.55	36.25
Yield Potential Index = (Ai x Aii) x (Bi x Bii) x (Ci x Cii)		213.33 X10 ⁴	812.31 X 10 ⁴	277.72 X 10 ⁴	281.27 X 10 ⁴	510.74 X 10 ⁴	389.60 X 10 ⁴	352.49 X 10 ⁴	302.93 X 10 ⁴	387.73 X 10 ⁴	195.68 X 10 ⁴
Yield Potential Ranking		9	1	8	7	2	3	5	6	4	10

Note: Calculation assuming 1 feet length at middle region of mature vine exhibited uniform and stable growth characteristics; Conversion to dried black pepper adopted for this calculation. 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'

4.3.2 Phenetic Analysis

4.3.2.1 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 4.4, while the data matrix for phenetic character states is shown in Table 4.5. Characteristics used were all tested and showed significant differences between at least two cultivars under the ANOVA test using SPSS.

Cluster analysis is based on measuring the similarity between the Operational Taxonomic Unit (OTU). In this study, ten OTUs, cultivar ‘Semongok Aman’ (SA), cultivar ‘Kuching’ (KCH), cultivar ‘Semongok Emas’ (SE), cultivar ‘Semongok Perak’ (SP), cultivar ‘Semongok 1’ (S1), cultivar ‘Nyerigai’ (NYE), cultivar ‘India’ (IND), cultivar ‘Lampung Daun Lebar’ (LDL), cultivar ‘Sarikei’ (SAR), and cultivar ‘Yong Petai’ (YP), were compared. Each OTU was 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 (Peres-Neto et al., 2003).

Table 4.4: Characteristics and character states used for phenetic analysis of the ten black pepper cultivars.

No.	Characteristics and character states
Leaf characteristics	
1	Leaf shape: 1. Elliptical; 2. Ovate; 3. Cordate; 4. Lanceolate
2	Leaf apex: 1. Mucronate; 2. Acute; 3. Acuminate
3	Leaf base: 1. Acute; 2. Oblique; 3. Rounded; 4. Cordate
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) cm: 1. <6; 2. 6-8; 3. 8-10; 4. 10-12; 5. >12
7	Blade length (L) cm: 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 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 yellow green); 2. Yellow-green group N144 series (Strong yellowish green); 3. Yellow-green group 145 series (Strong yellow green)
11	Inflorescence thickness- 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 flower per inflorescence: 1. <80; 2.80-90; 3. 90-100; 4.>100
13	Number of inflorescence (spike) per branch per node: 1. <20; 2. 20-30; 3. 30-40; 4.>40

Table 4.4 continued

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) (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 (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
Branch/ stem 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 node /foot of stem: 1. 1; 2. 2; 3. 3; 4. 4; 5.5
Shoot tip	
27	Anthocyanin: 1. Absent; 2. Present

*RHS colour codes used.

Table 4.5: Data matrix corresponding to Table 4.4.

CV.	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	1	1	1	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	2	3	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	1	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	1	2	2	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	1	2	2	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	4	3	3	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	1	2	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	1	2	1	4	3	2	5	4	3	3	2	3	1	4	2	3	1	3	1	2	3	3	5	2	5	4	2

Note: CV.- Cultivar, 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’. Symbol (-) indicates not detected. Number 1-27 represent characteristics as listed at Table 4.4.

Referring to the phenogram of Figure 4.27, black pepper cultivars were clustered into five groups, with a similarity index $\geq 85\%$. The high similarity among cultivar of black pepper is in accordance to study by Ho et al. (2005). The team reported black pepper cultivar were genetically closely related in their evaluation of genetic relatedness among black pepper (*Piper nigrum* L.) accessions using direct amplification of the mini satellite-region DNA (DAMD).

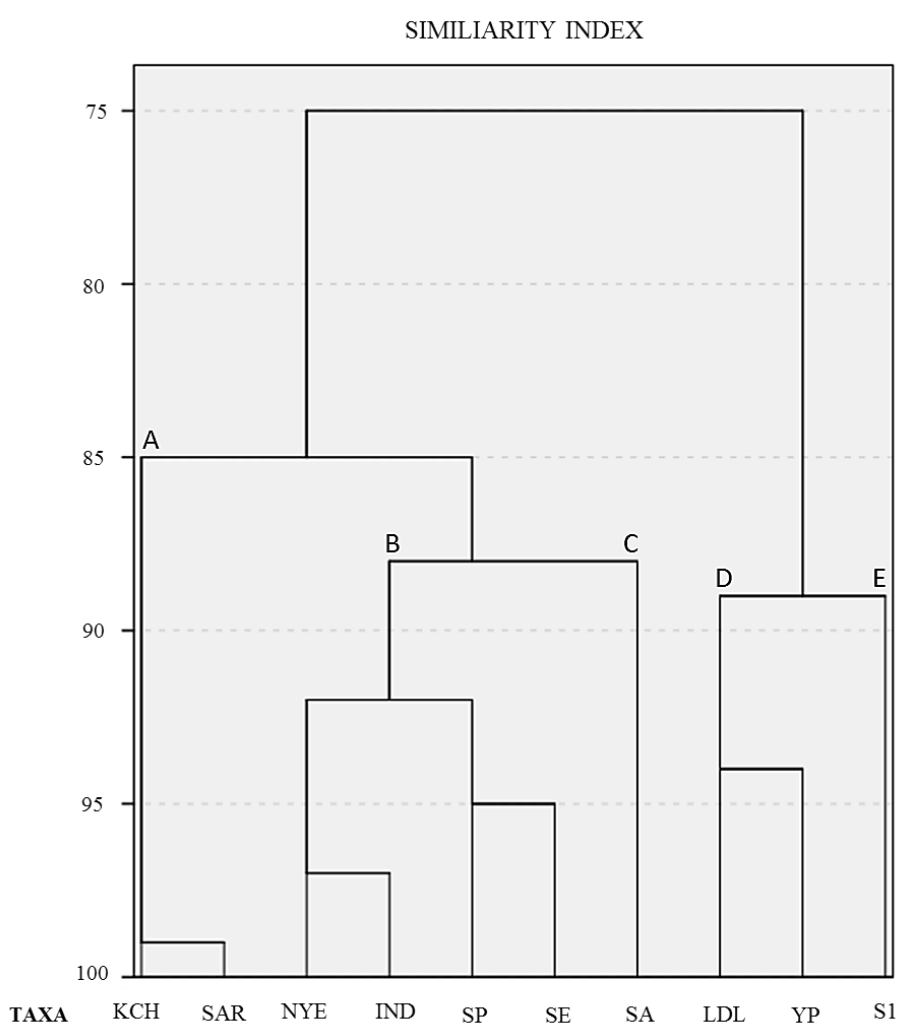


Figure 4.27: Phenogram presented based on average linkage (between groups) using Squared Euclidean Distance Method.

In this analysis, Cluster A comprised of two cultivars, ‘Kuching’ and ‘Sarikei’, with a similarity index as high as 99%. The two cultivars share 15 similar characteristics out of 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, comprised of four cultivars, ‘Nyerigai’, ‘India’, ‘Semongok Perak’, and ‘Semongok Emas’. The group is 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 hard dough stage), and Characteristic 23 (seed weight) are the important distinctive characteristics. Cluster C only consists of one cultivar, ‘Semongok Aman’. This cultivar is clustered alone with plenty distinct characteristics, even compared to the closest cluster (Cluster B), especially in Characteristic 2 (leaf apex), Characteristic 3 (leaf base), Characteristic 10 (inflorescence colour at 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 like Characteristic 8 (blade width-length ratio), Characteristic 10 (blade width-length ratio), Characteristic 11 (inflorescence thickness at withering stage), and Characteristic 14 (fruit spike length). Cultivar ‘Semongok 1’ is another cultivar clustered alone at cluster E. This cultivar shows 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 stem), if compared to the nearest cluster (Cluster D).

4.3.2.2 Principle Component Analysis

In this study, principle component analysis was performed using 27 morphological characteristics. Seven components were extracted as meaningful factors, with eigenvalues > 1 (Figure 4.28). These components explained 95.79% of the total variance, fulfilling the 95% confidence level mentioned by Jackson (1993) (Table 4.6). 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 (2014) explained that the PCA value can be categorized into three levels: the first level, with component value $X \geq 0.75$, has a very strong influence in the grouping; the second level, with a component value $0.50 \leq X < 0.75$, has a secondary influence in 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.

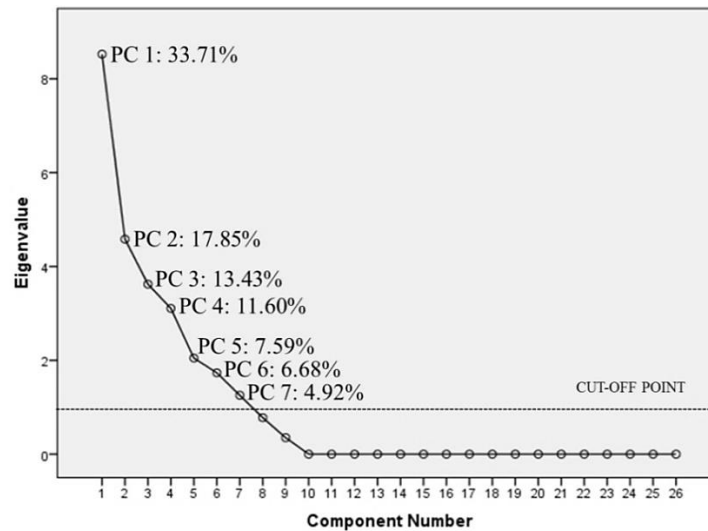


Figure 4.28: Scree plot showing cut-off point of extracted components for PCA of morphological characteristics. Component number 1-9 are principle component with variance while 10-26 are principle component with zero variance.

Table 4.6: 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

Note: PC- Principle component. PC1 (leaf area; inflorescence length; number of flower per inflorescence; fruit spike length; fruit size; number of inflorescence per branch), PC2 (leaf shape; leaf base; blade width; inflorescence thickness; anthocyanin; blade width-length ratio; number of nodes per foot of stem), PC3 (leaf colour; number of inflorescence per branch; fruit colour; seed weight; branch column type), PC4 (conversion rate- fresh to black; conversion rate- fresh to white; internode length), PC5 (inflorescence colour; internode length), PC6 (leaf apex; pericarp thickness; seed diameter) and PC7 (percent fruit set; seed diameter).

Based on PCA analysis (Table 4.7), 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 flower/inflorescence, fruit spike length, fruit size in diameter, fruit colour (hard dough stage), percent fruit set (%), conversion rate percent (fresh to black pepper), conversion rate percent (fresh to white pepper), pericarp thickness, seed weight, number of nodes per foot of stem, and anthocyanin colouration. This analysis also revealed that leaf, inflorescence, and fruit 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 other hand, the less important characteristics assisting in grouping are blade length and seed diameter, both with a PCA value lower than 0.60.

Table 4.7: Matrix component value for all the distinguishing characteristics.

	Principle Component (PC)						
	1	2	3	4	5	6	7
Leaf shape	.204	.807	-.101	.029	.078	.402	.043
Leaf apex	.279	.159	.213	-.455	-.151	.632	.432
Leaf base	.453	.696	-.107	-.167	-.367	.047	.360
LA (cm ²)	.868	.380	.072	-.166	-.080	.039	-.219
Leaf colour	.167	-.060	-.955	-.058	-.172	-.154	.017
Blade width (cm)	.538	.793	-.090	-.150	-.037	.113	-.036
Blade length (cm)	.531	.102	.497	-.056	.485	-.125	-.212
Blade width-length ratio	-.119	-.737	.167	.296	.261	-.337	.179
Inflorescence length (cm)	.948	.048	-.126	.154	-.026	.195	-.029
Inflorescence colour	-.011	-.125	-.171	-.033	.957	.019	-.150
Inflorescence thickness (mm)	-.220	.849	.334	-.191	-.093	.057	-.099
No. of flower/Inflorescence	.780	.393	.258	-.233	-.233	-.070	-.234
No. of Inflorescence/branch	-.621	-.155	-.523	.515	.020	-.045	-.220
Fruit spike length (mm)	.923	.150	-.193	.036	.132	.153	-.057
Fruit size in diameter (mm)	.848	.189	-.080	-.009	.298	.294	-.027
Fruit weight (single) (g)	.011	.343	-.355	-.689	.300	.035	.277
Fruit colour (hard dough stage)	.050	-.048	.932	.219	-.098	.105	-.023
Percent fruit set (%)	-.340	.060	-.196	.103	-.203	.020	.881
Conversion rate %(fresh to black pepper)	.240	.012	-.002	.922	.084	-.136	.209

Table 4.7 continued

Conversion rate % (fresh to white pepper)	-.189	-.231	.132	.824	.024	.439	.029
Pericarp thickness (mm)	.236	.276	.187	.056	.043	.890	-.033
Seed diameter (mm)	.423	.032	.092	.059	.477	.566	-.500
Seed weight (g)	-.169	-.279	.791	-.180	-.246	-.039	-.256
Branch column types	.122	-.357	.601	-.604	-.099	.341	.023
Internode length (cm)	-.167	.065	-.129	.713	-.628	-.076	.019
Number of node/foot of stem	-.496	-.789	.177	-.161	-.109	.148	-.185
Anthocyanin	.496	.789	-.177	.161	.109	-.148	.185

*Extraction Method: Principal Component Analysis.

*Rotation Method: Varimax with Kaiser Normalisation.

*Refer Appendix 1 for correlation matrix output.

This analysis helps to reveal diagnostic characteristics for black pepper cultivars. In PC1, the positive loading of characteristics, like leaf area, inflorescence length, number of flower per inflorescence, fruit spike length, and fruit size prove the proportional relationship among these characteristics, while the characteristic of number of inflorescence per branch has a negative loading in this PC. This explains that the cultivar has big leaf area, long inflorescence length, high number of flowers per inflorescence, long fruit spike length, and big fruit size, but low number of inflorescence per branch. This PC represents cv. ‘Yong Petai’ that showed plenty of unique characteristic for identification. PC2 has highlighted distinct characteristic of cv. ‘Semongok 1’. This PC showed a positive loading in characteristics like 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 stem. This PC reveals this cultivar has a very distinct leaf shape compared to other cultivars and the cultivar also tends to have a distinct leaf base, distinct leaf width, greater inflorescence thickness, and anthocyanin absent, but smaller width-length ratio and number of nodes per foot of stem. PC3 has a negative loading for two characteristics namely, leaf colour and number of inflorescence per branch, and positive loading for fruit colour, seed weight, and branch column type. This PC explains the unique qualitative characteristic of cv. 'Lampung Daun Lebar' that enables identification of this cultivar. PC4 most likely represents cv. 'Nyerigai'. This PC showed this cultivar has high conversion rate for both fresh to black or white, and also pretty high internode length. However, this cultivar has low fruit weight that attributed by negative loading in the analysis. PC5 showed high positive loading for inflorescence colour while negative loading for internode length. This PC represents cv. 'Semongok Perak' that showed distinct inflorescence colour, i.e. Green group 145 series, at the same time has low internode length. The sixth PC (PC6) only showed positive loading for three characteristics namely leaf apex, pericarp thickness and seed diameter. Cv. 'Semongok Aman' has fulfilled this PC, with rounded apex which is distinct among all cultivar and at the same time this cultivar has thick pericarp and big seed diameter. The last PC (PC7) showed only two meaningful components namely percent fruit set with positive loading and seed diameter with negative loading. Cv. 'India' has fulfilled the characteristic that has been extracted in this component. The principle component analysis is unable to extract interesting correlation on the morphological characteristics for cultivars like cv. 'Semongok Emas', cv. 'Sarikei' and cv. 'Kuching'. These cultivars have high similarity as proved in subchapter 4.2.2 Phenetic Analysis.

4.4 Conclusions

Qualitative and quantitative trait analysis and dichotomous key construction has assisted the diagnosis of ten important cultivars of black pepper in Malaysia. Besides cultivar identification, a yield potential formula based on morphological yield attributing factors has been created to evaluate yield potential among cultivars also, as outlined in Sub-chapter 4.3.1.3. This evaluation has concluded cv. ‘Kuching’ as high yield potential cultivar, followed by cv. ‘Semongok 1’ and cv. ‘Nyerigai’ at second and third position. The average yielder consists of cv. ‘Sarikei’ (4th position), cv. ‘India’ (5th position), cv. ‘Lampung Daun Lebar’ (6th position), cv. ‘Semongok Perak’ (7th position), cv. ‘Semongok Emas’ (8th position) and cv. ‘Semongok Aman’ (9th position) while the lowest rated cultivar is cv. ‘Yong Petai’.

Phenetic cluster analysis revealed that cultivars ‘Semongok Aman’ and ‘Semongok 1’ have 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 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. Cultivar ‘Lampung Daun Lebar’ and cultivar ‘Yong Petai’ have a similarity of 96%; however, the two showed distinctive differences on leaf width, leaf length-width ratio, spike thickness, and spike length characteristics. In Cluster A (Figure 4.25), cultivars ‘Kuching’ and ‘Sarikei’ showed the highest similarity index, thus are among the most difficult cultivars to diagnose morphological differences. 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, further discerning the distinctness of morphological characteristics among cultivars. This analysis has extracted seven meaningful principle components that assist identification of cv. 'Yong Petai', cv. 'Semongok 1', cv. 'Lampung Daun Lebar', cv. 'Nyerigai', cv. 'Semongok Perak', cv. 'Semongok Aman' and cv. 'India'. However, cultivars such as cv. 'Semongok Emas', cv. 'Sarikei' and cv. 'Kuching' are unable to determine through this analysis.

The findings of this study enable development of morphological marker for black pepper cultivar in Malaysia. This is prerequisite toward implementation of varietal regulation act in the country, at the same time served as important information for varietal improvement program in future. The overall phenetic analysis also showed that all the selected cultivars have distinct values enabling registration under the Malaysian Plant Variety Protection Act.

CHAPTER 5

PHYTOCHEMICAL CHARACTERIZATION OF BLACK PEPPER CULTIVARS

5.1 Introduction

Black pepper is not only valued for its aromatic and pungent properties, but also its medicinal properties (Krishnapura, 2009; Karsha & Lakshmi, 2010; Menghani et al., 2010; Butt et al., 2013; Meghwal & Goswami, 2013; Wong & Ling, 2014). The three main phytochemical compounds of black pepper are piperine, essential oil and oleoresin. The alkaloid compound, piperine, is the main compound that contributes to the pungency of black pepper (Krishnapura, 2009; Gorgani et al., 2017), while the aroma is attributed to the volatile oil found in the berry. Peppercorn has been shown to have positive effects on the digestive system (Bhat & Chandrasekhara, 1987; Platel & Srinivasan, 1996; Platel & Srinivasan, 2000). For example, pepper can influence intestinal motility, food transit time (Vazquez-Olivencia et al., 1992; Bajad et al., 2001a; Izzo et al., 2001; Platel and Srinivasan, 2001), gastric mucosa (Myers et al., 1987; Bai & Xu, 2000; Vasudevan et al., 2000; Ononiwu et al., 2002), absorptive function (Johri et al., 1992; Khajuria et al., 2002), and it contains antidiarrheal properties (Bajad et al., 2001b; Capasso et al., 2002).

The content of piperine found in berries or peppercorn extract ranges from 1.6% to 6.6% (Krishnamoorthy & Parthasarathy, 2009; Sruthi et al., 2013), essential oils 0.8%-3.6% (Schulz et al., 2005; Sruthi et al., 2013; Rezvanian et al., 2016), while oleoresin ranges from 6.19%-15.50% (Krishnamoorthy & Parthasarathy, 2009). In Malaysia,

analysis of piperine in black pepper berry was reported as 3.5% -7.81% (cv. ‘Kuching’), 3.4% - 5.88% (cv. ‘Semongok Emas’) and 5.4% - 6.8% (cv. ‘Semongok Aman’), while oleoresin for the three main cultivars were 11.00% -11.16%, 11.00% - 12.27% and 12.26% -15.50% respectively (Sim et al., 1996; Paulus, 2006). Sim et al. (1996) and Paulus (2006) also revealed that the volatile oil content of cv. ‘Kuching’, cv. ‘Semongok Emas’ and cv. ‘Semongok Aman’ were 2.8%-4.17%, 3.0%-6.50% and 3.8%-3.83%, respectively. However, the analysis was limited to only three common cultivars.

The constituents of phytochemicals of the berries have been intensively reported by Jirovetz et al. (2002); Nirmala Menon and Padmakumari (2005) and Wang et al. (2009). The volatile fraction of pepper oil is dominated by monoterpene hydrocarbons, sesquiterpenes, and oxygen-containing sesquiterpenes (Rmili et al., 2014), as listed in Table 5.1.

Table 5.1: The molecular structure and content of major monoterpene, sesquiterpenes and oxygen-containing sesquiterpenes in pepper berries.

Main Constituents of Volatile Oil	Molecular Formula	Content (%)
Monoterpene group		
Constituents: α -Pinene; β -Pinene; Limonene; Myrcene; α -Phellandrene; α -3-carene	$C_{10}H_{16}$	30-70%
Sesquiterpenes group		
Constituents: α -caryophyllene; β -caryophyllene; Germacrene D; (E)- β -Farnesene	$C_{15}H_{24}$	25-45%
Oxygen-containing Sesquiterpenes group		
Constituents: Caryophyllene oxide	$C_{15}H_{24}$	4-14%

This study focuses on the quantitative characterization of essential oil and piperine in peppercorn of Malaysian black pepper. The project outcome is crucial to improve pepper-based products, particularly in the pharmaceutical and nutraceutical industry.

5.2 Materials and Methods

5.2.1 Materials

The peppercorn samples were obtained from 10 cultivars as mentioned at chapter 3 and from five different farms located at Sarawak, namely Kampung Jagoi, Serikin; Kampung Karu, Padawan; Kampung Belawan, Sri Aman; Doha Kiasau, Tebedu and Agriculture Research Center (ARC) Semongok. Peppercorn collection was fixed at maturity of stage 12: ripe stage, as described by Sim (1979). To achieve a homogeneous sample to represent each variety, five multilocal samples of 200 gram each were mixed. In other word, five replications for each sample (cultivar) were achieved. The mixing was done to avoid locational and fruit maturity differences from influencing the accuracy of the analysis, as mentioned by Buckle et al. (1985) and Rezvanian et al. (2016).

Fresh samples were air-dried under the sun until the moisture content was below 13%. The dried peppercorns were grinded into a coarse powder (approximately particle size: 20 mesh) for extraction. Pure standard compounds for piperine, β -Caryophyllene, β -Pinene, Limonene, δ -3-Carene, α -Pinene, α -Humulene and Caryophyllene oxide were purchased from Sigma-Aldrich. A mass spectral database (HPCH2205.L and

Wiley7Nist05.L) were used to identify the composition of the rest of the chemical compounds.

5.2.2 Sample Preparation and GC Measurement

This analysis was performed at the accredited laboratory of Forest Research Institute Malaysia (FRIM) in 2017. The grounded berries samples were hydro distilled following the official ASTA method 5.0 (ASTA, 1997). The essential oils were subsequently analysed by using Gas Chromatography (Shimadzu GC-2010 Plus, fitted with HP-5MS), with temperature settings of program time - 3°C /min; initial temperature 60°C for 10 minutes; final temperature 230°C for 1 minute. The GC-MS analyses of the isolated essential oils were performed using Agilent 7890A/5975C, equipped with an HP-5MS column. The oven temperature was programmed from 60°C to 230°C at a heating rate of 3°C/min, and the final temperature was held for 1 min. The identification of compounds was based on relative retention time and mass spectra, which were compared by the mentioned pure standard compound, and also NBS75K and Wiley 138 library databases of the GC-MS system. The measurement of percentage composition was computed from the GC peak areas according to the 100% method without using any correction factors.

Aroma analysis was conducted also to compare the pleasant aroma index. The pleasant aroma index was based on concept by Schulz et al. (2005). The index was calculated via formula, (Aroma index = Pleasant Aroma Constituents Composition - Unpleasant Aroma Constituents Composition). The ranking based on pleasant aroma index among all the cultivars were the ultimate outcome of this study.

5.2.3 HPLC Analysis of Piperine

A total of 0.5g of sieved pepper powder (500 μm) was mixed with 5 mL of methanol, and the mixture was ultra-sonicated for 15 minutes. The resulting solution was filtered using a 0.45 μm syringe filter. The solution was diluted 20 times before analysis. The sample was analysed using HPLC system (Waters 2535 quaternary gradient module, Waters 2707 Auto sampler and Waters 2998 photodiode array detector), with reversed-phase column (Phenomenex Luna C18 column, 4.6 mm i.d. x 250 mm) coated with 184 silicone. For elution of the constituents, two solvents denoted as A (0.1% aqueous formic acid) and B (acetonitrile) were employed. The flow rate used was 1.0mL/min, and the injection volume was 10 μL . The retention times and UV spectra of the chromatographic peak for piperine were analysed in each of the samples. The gradient HPLC for the analysis as illustrated at Table 5.2.

Table 5.2: The gradient HPLC for the analysis.

Time (min)	%A	%B
0	40.00	60.00
15	30.00	70.00
17	0.00	100.00
25	0.00	100.00
33	40.00	60.00

Note: A= 0.1% aqueous formic acid; B=acetonitrile.

5.2.4 Statistical Analysis

Data were analyzed using one way analysis of variance (ANOVA) with the SPSS software (version 15, SPSS Inc., Chicago, USA). The DUNCAN Test, at $\alpha = 0.05$ level of significance was done to compare the means. All quantitative data were expressed as the mean $\pm$ standard deviation (SD). Principle component analysis (PCA) was performed on the essential oil constituents to identify the correlation among the constituents. The analysis is based on Rotated Component Matrix and Rotation Method adopted is Varimax with Kaiser Normalization. PCA extracted the valuable information from a multivariate data table and express this information as new variables (Jolliffe, 2002).

5.3 Results and Discussion

5.3.1 Essential Oil Analysis

In quantification analysis of essential oil among the cultivars, the ANOVA test showed eight groups that differ significantly in percentage content (Table 5.3). Cv. 'Semongok Aman' with 2.50% content is the highest yielder. They are followed by cv. 'Semongok 1' (2.26%), cv. 'India' (2.28%), and cv. 'Lampung Daun Lebar' (2.24%). The three cultivars with the second-best yields show no significant difference in essential oil. While, cv. 'Semongok Emas' (1.92%), cv. 'Yong Petai' (1.67%), cv. 'Semongok Perak' (1.25%), cv. 'Kuching' (1.18%) and cv. 'Sarikei' (1.10%) all contain less essential oil. The least essential oil cultivar is cv. 'Nyerigai', with a per cent yield as low as 0.54%. Essential oil contents analysis of black pepper reported 0.80-3.60 (mL/100 g) for commercial black pepper conducted in Germany (Schulz et al., 2005), 1.6%-3.2% in India (Sruthi et al.,

2013), 0.76%-1.06% in Peninsular Malaysia (Rezvanian et al., 2016) and 0.75%-1.90% in Brazil (Martinelli et al., 2017), that showed variability.

Table 5.3: Percent content of essential oil in peppercorn of the ten cultivars.

Variety	Essential oil content (%)	GC Chromatogram
SA	2.50±0.090 ^a	Appendix 2
KCH	1.18±0.016 ^f	Appendix 3
SE	1.92±0.010 ^c	Appendix 4
SP	1.25±0.009 ^e	Appendix 5
S1	2.26±0.009 ^b	Appendix 6
NYE	0.54±0.012 ^h	Appendix 7
IND	2.28±0.017 ^b	Appendix 8
LDL	2.24±0.012 ^b	Appendix 9
SAR	1.10±0.089 ^g	Appendix 10
YP	1.67±0.015 ^d	Appendix 11

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

The quantification estimation of essential oil constituents has also been studied in this project. A total of thirty one constituents (Table 5.4) have been identified in essential oil of black pepper cultivars, which constituted mainly by monoterpene hydrocarbons, sesquiterpene hydrocarbons and oxygenated terpenes. Analysis showed the main constituent of essential oil are β -Caryophyllene (17.42%-42.97%), β -Pinene (7.69%-21.11%), Limonene (9.48%-15.88%), δ -3-Carene (2.57%-20.58%), α -Pinene (2.82%-4.80%), α -Humulene (1.84%-3.23%) and δ -Elemene (0.29%-5.50%). This is in accordance to study done by Jagella and Grosch (1999); Jirovetz et al. (2002); Nirmala Menon and

Padmakumari (2005); Wang et al. (2009) and Rmili et al. (2014). These constituents generally give citrusy, woody, and floral notes (Jirovetz et al., 2002). In later year, Meghwal and Goswami (2013) has specifically analyse the aroma types, such as α -terpineol (Floral), Acetophenone (Irritant, sharp), Hexonal (Green apple), Nerol (Fresh, Floral, Herbal), Nerolidol (Mild spicy, Rooty), 1,8-cineol (Camphory), Dihydrocarveol (Warm, Woody), Citral (Citrusy), α -pinene (Terperic, Oxidised) and Piperolnol (Sweet, Floral).

Table 5.4: Essential oil constituent composition of the ten cultivars.

No.	Chemical name	% Composition									
		SA	KCH	SE	SP	S1	NYE	IND	LDL	SAR	YP
1	α -Thujene	1.09	0.34	0.96	0.62	0.42	0.06	0.99	1.50	0.08	1.34
2	α -Pinene	3.80	4.55	3.59	2.82	3.01	4.80	3.02	4.42	3.54	4.48
3	β -Pinene	18.99	13.07	13.75	9.19	13.48	9.09	16.58	21.11	7.69	18.84
4	Myrcene	1.17	2.00	1.55	1.22	1.17	2.10	1.05	1.32	1.60	1.69
5	α -Phellandrene	0.64	0.02	-	0.42	0.20	-	0.30	0.39	-	-
6	δ -3-Carene	6.22	16.93	17.62	7.09	2.57	20.58	4.75	2.41	20.29	11.66
7	Limonene	12.01	15.88	10.63	14.19	12.50	16.64	9.48	14.80	12.93	12.09
8	γ -Terpinene	0.24	0.13	0.38	1.07	0.03	-	-	1.02	0.10	0.33
9	Terpinolene	0.31	0.68	0.89	0.65	0.15	0.62	-	0.47	0.93	0.55
10	Linalool	0.26	0.39	0.14	0.42	0.10	-	0.79	0.54	0.50	0.92
11	Terpinen-4-ol	0.26	Trace	0.34	3.34	0.29	-	0.69	1.13	-	1.08
12	δ -Elemene	2.12	2.31	2.05	0.29	1.21	3.09	1.64	0.46	5.50	2.12
13	α -Cubebene	0.29	0.13	0.21	0.28	0.32	0.21	0.37	0.61	-	0.25
14	α -Copaene	0.29	1.95	3.70	4.99	5.10	2.13	4.63	-	2.86	3.41
15	β -Elemene	0.51	0.53	0.38	0.58	-	3.09	1.95	-	5.50	0.53
16	β -Caryophyllene	40.87	34.21	33.22	32.45	42.97	29.51	35.11	17.42	36.56	29.97
17	γ -Elemene	0.99	0.01	0.01	0.70	-	0.12	0.35	-	-	0.19
18	α -Humulene	2.43	1.84	1.98	2.11	2.59	1.96	2.47	3.23	Trace	1.84
19	(<i>E</i>)- β -Farnesene	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	2.14	Trace
20	Germacrene D	0.08	0.08	2.60	0.29	0.25	0.12	-	3.27	0.12	0.36
21	β -Selinene	0.60	0.25	-	0.50	0.52	0.61	3.37	-	0.61	0.41
22	α -Selinene	0.45	0.36	0.15	0.48	0.51	0.61	3.39	-	0.75	0.28

Note: SA- cv. 'Semongok Aman', KCH- cv. 'Kuching', SE- cv. 'Semongok Emas', SP- cv. 'Semongok Perak', S1- cv. 'Semongok 1', NYE- cv. 'Nyerigai', IND- cv. 'India', LDL- cv. 'Lampung Daun Lebar', SAR- cv. 'Sarikei' and YP- cv. 'Yong Petai'. Symbol (-) indicate not detected.

Table 5.4 continued

No.	Chemical name	% Composition									
		SA	KCH	SE	SP	S1	NYE	IND	LDL	SAR	YP
23	β -Bisabolene	1.95	0.53	1.99	2.03	4.96	0.40	-	4.95	-	1.20
24	α -Cadinene	-	0.84	1.70	2.92	3.61	1.51	2.96	-	1.59	1.83
25	Germacrene B	1.52	0.26	-	4.86	-	-	-	-	-	-
26	(E)-Nerolidol	2.15	0.57	-	0.98	-	-	-	-	-	-
27	Caryophyllene oxide	1.18	1.37	0.10	1.21	1.62	2.66	3.06	-	0.35	1.56
28	α -Zingiberene	-	-	-	-	-	-	-	4.04		-
29	(E)- γ -Bisabolene	-	-	-	-	-	-	-	Trace		-
30	Herdicaryol	-	-	-	-	-	-	-	5.53		-
31	α -Bisabolol	-	-	-	-	-	-	-	2.94		-

Note: SA- cv. 'Semongok Aman', KCH- cv. 'Kuching', SE- cv. 'Semongok Emas', SP- cv. 'Semongok Perak', S1- cv. 'Semongok 1', NYE- cv. 'Nyerigai', IND- cv. 'India', LDL- cv. 'Lampung Daun Lebar', SAR- cv. 'Sarikei' and YP- cv. 'Yong Petai'. Symbol (-) indicate not detected.

In this study, principle component analysis (PCA) has been conducted to analyse the correlation of essential oil constituent among the ten black pepper cultivars. The analysis was performed using 28 constituents as characters, namely α -Thujene, β -Pinene, α -Phellandrene, δ -3-Carene, γ -Terpinene, Terpinolene, Linalool, Terpinen-4-ol, δ -Elemene, α -Cubebene, α -Copaene, β -Elemene, γ -Elemene, α -Humulene, (E)- β -Farnesene, Germacrene D, β -Selinene, α -Selinene, β -Bisabolene, α -Cadinene, Germacrene B, (E)-Nerolidol, Caryophyllene oxide, α -Zingiberene, (E)- γ -Bisabolene, Herdicaryol and α -Bisabolol (Table 5.5). The remaining three constituents, α -Pinene, Myrcene Limonene and β -Caryophyllene were excluded from the analysis because all the cultivars fall under same character state (Table 5.6).

Table 5.5: Characteristics and character states of essential oil constituent of the ten cultivars for the PCA analysis.

No.	Characteristics and character states
1	α -Thujene: 1. <1.5; 2. 1.5-3.0; 3. >3.0
2	α -Pinene: 1. <0.70; 2. 0.70-1.40; 3. >1.40
3	β -Pinene: 1. <4.5; 2. 4.5-9.0; 3. >9.0
4	Myrcene: 1. <0.30; 2. 0.30-0.60; 3. >0.60
5	α -Phellandrene: 1. <0.20; 2. 0.20-0.40; 3. >0.40
6	δ -3-Carene: 1. <6.0; 2. 6.0-12.0; 3. >12.0
7	Limonene: 1. <2.40; 2. 2.40-4.80; 3. >4.80
8	γ -Terpinene: 1. <0.30; 2. 0.30-0.60; 3. >0.60
9	Terpinolene: 1. <0.26 ; 2. 0.26-0.52; 3. >0.52
10	Linalool: 1. <0.27; 2. 0.27-0.54; 3. >0.54
11	Terpinen-4-ol: 1. <0.36; 2. 0.36-0.72; 3. >0.72
12	δ -Elemene: 1. <1.74 ; 2. 1.74-3.48; 3. >3.48
13	α -Cubebene: 1. <0.20; 2. 0.20-0.40; 3. >0.40
14	α -Copaene: 1. <1.60 ; 2. 1.60-3.20; 3. >3.20
15	β -Elemene: 1. <1.80 ; 2. 1.80-3.60; 3. >3.60
16	β -Caryophyllene: 1. <8.52; 2. 8.52-17.04; 3. >17.04
17	γ -Elemene: 1. <0.33; 2. 0.33-0.66; 3. >0.66
18	α -Humulene: 1. <1.08; 2. 1.08-2.16; 3. >2.16
19	(E)- β -Farnesene: 1. <0.71; 2. 0.71-1.42; 3. >1.42
20	Germacrene D: 1. <1.09; 2. 1.09-2.18; 3. >2.18
21	β -Selinene: 1. <1.12 ; 2. 1.12-2.24; 3. >2.24
22	α -Selinene: 1. <1.13; 2. 1.13-2.26; 3. >2.26
23	β -Bisabolene: 1. <1.65; 2. 1.65-3.3 ; 3. >3.3
24	α -Cadinene: 1. <0.99; 2. 0.99-1.97; 3. >1.97
25	Germacrene B: 1. <1.62; 2. 1.62-3.24; 3. >3.24
26	(E)-Nerolidol: 1. <0.72; 2. 0.72-1.43; 3. >1.43
27	Caryophyllene oxide: 1. <1.02; 2. 1.02-2.04 ; 3. >2.04
28	α -Zingiberene: 1. Available; 2. Not available
29	(E)- γ -Bisabolene: 1. Available; 2. Not available
30	Herdicaryol: 1. Available; 2. Not available
31	α -Bisabolol: 1. Available; 2. Not available

Table 5.6: Data matrix corresponding to Table 5.5.

CV.	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	28	29	30	31	
SA	1	3	3	3	3	2	3	1	2	1	1	2	2	1	3	3	3	3	1	1	1	1	2	-	1	3	2	-	-	-	-	
KCH	3	3	3	3	1	3	3	1	3	2	1	2	1	2	3	3	1	2	1	1	1	1	1	1	1	1	2	-	-	-	-	
SE	1	3	3	3	-	3	3	2	3	3	1	2	2	3	1	3	1	2	1	3	-	1	2	2	-	-	1	-	-	-	-	
SP	1	3	3	3	3	2	3	3	3	2	3	1	2	3	1	3	3	2	1	1	1	1	2	3	3	2	2	-	-	-	-	
S1	1	3	3	3	2	1	3	1	1	1	1	1	2	3	-	3	-	3	1	1	1	1	3	3	-	-	2	-	-	-	-	
NYE	1	3	3	3	-	3	3	-	3	-	-	2	2	2	2	3	1	2	1	1	1	1	1	1	1	-	-	3	-	-	-	-
IND	1	3	3	3	2	1	3	-	-	3	2	1	2	3	2	3	2	3	1	-	3	3	-	3	-	-	3	-	-	-	-	-
LDL	2	3	3	3	2	1	3	3	2	2	3	1	3	-	-	3	-	3	1	3	-	-	3		-	-		1	1	1	1	
SAR	1	3	2	3	-	3	3	1	3	2	-	3	-	2	3	3	-	1	3	1	1	1		2	-	-	1	-	-	-	-	
YP	1	3	3	3	-	2	3	2	3	3	3	2	2	3	1	3	1	2	1	1	1	1	1	2	-	-	2	-	-	-	-	

Note: CV.- Cultivars, 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’. Symbol (-) indicates not detected. Number 1-31 represent characteristics as listed at Table 5.5.

Six components, namely PC 1 (α -Thujene; α -Zingiberene; (E)- γ -Bisabolene; Herdicaryol; α -Bisabolol), PC 2 (β -Pinene; β -Elemene; (E)- β -Farnesene; δ -Elemene), PC 3 (Terpinolene; δ -3-Carene; δ -Elemene; α -Humulene; β -Selinene; α -Selinene), PC 4 (γ -Elemene; (E)- Nerolidol; α -Phellandrene; Germacrene B), PC 5 (Linalool; β -Selinene; α -Selinene; Caryophyllene oxide; β -Bisabolene) and PC 6 (α -Copaene, α -Copaene, Germacrene B and Terpinen-4-ol) were extracted as meaningful factors, with eigenvalues > 1 (Figure 5.1). These components explained 93.20% of the total variance (Table 5.7). The first principle component (PC 1) explained 33.96% of the total variation, the second component (PC 2) explained 19.32% of the variation, and the third component (PC 3) explained 13.25% of the variation. Whilst, PC 4 to PC 7 responsible for 26.67% of the variation, with weightage of 10.76%, 9.36% and 6.55% respectively.

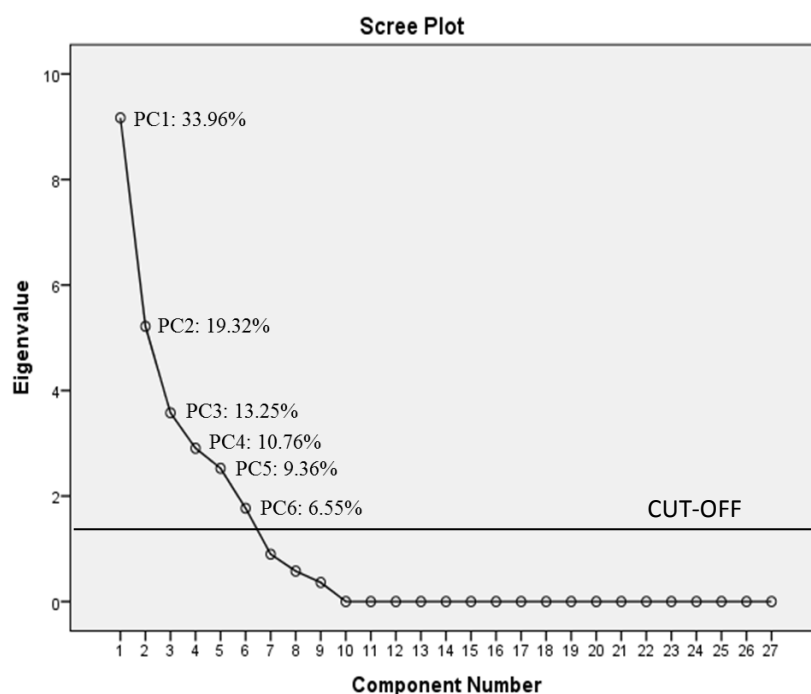


Figure 5.1: Scree plot showing cut-off point of extracted components for PCA of phytochemical analysis.

Table 5.7: Total variance obtained using Principle Component Analysis (PCA) for the essential oil constituent analysis.

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	Variance (%)	Cumulative (%)	Total	Variance (%)	Cumulative (%)	Total	Variance (%)	Cumulative (%)
PC 1	9.169	33.960	33.960	9.169	33.960	33.960	7.906	29.282	29.282
PC 2	5.216	19.320	53.280	5.216	19.320	53.280	4.296	15.913	45.194
PC 3	3.577	13.248	66.527	3.577	13.248	66.527	4.148	15.362	60.556
PC 4	2.906	10.763	77.290	2.906	10.763	77.290	3.174	11.755	72.311
PC 5	2.526	9.355	86.645	2.526	9.355	86.645	2.852	10.562	82.872
PC 6	1.768	6.550	93.195	1.768	6.550	93.195	2.787	10.322	93.195

Note: PC1-PC6 were extracted as meaningful factors.

PCA value can be categorized into three levels. The first level, with component value $X \geq 0.75$, has a very strong influence in the grouping; The second level, with a component value $0.50 \leq X < 0.75$, has a secondary influence in the separation of OUT and the third level, with the component value less than 0.50, has the least influence on separation at a minimal level (Pasagi et al., 2014). Matrix component value that has been generated (Table 5.8) showed some interesting correlation among the essential oil constituents that could assist identification of black pepper cultivars.

PC 1 showed strong positive correlation among α -Thujene, α -Zingiberene, (E)- γ -Bisabolene, Herdicaryol and α -Bisabolol, with PCA value of 0.984 respectively. Constituents such as γ -Terpinene, Terpinen-4-ol, α -Cubebene, Germacrene D and β -Bisabolene also showed second level of positive correlation. The strong positive correlation means all the constituents involved has high percentage of composition. This PC represents cv. 'Lampung Daun Lebar' that showed uniqueness in constituent composition compared to others cultivar. PC 2 has high positive loading for β -Pinene but high negative loading for β -Elemene and (E)- β -Farnesene. Negative loading at second level also recorded for δ -Elemene. This PC illustrated unique constituent composition of cv. 'Semongok 1'. Besides cv. 'Semongok 1' there are no other cultivars showed high β -Pinene, while both β -Elemene and (E)- β -Farnesene absent. PC 3 is best represents cv. 'India'. This PC showed negative loading for Terpinolene (first level), δ -3-Carene and δ -Elemene while positive loading for α -Humulene, β -Selinene and α -Selinene. In other words, peppercorn of cv. 'India' has relatively low content of Terpinolene, δ -3-Carene and δ -Elemene while relatively high for α -Humulene, β -Selinene and α -Selinene, which is

unique compare to others cultivars. The forth PC, PC4 showed positive first level loading for γ -Elemene, (E)-Nerolidol and α -Phellandrene while Germacrene B has second level positive loading. This composition is unique to cv. 'Semongok Perak'. PC 5 has highlighted the uniqueness of cv. 'Yong Petai' constituent composition. This cultivar showed relatively high Linalool, β -Selinene, α -Selinene and Caryophyllene oxide content however relatively low in β -Bisabolene. The last PC (PC 6) even though with low percentage of variance (6.55% only), but still representative to cv. 'Kuching' that showed availability (PC positive loading) of α -Copaene, α -Copaene, Germacrene B and Terpinen-4-ol at the same time, which is rare in other cultivars. This analysis however is unable to detect constituent composition uniqueness for cv. Semongok Aman, cv. Semongok Emas, cv. Nyerigai and cv. Sarikei, indicating the four cultivars identification through phytochemical constituent composition is unachievable.

Table 5.8: Matrix component value for all the distinguishing characteristics of essential oil constituent.

	Components					
	1	2	3	4	5	6
α -Thujene	.984	.049	.091	-.053	.019	-.125
β -Pinene	.051	.980	.165	.007	.054	.032
α -Phellandrene	.101	-.496	.248	.803	-.155	.107
δ -3-Carene	-.402	-.223	-.794	-.156	-.069	-.227
γ -Terpinene	.714	.173	-.209	.289	-.098	.475
Terpinolene	-.068	-.093	-.986	-.034	.046	.033
Linalool	.060	-.078	-.027	-.146	.909	.202
Terpinen-4-ol	.563	.233	-.066	.213	.418	.512
δ -Elemene	-.339	-.550	-.556	-.153	-.094	-.420
α -Cubebene	.681	.472	.324	.073	-.031	.134
α -Copaene	-.494	.115	.099	-.315	.064	.787
β -Elemene	-.187	-.849	.053	-.109	.347	-.097
γ -Elemene	-.167	.173	.142	.931	.085	.101
α -Humulene	.261	.561	.745	.164	-.061	-.174
(E)- β -Farnesene	-.051	-.980	-.165	-.007	-.054	-.032
Germacrene D	.681	.138	-.082	-.314	-.310	.037
β -Selinene	-.173	-.042	.660	-.040	.628	.101
α -Selinene	-.173	-.042	.660	-.040	.628	.101
β -Bisabolene	.554	.336	.272	.058	-.601	.241
α -Cadinene	-.370	-.086	.401	-.018	.094	.790
Germacrene B	.014	.116	-.271	.624	.065	.677
(E)-Nerolidol	-.136	.183	-.003	.899	-.218	-.237
Caryophyllene oxide	.343	.418	.387	.041	.593	-.179
α -Zingiberene	.984	.049	.091	-.053	.019	-.125
(E)- γ -Bisabolene	.984	.049	.091	-.053	.019	-.125
Herdicaryol	.984	.049	.091	-.053	.019	-.125
α -Bisabolol	.984	.049	.091	-.053	.019	-.125

Note: 1st level PCA- Bolded black font; 2nd level PCA- Normal font; 3rd level PCA- Grey coloured font.

*Extraction Method: Principal Component Analysis.

*Rotation Method: Varimax with Kaiser Normalisation.

*Refer Appendix 12 for correlation matrix output.

Based on the analysis of essential oil, the major monoterpene hydrocarbons compositions found in peppercorn are α -Pinene, β -Pinene, Limonene, δ -3-Carene, α -Thujene, Myrcene, α -phellandrene, Terpinolene, γ -Terpinene, Terpinen-4-ol and Linalool. An optimal aroma is obtained if the content of monoterpenes (except α - and β - pinene) is high and at the same time the amount of pinenes is low (Schulz et al., 2005). Aroma analysis based on monoterpene content (Table 5.9) reveals that cv. 'Nyerigai' has highest contain compared with the others with index of 26.11% (percent composition of pleasant aroma attributed monoterpenen), followed by cv. 'Sarikei' (25.20%) and cv. 'Kuching' (18.75%). Whilst, cv. 'Lampung Daun Lebar' (-11.95%), cv. 'India' (-1.55%) and cv. 'Semongok Aman' (-0.59%) all scored negative aroma index based on monoterpene content evaluation. In other word, cv. 'Nyerigai', cv. 'Sarikei' and cv. 'Kuching' have more pleasant aroma compared to other cultivars, while cv. 'Lampung Daun Lebar', cv. 'India' and cv. 'Semongok Aman' have less favourable aroma. This finding is very important to assist black pepper product diversification and marketing classification.

Table 5.9: Aroma analysis based on monoterpene constituents in essential oil of the ten cultivars.

No.	Monoterpene constituents	SA	KCH	SE	SP	S1	NYE	IND	LDL	SAR	YP
Pleasant aroma (Schulz et al., 2005)											
1	α -Thujene	1.09	0.34	0.96	0.62	0.42	0.06	0.99	1.50	0.08	1.34
2	Myrcene	1.17	2.00	1.55	1.22	1.17	2.10	1.05	1.32	1.60	1.69
3	α -Phellandrene	0.64	0.02	-	0.42	0.20	-	0.30	0.39	-	-
4	δ -3-Carene	6.22	16.93	17.62	7.09	2.57	20.58	4.75	2.41	20.29	11.66
5	Limonene	12.01	15.88	10.63	14.19	12.50	16.64	9.48	14.80	12.93	12.09
6	γ -Terpinene	0.24	0.13	0.38	1.07	0.03	-	-	1.02	0.10	0.33
7	Terpinolene	0.31	0.68	0.89	0.65	0.15	0.62	-	0.47	0.93	0.55
8	Linalool	0.26	0.39	0.14	0.42	0.10	-	0.79	0.54	0.50	0.92
9	Terpinen-4-ol	0.26	Trace	0.34	3.34	0.29	-	0.69	1.13	-	1.08
Total Percentage		22.20	36.37	32.51	29.02	17.43	40.00	18.05	13.58	36.43	29.66
Unpleasant aroma (Schulz et al., 2005)											
10	α -Pinene	3.80	4.55	3.59	2.82	3.01	4.80	3.02	4.42	3.54	4.48
11	β -Pinene	18.99	13.07	13.75	9.19	13.48	9.09	16.58	21.11	7.69	18.84
Total Percentage		22.20	17.62	17.34	12.01	16.49	13.89	19.60	25.53	11.23	23.32
Aroma index (%Pleasant aroma- %Unpleasant aroma)											
		-0.59	18.75	15.17	17.01	0.94	26.11	-1.55	-11.95	25.20	6.34

Note: SA- cv. 'Semongok Aman', KCH- cv. 'Kuching', SE- cv. 'Semongok Emas', SP- cv. 'Semongok Perak', S1- cv. 'Semongok 1', NYE- cv. 'Nyerigai', IND- cv. 'India', LDL- cv. 'Lampung Daun Lebar', SAR- cv. 'Sarikei' and YP- cv. 'Yong Petai'. Symbol (-) indicates not detected.

Essential oil consists mainly of monoterpenes, sesquiterpenes, and their derivatives that are responsible for the characteristic aroma, and imparts the identifying flavor and odor most closely associated with the plant itself. Essential oil has been widely used in sanitary products, cosmetic, perfume and make-up industries. The oil also used in food and beverage industries for fragrance purpose. Besides, the oil is important as warming and energizing oil to heal respiratory infections, and for soothing muscular aches and pains (Govindarajan, 1977; Yanishlieva et al., 2006). Other therapeutic properties include antibacterial, antifungal, antioxidant and insecticidal agents (Charles & Simon, 1990; Bakkali et al., 2008). With wide range of usage, essential oil industry has high investment potential. This study has identified cv. 'Semongok Aman' as the most viable cultivar for the production of essential oil. This is followed by cv. 'India', cv. 'Semongok 1' and cv. 'Lampung Daun Lebar'. In other words, these are the suitable cultivars to be invested for the purpose of essential oil extraction.

5.3.2 Piperine Analysis

Quantitative estimation of piperine in peppercorn extract was analysed in this study. Malaysian peppercorn piperine content was proven ranged from 1.58 - 4.25 %. DUNCAN test was performed to reveal the mean content different among ten cultivars of black pepper, as illustrated at Table 5.10. The results show that the piperine content (in percentage) for cv. 'Semongok Aman' (4.25%) and cv. 'Lampung Daun Lebar' (4.20%) is significantly higher compared to other cultivars. Cultivars like cv. 'Kuching', cv. 'Semongok 1', cv. 'India', cv. 'Sarikei', and cv. 'Nyerigai', with yields of 3.65%, 3.51%, 3.42%, 3.10% and 3.09% is the second-best group. The third-ranking group are cv.

‘Semongok Perak’ (2.54%) and cv. ‘Yong Petai’ (2.44%), which all has a slightly lower yield than the second group. Whilst, cv. ‘Semongok Emas’ with a content level of 1.58% yields the least among all cultivars. Rezvanian et al. (2016) reported piperine yields of peppercorn collected from Johor, Melacca and Sarawak is between 4.75%-5.85%. However, the evaluations do not separate the different cultivars. Sri Lankan research conducted in 1983 reported a piperine content range of 2-7% for Indian and Malaysian *P. nigrum* fruits, and 7-15% for Sri Lankan pepper (Jansz et al., 1983). More recently, a study conducted in India reported 2.49% - 4.49% of piperine content in black pepper (Sruthi et al., 2013). This revealed variation on piperine content between Malaysian cultivars and cultivars in other country. However, the analysis outcome may affect by locational differences, cultivar, agro climatic variation, ripening stages and method of oil extraction (Buckle et al., 1985; Rezvanian et al., 2016; Zachariah & Parthasarathy, 2008).

Table 5.10: Percent content of piperine in peppercorn of the ten cultivars.

Variety	Piperine content (%)	HPLC chromatogram
SA	4.25±0.398 ^a	Appendix 13
KCH	3.65±0.327 ^b	Appendix 14
SE	1.58±0.008 ^d	Appendix 15
SP	2.54±0.058 ^c	Appendix 16
S1	3.51±0.180 ^b	Appendix 17
NYE	3.10±0.257 ^b	Appendix 18
IND	3.42±0.338 ^b	Appendix 19
LDL	4.20±0.134 ^a	Appendix 20
SAR	3.09±0.207 ^b	Appendix 21
YP	2.44±1.162 ^c	Appendix 22

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

Piperine is responsible for the pungency of black pepper (Krishnapura, 2009; Gorgani et al., 2017). This study reveals that cv. 'Semongok Aman' and cv. 'Lampung Daun Lebar' produce more pungent peppercorn while cv. 'Semongok Emas' produces the least pungent peppercorn among the cultivars. Beside pungent property, the therapeutic properties of piperine are abundant (Gorgani et al., 2017). The major one includes immunomodulatory, anti-carcinogenic, stimulatory, hepatoprotective, anti-inflammatory by Darshan and Doreswamy (2004), antimicrobial by Yang et al. (2002), antiulcer activities by Bai and Xu (2000), biotransformative effects by Atal et al. (1985) and Wu (2007), protective effect against radiation by Raman and Gaikar (2002b), increases pancreatic lipase activity and stimulates pancreatic amylase, trypsin, and chymotrypsin by Platel and Srinivasan (2000) and reduction of blood cholesterol, triglycerides, and glucose by Mueller and Hingst (2013). The information of piperine yield on different cultivars is very crucial for pharmacological industry in ensuring better viability. This study has revealed cv. 'Semongok Aman' and cv. 'Lampung Daun Lebar' as favourable cultivar for this industry based on piperine content analysis.

5.4 Conclusions

GCMS and HPLC analysis reveal the composition of major phytochemical in peppercorn extracted from different cultivars. Cv. 'Semongok Aman' was found to have the highest content of piperine (4.25%) and essential oil (2.50%). However cv. 'Lampung Daun Lebar' is the only cultivar with relatively high content for all three components, i.e. piperine (4.20%), essential oil (2.24%) and oleoresin (11.13%). The constituent of volatile oil was also quantified. Important constituents identified are β -Caryophyllene (17.42%-

42.97%), β -Pinene (7.69%-21.11%), Limonene (9.48%-15.88%), δ -3-Carene (2.57%-20.58%), α -Pinene (2.82%-4.80%), α -Humulene (1.84%-3.23%) and δ -Elemene (0.29%-5.50%). Principle component analysis that has been conducted managed to detect the essential oil constituent uniqueness for cultivars such as cv. 'Lampung Daun Lebar', cv. 'Semongok 1', cv. 'India', cv. 'Semongok Perak', cv. 'Yong Petai' and cv. 'Kuching', as explained in sub-chapter 5.3.2. This study also revealed that peppercorn of cv. 'Nyerigai', cv. 'Sarikei' and cv. 'Kuching' have more pleasant aroma based on the monoterpenes content analysis. Adversely, cv. 'Lampung Daun Lebar', cv. 'India' and cv. 'Semongok Aman' were categorized as less favourable aroma group based on monoterpene index.

Even though sampling method in this study has minimized the factor contributing to variability of analysis, phytochemical content affected by many factors such as locational differences, cultivar, agro climatic variation, ripening stages and method of oil extraction (Buckle et al., 1985; Zachariah & Parthasarathy, 2008; Rezvanian et al., 2016). The legitimacy of the phytochemical analysis requires repeated verification.

CHAPTER 6

PHYSIOLOGICAL AND FLORAL BIOLOGY OF BLACK PEPPER CULTIVARS

6.1 Introduction

Crop productivity is mainly controlled by genetic factor (Poehlman & Sleeper, 1995; Dennis et al., 2007) but subject to environmental factor to reach its genetic potential. Generally, productivity related traits that are influenced by genetic factors are gas exchange performance that constituted by net photosynthesis rate (A), stomatal conductance (g_s) and transpiration rate (E) and also biochemical constituents and pathway of plant (Poehlman & Sleeper, 1995). Photosynthetic performance has been proven positively correlated to yield and growth performance of crop (Murata, 1981; Ignacimuthu & Babu, 1989; El Sharkawy et al., 1993; De Tafur et al., 1997; Conforto et al., 2005; Long et al., 2006b). In black pepper, the positive correlation particularly on yield has been proven by Mathai (1983) and also Krishnamurthy and Chempakam (2009). Besides net photosynthesis rate, the leaf stomatal conductance (Ishihara & Saito, 1987; Hirasawa et al., 1988; Kusumi et al., 2012; Golam Sarwar et al., 2013; Sabagh et al., 2017) and transpiration rate (De Wit, 1958; Smeal et al., 1991; Ben-Gal & Shani, 2002; Madrid, 2013) were among the physiological yield attributing factors that related to plant productivity.

Varietal difference contributed to variation in physiological performance of crop (Thorne, 1963; Dornhoff & Shibles, 1970; Hirasawa et al., 2010). In black pepper, the physiological characterization among varieties was reported by Krishnamurthy and

Chempakam (2009) in India. They mentioned that the photosynthetic differences were observed between high and low yielding variety. However, there is no report on physiological comparison of black pepper cultivar in Malaysia so far. This particular study emphasized on physiological assessment to compare the yield potential of different cultivars. The Ten important cultivars were compared based on gas exchange performance that covering net photosynthesis rate, stomatal conductance and transpiration rate study. At the same time leaf area index (LAI) were also evaluated. The correlation between the yields attributing physiology parameters were also studied to reveal interaction that influence the productivity of black pepper. This study ultimate goal is to provide the black pepper farmer with yield potential particulars on each recommended cultivars.

In floral biology study, hybridization ensures a wide variety exists among the cultivars of pepper ensuring quality attributes and yield. High yielding pepper cultivars are needed to compensate high production cost constraints in Malaysia (Sim, 1993). Intervarietal hybridization has become the main thrust in the pepper breeding program since it was initiated in Agriculture Research Centre, Semongok of Sarawak in 1957. However, the breeding work is limited in achievement so far, with only one promising hybrid developed from crosses between ‘Balankotta’ and ‘Kuching’. The hybrid, named ‘Semongok Emas’ was released to farmers in 1991 (Sim, 1993). In India, two achievements through conventional breeding have been reported so far, i.e. ‘Panniyur 1’ and ‘Panniyur 3’, both from crosses between ‘Uthiaranotta’ and ‘Cheriyakaniyakadan’ (Ravindran et al., 1981). Limited achievement of conventional pepper breeding is possibly due to lack of fundamental information on the nature of the reproductive biology, particularly on pollen viability and stigma receptivity.

Ravindran et al. (1981), Sim (1985) and Chen et al. (2009) have reported similar artificial pollination procedures for pepper. In general, the artificial hybridization was carried out by inseminating the pollen suspension of the male plant onto the stigma of female plant. However, in these reports, there is no specification concerning the viable stage of pollen for collection and the receptive stage of the stigma. So, there is some doubt regarding the reliability of the procedure and the technique of artificial pollination. In addition, intricacy in artificial pollination of pepper is perhaps due to the catkin type of inflorescent, minute sized flower and lack of uniformity in emergence of anther and time of anthesis. A fundamental study in floral biology of the pepper was needed to ensure efficiency of conventional breeding. The objectives of this study were to determine the time of anthesis, the nature of pollen viability and the stigma receptivity of the pepper, while the aim was to improve the efficiency of conventional breeding.

6.2 Materials and Methods

6.2.1 Physiological Study

6.2.1.1 Gas Exchange Evaluation

6.2.1.1.1 Material Preparation

The experiment was conducted in month of July in 2018 at black pepper farm located in Kampung Belawan, Sri Aman as all the ten varieties were cultivated in this area. The cultivars selected for the assessments include the 10 cultivars as mentioned at chapter 3. All pepper vines involved in this study were aged three years and above, at fruit bearing maturity stage. The vines were maintained with standardized practice for more than six

months following standard practice as described by Paulus et al. (2011b), before the assessment. The assessment targeted on fully expanded young leaf positioned second to third leaf from distal end of plagiotropic branches (Figure 6.1). Selected leaf must be highly vigour and leaf surface free from dust contamination.

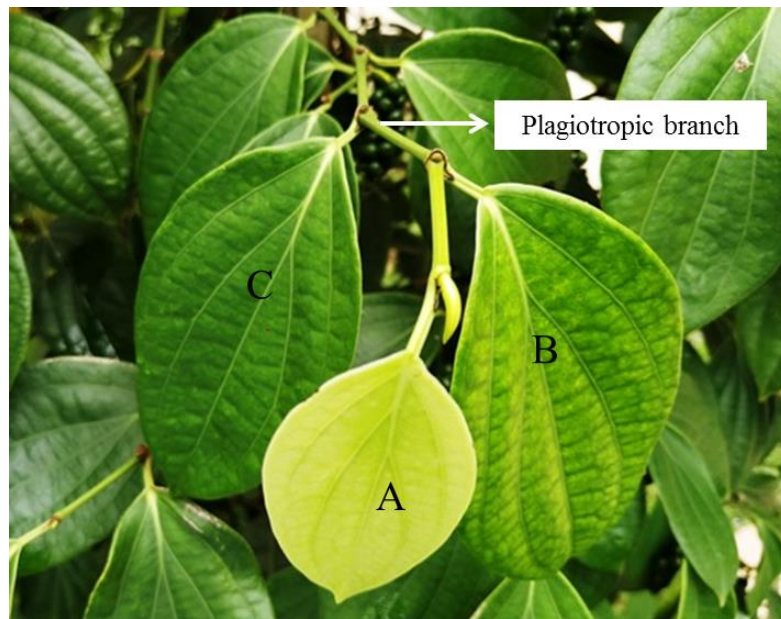


Figure 6.1: Plagiotropic branch of black pepper. A. 1st leaf; B. 2nd leaf and C. 3rd leaf.

6.2.1.1.2 Experimental Design for Gas Exchange Assessments

Gas exchange parameters including photosynthetic rate, transpiration rate and stomatal conductance rate were determined in this study. All the measurements were done using a Portable Photosynthesis System (LI-COR LI-6400 Infrared Gas Analyzer, Lincoln, Nebraska, USA). Measurements of net photosynthesis on an area basis (A) ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), leaf stomatal conductance (g_s) ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) and transpiration rate (E) ($\text{mmol H}_2\text{O}$

$\text{m}^{-2}\text{s}^{-1}$) of fifteen (15) different leaves per variety were monitored. Light intensity (Photosynthetically active radiation, PAR) within the sampling chamber was set to PAR at $1500 \mu\text{mol m}^{-2}\text{s}^{-1}$ which was presumed to be the intensity where photosynthetic rates for black pepper would be maximal. The CO_2 flow into the chamber was maintained at a concentration of $400 \mu\text{mol mol}^{-1}$ (Sulok et al., 2014). The humidity flow into the chamber was fixed at $500 \mu\text{mol s}^{-1}$.

The treatments for this experiment is based on cultivar difference: (A) SA – fully expanded young leaf of cv. ‘Semongok Aman’; (B) KCH – fully expanded young leaf of cv. ‘Kuching’; (C) SE – fully expanded young leaf of cv. ‘Semongok Emas’; (D) SP – fully expanded young leaf of cv. ‘Semongok Perak’; (E) S1 – fully expanded young leaf of cv. ‘Semongok 1’; (F) NYE – fully expanded young leaf of cv. ‘Nyerigai’; (G) IND – fully expanded young leaf of cv. ‘India’; (H) LDL – fully expanded young leaf of cv. ‘Lampung Daun Lebar’; (I) SAR – fully expanded young leaf of cv. ‘Sarikei’ and (J) YP – fully expanded young leaf of cv. ‘Yong Petai’. All treatments were replicated 15 times for each farm, giving a total of 45 leaves altogether being assessed for each cultivar. Measurement was done between 1000 to 1200 h during stable saturating light conditions (Johnson & Murchie, 2011). The ambient temperature as long as in between $30\text{--}35^\circ\text{C}$ is acceptable, since optimum temperature for C3 photosynthesis is broad, in the range of $10\text{--}35^\circ\text{C}$ (Yamori et al., 2014).

6.2.1.1.3 Data Analysis

Gas exchange data were analyzed using one-way analysis of variance (ANOVA) with the SPSS software (version 15). The DUNCAN Test, at $\alpha = 0.05$ level of significance was done to compare the means and to determine whether there were any differences among treatments.

6.2.1.2 Leaf Area Index

6.2.1.2.1 Materials Preparation

The black pepper cultivars selected for the assessments include the 10 cultivars as mentioned at chapter 3. The assessment was carried out concurrent to gas exchange assessment on same vine and same plot, located at Kampung Belawan, Sri Aman.

6.2.1.2.2 Experiment Design for Leaf Area Index Assessment

Indirect measurement method was adopted to assess leaf area index (LAI) of black pepper cultivars by using Plant Canopy Analyser, LAI-2000 (LI-COR Inc., Lincoln, NE, USA). These methods are non-destructive, adopting principle of transmission of radiation through the canopy (Anderson, 1971; Ross, 1981) and based on a statistical approach to foliar element distribution and arrangement in the canopy (Jones, 1992). Measurements of LAI based on fraction of transmitted radiation that passes through a plant canopy in five zenith angles simultaneously, through a fish-eye light sensor (Bréda, 2003). The treatments were designed based on cultivar difference: (A) SA – matured vine of cv. ‘Semongok

Aman’; (B) KCH – matured vine of cv. ‘Kuching’; (C) SE – matured vine of cv. ‘Semongok Emas’; (D) SP – matured vine of cv. ‘Semongok Perak’; (E) S1 – matured vine of cv. ‘Semongok 1’; (F) NYE – matured vine of cv. ‘Nyerigai’; (G) IND – matured vine of cv. ‘India’; (H) LDL – matured vine of cv. ‘Lampung Daun Lebar’; (I) SAR – matured vine of cv. ‘Sarikei’; (J) YP – matured vine of cv. ‘Yong Petai’. All treatments were replicated 5 times at three different farms, giving a total of 15 vines altogether being assessed in this study. Measurements were done between 1000 to 1200 h under stable saturating light conditions. Data collection was completed in single day to avoid variability caused by sun light intensity uniformity factor.

6.2.1.2.3 Data Analysis

LAI were analyzed using one-way analysis of variance (ANOVA) with the SPSS software (version 15). The DUNCAN Test, at $\alpha = 0.05$ level of significance was done to compare the means and to determine whether there were any differences among treatments.

6.2.2 Floral Biology Study

6.2.2.1 Material Preparation

The experiment was carried out from March to July 2016. The plant materials used in this study were obtained from a plant house at the Malaysian Pepper Board and a pepper germplasm collection plot located at the Agriculture Research Center, Semongok. Ten pepper cultivars as mentioned at chapter 3 were evaluated. For each cultivar, ten plants

were planted in pots sized ten inches in diameter, with a potting mixture combination of topsoil, sand and peat moss at 1:1:1 ratio, to ensure a sufficient supply of research materials. The planting procedure adopted the standard practice as described by Paulus et al. (2011b) in 'Pepper Production Technology in Malaysia'.

6.2.2.2 Experiment Design and Data analysis

6.2.2.2.1 Pollen Morphology Study

Palynology study is crucial for identification of pollen. For this study, only cultivar 'Semongok Aman' was used. Previous research has proven the unlikeliness of morphological differences among pepper cultivars' pollen (Iljas, 1960; Martin & Gregory, 1962; Rahiman, 1981; Ravindran et al., 2000a). Anthers were collected from the mature flowers and stored in a plastic centrifuge tube filled with 70% alcohol. The collected anthers were crushed with a glass rod and the solution was filtered through fine meshes with a 0.32 mm aperture to collect pollen grains. The pollen grains were prepared for light and scanning electron microscopy (SEM) by the standard method as described by Arora and Modi (2008). For the SEM study, pollen grains were suspended in a drop of ethanol and directly transpired with a fine pipette to a metallic stub using double sided cello tape and coated with gold palladium in a sputtering chamber. The SEM examination was carried out on a LEO electron microscope (Model LEO 430) at Universiti Malaysia Sarawak. The terminology of the pollen characteristics and analysis used is in accordance with Bhattacharya et al. (2006) and Agashe (2006).

6.2.2.2.2 Anther Dehiscence

Observation of anther dehiscence was carried out in the first five months of 2016 at the plant house, Malaysian Pepper Board. The objective of this observation is to reveal the estimated time, to ease tediousness in checking the anthesis time. Normally, pollen dispersal under field conditions can be erratic over time and dependent on relative humidity and temperature (Yates & Darrel, 1993). In each month, ten days were randomly selected for carrying out the observation, regardless of weather condition. The anthers selected for the study were at the pre-dehiscence stage. Only two pair of anthers from single flower were examined per time. Observation, with one hour intervals each time, was carried out starting from early morning until anther dehiscence was noticed. The preliminary judgment on anther dehiscence was made on the basis that whitish powder-like pollens were seen by naked eyes. Then, the released pollens were confirmed through stereomicroscope observation.

6.2.2.2.3 Pollen Viability Study

Ten cultivars as mentioned at chapter 3 were evaluated in this study. Based on the finding of the anther dehiscence study, the pollen was collected at five plant growth stages to test for viability:

- Stage 1: Right after anther dehiscence;
- Stage 2: Five hours after anther dehiscence;
- Stage 3: Ten hours after anther dehiscence;
- Stage 4: Fifteen hours after anther dehiscence; and

- Stage 5: Twenty hours after anther dehisced.

The test was carried out *in vitro*. A moist condition for pollen germination was created by placing a glass slide on top of bent glass tubing, which was placed inside a petri dish with small amount of water added. This created a moist environment to maintain humidity of the medium to promote germination. Chen et al. (2009) in 2009, published the germination medium protocol for pollen viability test on *P. colubrinum*, which was then adapted to *P. nigrum* pollen germination. The optimized liquid medium consisted of 10% sucrose, 100 mg/L boric acid, and 300 mg/L calcium nitrate.

To initiate the observation, pollen grains at the desired stage were carefully transferred onto the liquid medium. The pollen of *P. nigrum* takes at least six hours to initiate germination following the methods by Chen et al. (2009). After six hours of treatment, the glass slide was taken out of the petri dish, and the germinated pollen were then fixed with Carnoy's fixative and stained with Safranin. Pollen grains were observable under the compound microscope (LEICA model DM 4000B) with 400X magnification at Malaysian Pepper Board central laboratory. Percentage of the germinated pollen was counted via microscope and the length of the pollen tube was measured via a reticule (built-in scale on eyepiece). For all five stages of anther dehiscence, one hundred grains of pollen for three replicated treatments of the ten cultivars were counted to obtain the percentage of pollen germination. For pollen tube length evaluation, 30 germinated pollen grains from three replications were calculated. Both the germination percentage and the

pollen tube length were evaluated to indicate the level of pollen viability. The data were analyzed by one-way ANOVA to identify the most viable stage of pollen.

6.2.2.2.4 Stigma Receptivity Study

This study was performed on the ten cultivars to check the receptivity difference. The receptivity is indicated by production of peroxidase on stigmatic surfaces. The production of peroxidase is indicated by a formation of blue dot on stigmatic surfaces (Chen et al., 2009). The dissolved Peroxtesmo KO paper is needed because the stigma of pepper is so minute (about one millimeter in size) and fragile that the test cannot be done with direct contact of the stigma with Peroxtesmo KO paper. The Peroxtesmo KO solution was prepared with a concentration of 5 paper/1 mL of distilled water. Observation must be done immediately within five minutes, after the treatment using the Fluorescent Stereo Microscope Leica M165 FC at Malaysian Pepper Board central laboratory. The stigma at three distinctive stages was sampled for observation, i.e.

- Stage 1: First appearance of stigma (Day 1 of emergence);
- Stage 2: Elongation and spreading of stigma (Day 2-3 of emergence); and
- Stage 3: Complete emergence and wide spreading of stigma (Day 4-6 of emergence).

The description on stages of stigma was based on description by Sim in 1979. For each cultivar and each stage of stigma, 30 stigmas per inflorescence were collected for the test. One-way ANOVA statistical analysis was carried out to determine the most receptive stage of the stigma for the ten cultivars studied.

6.3 Results and Discussion

6.3.1 Physiological Study

6.3.1.1 Gas Exchange Evaluation

Gas exchange for the ten black pepper cultivars as mentioned at chapter 3 has been evaluated by using portable photosynthesis system, with aim to compare yield performance based on physiological characteristics. DUNCAN analysis (Figure 6.2) showed Malaysian black pepper photosynthetic performance ranged from $9.956 \mu\text{molCO}_2 \text{ m}^{-2}\text{s}^{-1}$ to $13.074 \mu\text{molCO}_2 \text{ m}^{-2}\text{s}^{-1}$. Five significantly different groups have been identified from the data analysis. The first group consisted of cultivar 'Kuching' ($13.074 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) while the second group constituted by cv. 'India' ($11.831 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) and cv. 'Nyerigai' ($11.175 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$). The average performers are cv. 'Semongok 1' ($11.074 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$), cv. 'Semongok Emas' ($10.827 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) and cv. 'Semongok Aman' ($10.691 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$), that ranked in third group. Photosynthetic performance of cv. 'Semongok Perak' ($10.333 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) falls in fourth group, with slightly lower performance compare to cv. 'Semongok Emas' and cv. 'Semongok Aman'. DUNCAN analysis revealed the poorest performing group consisting of cv. 'Yong Petai' ($10.054 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$), cv. 'Lampung Daun Lebar' ($10.004 \mu\text{molCO}_2 \text{ m}^{-2}\text{s}^{-1}$) and cv. 'Sarikei' ($9.956 \mu\text{molCO}_2 \text{ m}^{-2}\text{s}^{-1}$).

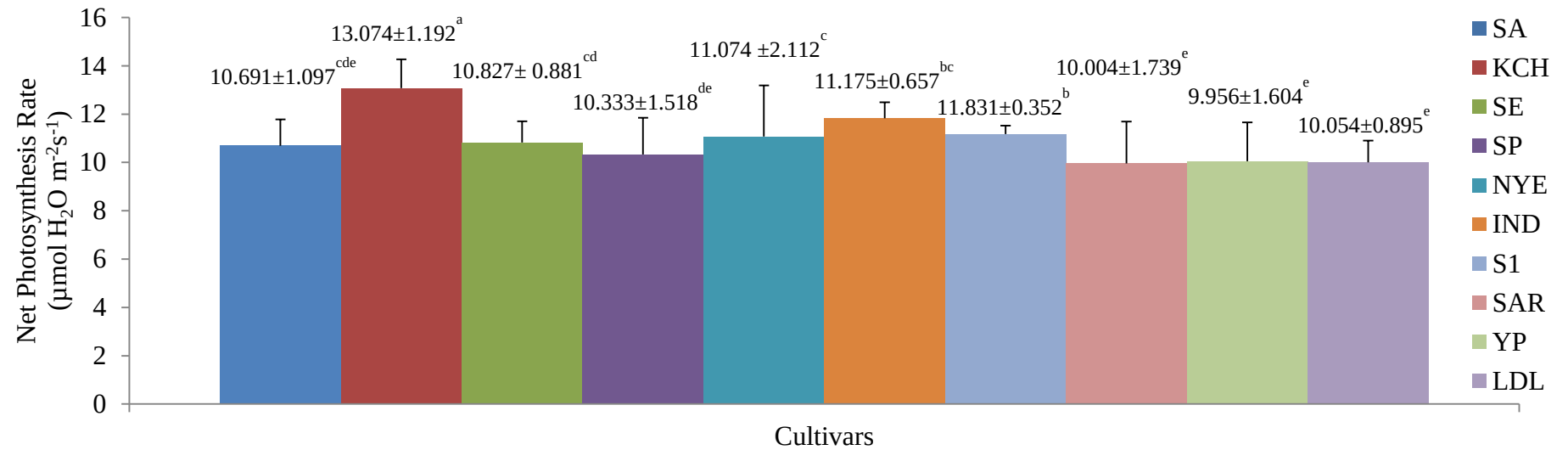


Figure 6.2: Photosynthetic performance for ten black pepper cultivars. SA - ‘Semongok Aman’, KCH - ‘Kuching’, SE - ‘Semongok Emas’, SP - ‘Semongok Perak’, S1 - ‘Semongok 1’, NYE - ‘Nyerigai’, IND - ‘India’, LDL - ‘Lampung Daun Lebar’, SAR - ‘Sarikei’ and YP - ‘Yong Petai’. Means followed by the different superscript letter are significantly different at $P \leq 0.05$, analysed by DUNCAN test.

Leaf stomatal conductance (g_s) has also been evaluated in this study. DUNCAN analysis showed four significant groups among cultivars in their stomatal conductance performance (Figure 6.3). Data showed stomatal conductance of black pepper cultivars ranged 0.112 mol H₂O m⁻²s⁻¹ to 0.237 mol H₂O m⁻²s⁻¹ (Figure 6.3). Cv. ‘Kuching’ (0.237 mol H₂O m⁻²s⁻¹) and cv. ‘India’ (0.219 mol H₂O m⁻²s⁻¹), both ranked in first group. This is followed by cv. ‘Lampung Daun Lebar’ (0.189 mol H₂O m⁻²s⁻¹), cv. ‘Nyerigai’ (0.188 mol H₂O m⁻²s⁻¹), cv. ‘Semongok Emas’ (0.183 mol H₂O m⁻²s⁻¹), cv. ‘Semongok Perak’ (0.179 mol H₂O m⁻²s⁻¹) and cv. ‘Semongok Aman’ (0.172 mol H₂O m⁻²s⁻¹), in second significant group. In third group, cv. ‘Yong Petai’ (0.138 mol H₂O m⁻²s⁻¹) and cv. ‘Sarikei’ (0.124 mol H₂O m⁻²s⁻¹) were the members. Cv. ‘Yong Petai’ recorded 0.124 mol H₂O m⁻²s⁻¹, among the lowest stomatal conductance rate.

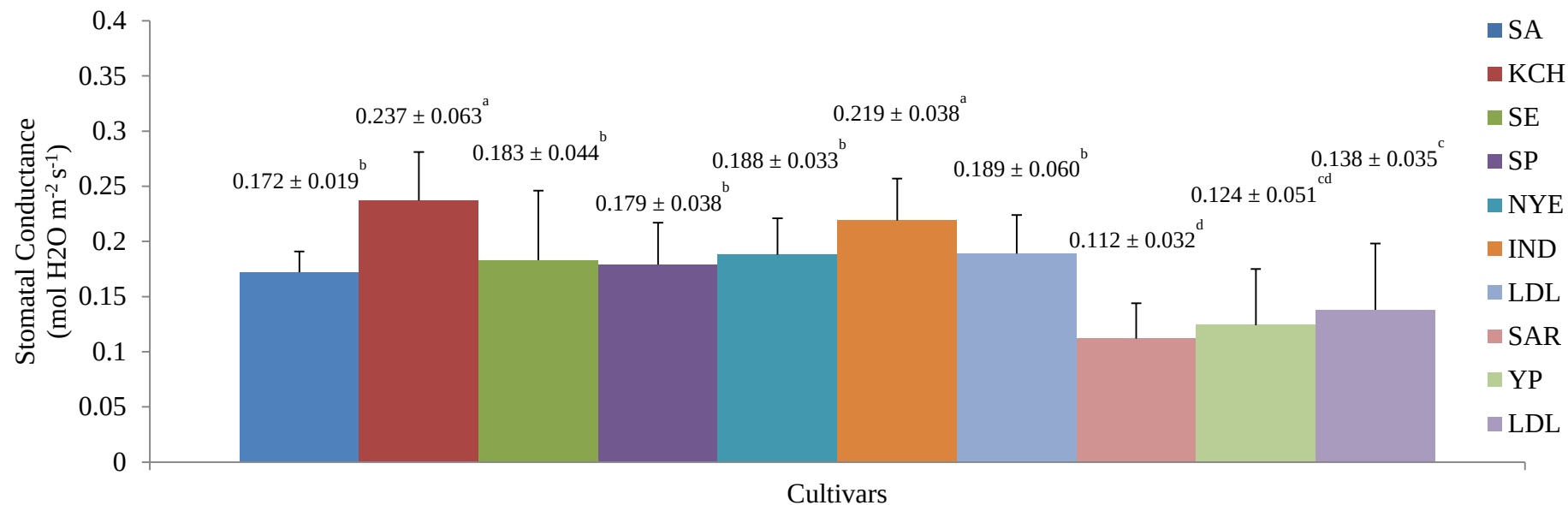


Figure 6.3: Leaf stomatal conductance evaluation for ten black pepper cultivars. SA - ‘Semongok Aman’, KCH - ‘Kuching’, SE - ‘Semongok Emas’, SP - ‘Semongok Perak’, S1 - ‘Semongok 1’, NYE - ‘Nyerigai’, IND - ‘India’, LDL - ‘Lampung Daun Lebar’, SAR - ‘Sarikei’ and YP - ‘Yong Petai’. Means followed by the different superscript letter are significantly different at $P \leq 0.05$, analysed by DUNCAN test.

Another important gas exchange parameter is transpiration rate (E). The transpiration rate of black pepper cultivars that has been recorded ranging from 2.140 mmol H₂O m⁻²s⁻¹ to 4.269 mmol H₂O m⁻²s⁻¹ (Figure 6.4). DUNCAN analysis showed significantly different in statistical analysis of transpiration rate among black pepper cultivars. Three significant groups were identified. Cv. 'Kuching' (4.269 mmol H₂O m⁻²s⁻¹) and cv. 'Nyerigai' (4.050 mmol H₂O m⁻²s⁻¹) both have the highest transpiration rate, fall under first group. This is followed by cv. 'India' (3.827 mmol H₂O m⁻²s⁻¹), that slightly lower than cv. 'Nyerigai' (4.050 mmol H₂O m⁻²s⁻¹). Thus, cv. 'Nyerigai' also falls under second group complemented by cv. 'India'. Cv. 'Semongok 1' with transpiration rate of 3.687 mmol H₂O m⁻²s⁻¹ falls under group three (together with cv. 'India') and group four (together with cv. 'Semongok Emas'). Cv. 'Semongok Emas' (3.400 mmol H₂O m⁻²s⁻¹) with slightly higher transpiration rate compared to cv. 'Semongok 1', also constituted group fifth that joined by cv. 'Semongok Perak' (3.330 mmol H₂O m⁻²s⁻¹) and cv. 'Semongok Aman' (3.325 mmol H₂O m⁻²s⁻¹). The sixth group, with the lowest transpiration rate is constituted by cv. 'Yong Petai' (2.251 mmol H₂O m⁻²s⁻¹), cv. 'Sarikei' (2.192 mmol H₂O m⁻²s⁻¹) and cv. 'Lampung Daun Lebar' (2.140 mmol H₂O m⁻²s⁻¹).

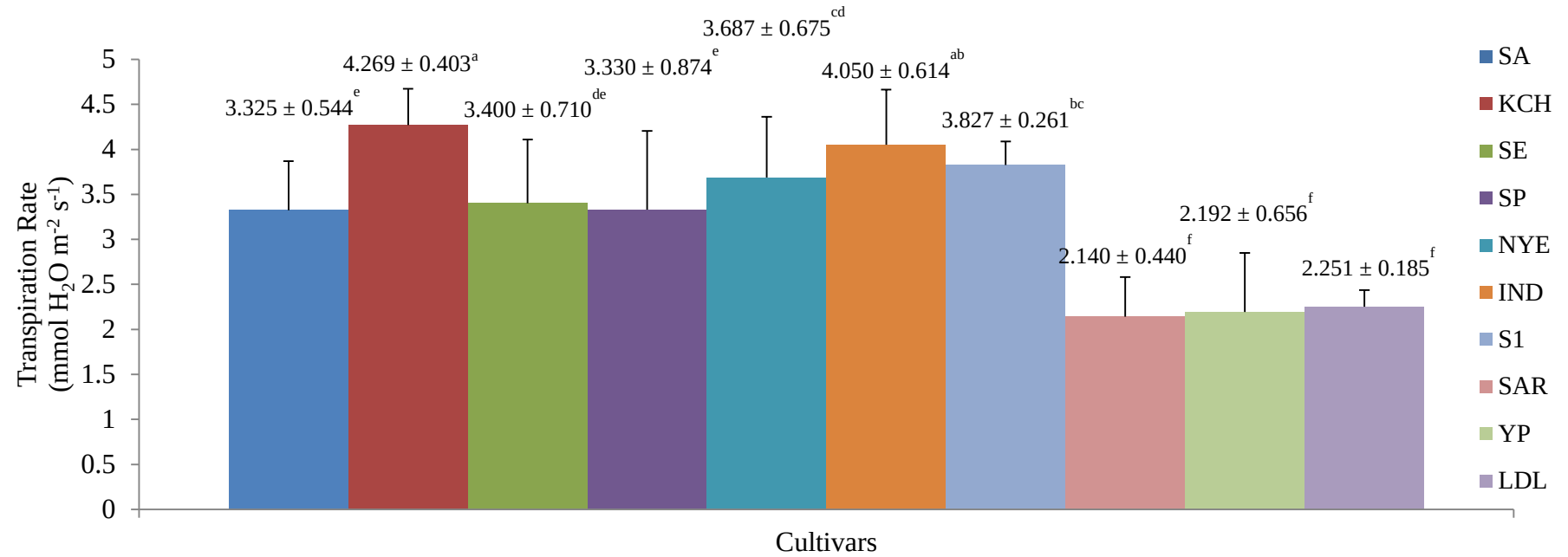


Figure 6.4: Transpiration rate evaluation for ten black pepper cultivars. SA - ‘Semongok Aman’, KCH - ‘Kuching’, SE - ‘Semongok Emas’, SP - ‘Semongok Perak’, S1 - ‘Semongok 1’, NYE - ‘Nyerigai’, IND - ‘India’, LDL - ‘Lampung Daun Lebar’, SAR - ‘Sarikei’ and YP - ‘Yong Petai’. Means followed by the different superscript letter are significantly different at $P \leq 0.05$, analysed by DUNCAN test.

This study also revealed the close relationship between net photosynthesis rate (A) and leaf stomatal conductance (g_s) (Figure 6.5) that shows a polynomial cubic regression line of zero intercept with $r^2=0.8234$ indicating that higher stomatal conductance increased photosynthesis activity in black pepper. This is on par with assumption made by Lawson and Blatt (2014) as reported in a review paper entitled ‘Stomatal Size, Speed, and Responsiveness Impact on Photosynthesis and Water Use Efficiency’ and opinion by Flexas et al. (2013) as mentioned in paper entitled ‘Diffusional Conductance to CO_2 as a Target for Increasing Photosynthesis and Photosynthetic Water-Use Efficiency’. The transpiration rate of black pepper also showed strong correlation with stomatal conductance ($r^2=0.9594$) (Figure 6.6) and photosynthesis rate (A) ($r^2=0.9051$) (Figure 6.7) regardless of cultivars difference. None of the cultivar showed adverse correlation.

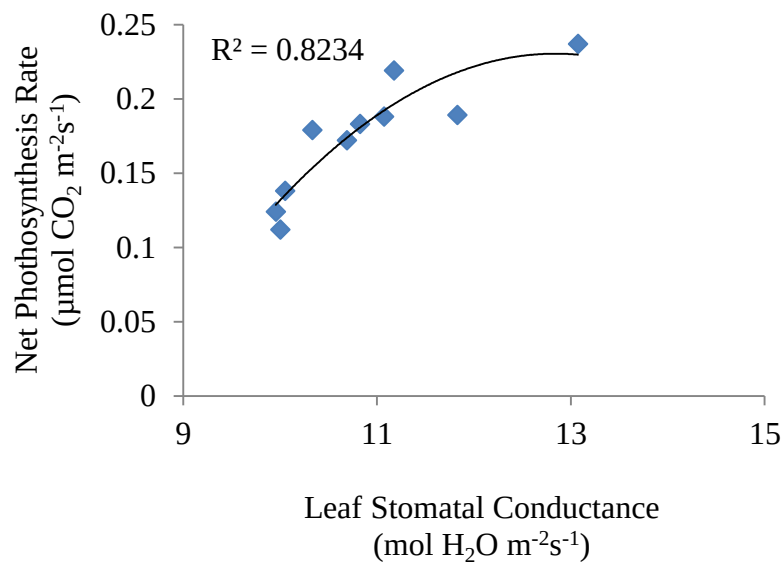


Figure 6.5: Relationship between net photosynthesis rate and leaf stomatal conductance for the ten black pepper cultivars. Square dot represent key value for cultivar, means $\pm$ S.E. and the regression line (continuous) is shown.

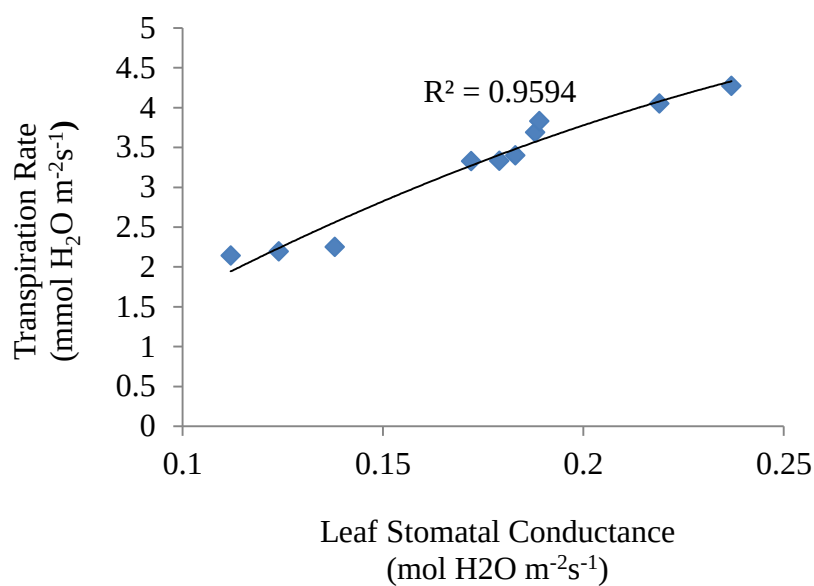


Figure 6.6: Relationship between transpiration rate and leaf stomatal conductance for the ten black pepper cultivars. Square dot represent key value for cultivar, means $\pm$ S.E. and the regression line (continuous) is shown.

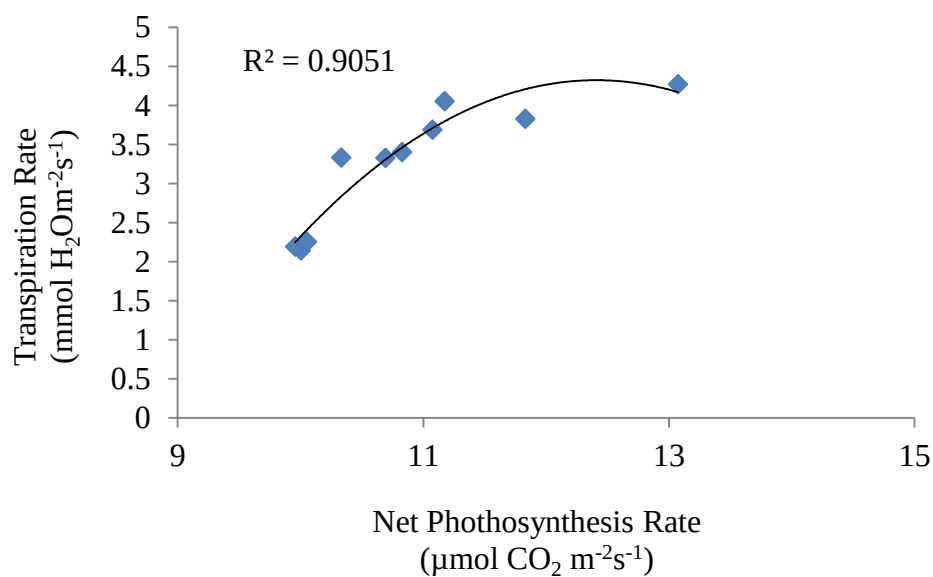


Figure 6.7: Relationship between transpiration rate and net photosynthesis rate for the ten black pepper cultivars. Square dot represent key value for cultivar, means $\pm$ S.E. and the regression line (continuous) is shown.

Photosynthetic performance positively correlated to yield (Long et al., 2006b) and growth performance (Murata, 1981) of crop. In black pepper, the positive correlation particularly on yield has been proven by Mathai (1983) and (Khrisnamurthy & Chempakam, 2009). This is also supported by others researcher that studied on rubber (Conforto et al., 2005), cassave (El Sharkawky et al., 1993; De Tafur et al., 1997) and mung bean (Ignacimuthu & Babu, 1989).

The yield performance of black pepper is dependent on the formation of lateral branches or the fruiting branches (Mathai, 1983; Sim & Rosmah, 2011). The canopy with relatively higher photosynthesis rate promoted growth and development of productive laterals and sustained relatively large number of fruit spike (Mathai, 1983). This is further confirmed by Khrisnamurthy and Chempakam (2009) in their gas exchange study on low and high yielder of Indian black pepper cultivars. They proved that high yielder cultivars like ‘Panniyur-1’, ‘OPKM’, ‘HP780’, ‘1041’ and ‘HP813’ have relatively high gas exchange rate compare to low yielder cultivars. Based on photosynthesis rate, cultivar cv. ‘Kuching’, cv. ‘India’ and cv. ‘Nyerigai’ are the three most promising cultivar while cv. ‘Sarikei’, cv. ‘Lampung Daun Lebar’ and cv. ‘Yong Petai’ are less promising, in term of yield potential. Cultivars like cv. ‘Semongok 1’, cv. ‘Semongok Emas’, cv. ‘Semongok Aman’ and cv. ‘Semongok Perak’ are considered average yielder. These analysis outcomes are partially supported by yield potential study in sub-chapter 4.3.1.3 that based on morphological yield attributing characters (consists of inflorescence, fruit set, fruit conversion rate and percent flowering parameters). The study in Chapter 4.0 has proved cv. ‘Kuching, cv. ‘Semongok 1’ and cv. ‘Nyerigai’ as top yielding cultivars while cv. ‘Semongok Aman’, cv. ‘Semongok Emas’ and cv. ‘Yong Petai’ ranked lowest three in the

evaluation. Cv. 'Semongok 1' has high yield index based on morphological yield character (ranked second in morphological assessment) likes long spike (high number of flower per spike) and berry with high conversion rate (fresh to dry), however photosynthetic assessment showed lower rate. This outcome showed contradicting conclusion between morphological based yield estimation and physiological based yield estimation. The contradict outcome also observed on cv. 'India', cv. 'Semongok Aman' and cv. 'Semongok Emas'. However, the morphological based yield potential formula has not include leaf parameters in the evaluation despite the fact that leaf parameters are the main factor influencing photosynthetic performance in physiological study (Hedden, 2003; Peng et al., 2008; Zhu et al., 2010). This could be the factor contributed to the unparalleled outcome.

6.3.1.2 Leaf Area Index (LAI) Analysis

Leaf area index defined as total one-sided area of leaf tissue per unit ground surface (Watson, 1947). LAI is an important parameter in crop growth (Patil et al., 2018). The leafiness of the crop was reflected and leafiness in one way reflects the photosynthetic capability of the crop (Patil et al., 2018). The linear correlation between LAI and crop growth is generally supported by Monteith (1977); Hirose et al. (1997); De Jong and Ryugo (1998); Friedrich and Fischer (2000); Guo and Sun, (2001); Lakso and Flore (2003); Fischer (2011) and Senyigit et al. (2013). In black pepper, positive correlation between vine production and LAI has been reported by Varughese and Ghawas (1993) and Yap (2012). Besides, leaf area density is significantly correlated with radiation interception and light environment in the fruit zone (Dokoozlian & Kliewer, 1995) that linked to vine productivity.

Leaf Area Index for the ten cultivars at mature stage has been assessed via Plant Canopy Analyser, LAI-2000 (LI-COR Inc., Lincoln, NE, USA). DUNCAN analysis on LAI showed trivial variations for the ten cultivars that have been selected for assessment (Table 6.1). The ten cultivars were only divided into two significantly difference groups. Cv. ‘Semongok Emas’ has the highest index among all cultivar, i.e. 6.786 thus under first group. This is followed by cv. ‘Yong Petai’ with index of 6.385 that share weightage in first and second group. The rest of the cultivars i.e. cv. Kuching (6.290), cv. Nyerigai (6.287), cv. Semongok 1 (6.271), cv. Semongok Aman (6.225), cv. India (6.150), cv. Lampung Daun Lebar (6.142), cv. Semongok Perak (6.130) and cv. Sarikei (6.095) fall under second group, with insignificant variation. Thus, data analysis suggested ‘Semongok Emas’ and cv. ‘Yong Petai’ as promising cultivars in term of yield potential based on LAI index. However, the strong positive correlation between LAI and net photosynthesis rate (A) (Figure 6.8) that shows a polynomial cubic regression line of zero intercept with $r^2=0.9781$, indicating that the LAI index and photosynthetic performance in black pepper cultivar are parallel. Generally, the LAI index among cultivars still followed the pattern exhibited in gas exchange parameters.

Table 6.1: LAI for ten black pepper cultivars.

Cultivars	Leaf Area Index (LAI)
cv. 'Semongok Aman'	6.225 $\pm$ 0.410 ^b
cv. 'Kuching'	6.290 $\pm$ 0.271 ^b
cv. 'Semongok Emas'	6.786 $\pm$ 0.839 ^a
cv. 'Semongok Perak'	6.130 $\pm$ 0.228 ^b
cv. 'Semongok1'	6.271 $\pm$ 0.707 ^b
cv. 'Nyerigai'	6.287 $\pm$ 0.808 ^b
cv. 'India'	6.150 $\pm$ 0.122 ^b
cv. 'Lampung Daun Lebar'	6.142 $\pm$ 0.090 ^b
cv. 'Sarikei'	6.095 $\pm$ 0.587 ^b
cv. 'Yong Petai'	6.385 $\pm$ 0.262 ^{ab}

Note: Means followed by the different superscript letter are significantly different at $P \leq 0.05$, analysed by DUNCAN test.

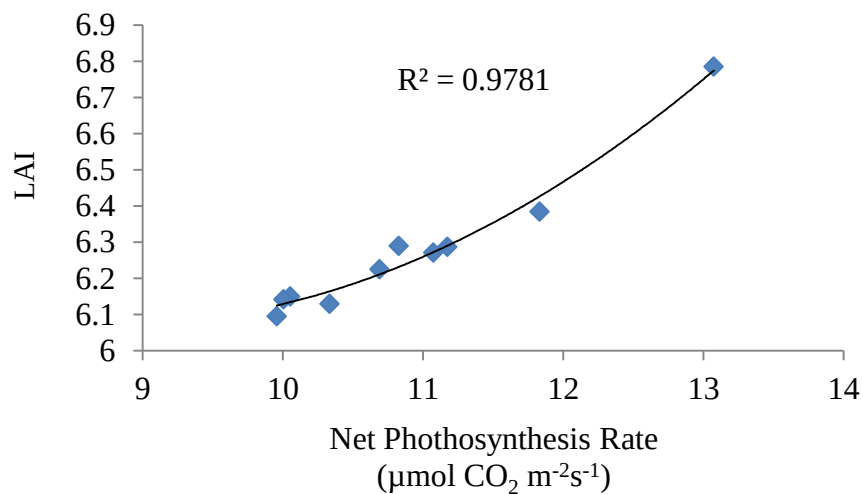


Figure 6.8: Relationship between LAI and net photosynthesis rate in black pepper cultivars. Square dot represent key value for cultivar, means $\pm$ S.E. and the regression line (continuous) is shown.

6.3.2 Floral Biology Study

6.3.2.1 Pollen Morphology Study

Pollen morphology of the pepper cultivar ‘Semongok Aman’ was studied via SEM observation with an aim to precisely collect the right pollen for anther dehiscence and viability study. SEM observation showed pollen grain size is about <10 µm in diameter, categorised under myosotis, spherical shaped, radially symmetrical and with irregular pinulose sculpturing (Figure 6.9). From previous studies, *Piper nigrum* exhibits much uniformity in pollen morphology (Iljas, 1960; Martin & Gregory, 1962; Rahiman, 1981; Ravindran et. al, 2000c). Thus, only one cultivar, ‘Semongok Aman’, was selected for this study.

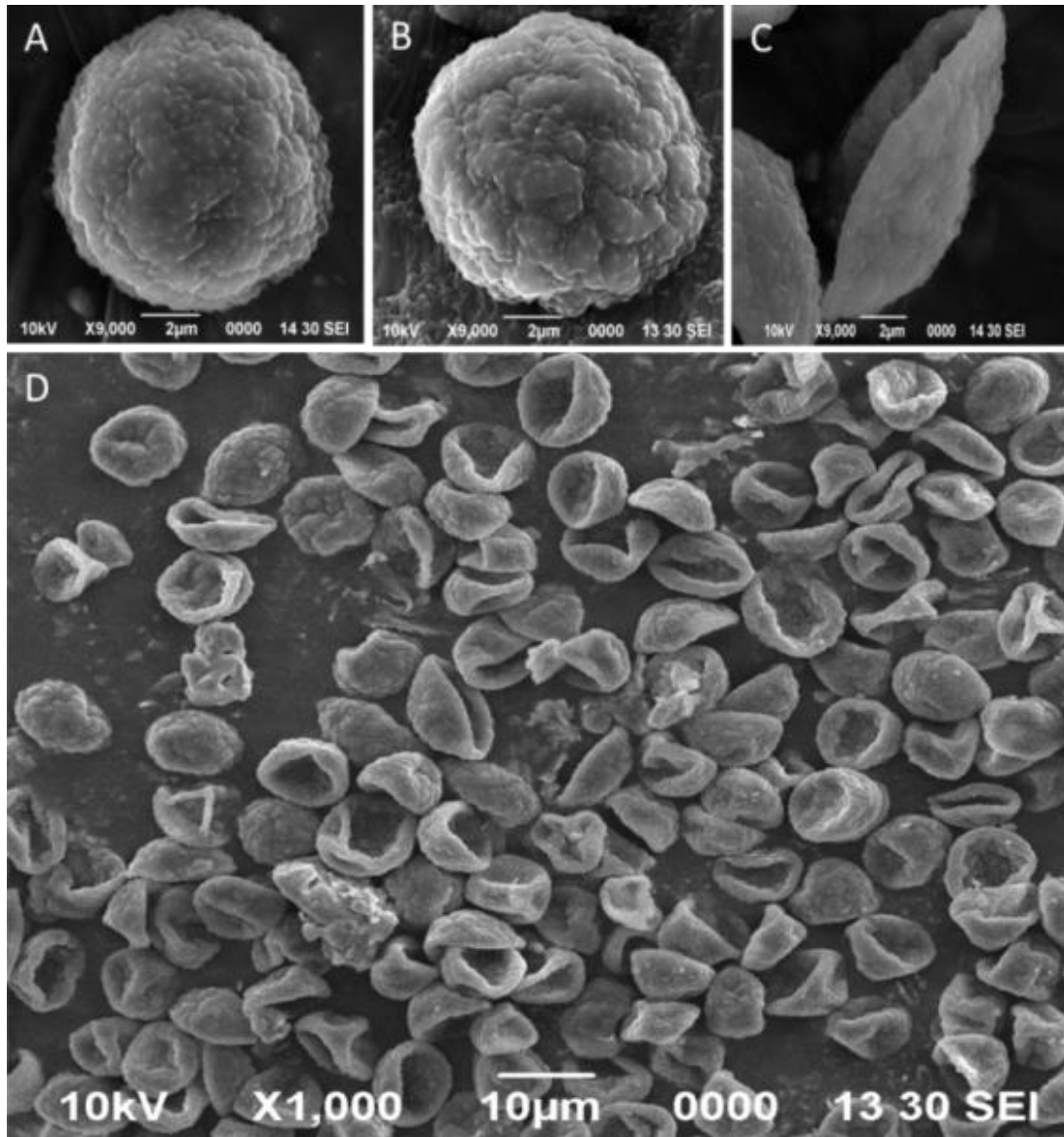


Figure 6.9: SEM micrographs showing size, shape and sculpture of pepper cultivar ‘SA’ pollen grains. A and B. Viable pollen with turgid appearance. C. Unviable pollen with flattened appearance. D. Mixture of viable and unviable pollens.

6.3.2.2 Anther Dehiscence

Investigation on anther dehiscence was done using cultivar ‘Semongok Aman’, from March to July 2016. Results showed the median time of anther dehiscence was between 2200 h - 2400 h. Observation showed months of March and April showed high variation in the median time of anther dehiscence, ranging from 2230 h (March 2016) to 2225 h (April 2016) (Figure 6.10), whilst in May, June, and July 2016, the medians time recorded were 2315 h, 2345 h, and 2350 h, respectively. There were trivial variations in the time of anther dehiscence for the months of May, June, and July when compared to the months of March and April (Figure 6.10). However, statistical analysis proved the variation was a non-significant difference at ($P \leq 0.05$).

Based on the rainfall data in 2016 by Malaysian Meteorological Department, the months of March and April have higher rainfall compared to the subsequent months of May, June, and July. Reduced rainfall between May and July may introduce higher temperatures and lower relative humidity to the environment. Thus, the delayed in anther dehiscence for the three months is most likely due to higher temperatures and lower RH factors. This finding is supported by Sato et al. (2000), who suggested that high temperature exposure could inhibit the anther dehiscence process. Other researchers (Sharp & Chisman, 1961; Jovanovic & Tucovis, 1975; Yates & Darrel, 1993) also reported the adverse effects of elevated temperatures and low RH influences on anther dehiscence in several crops that eventually affect the fruit set.

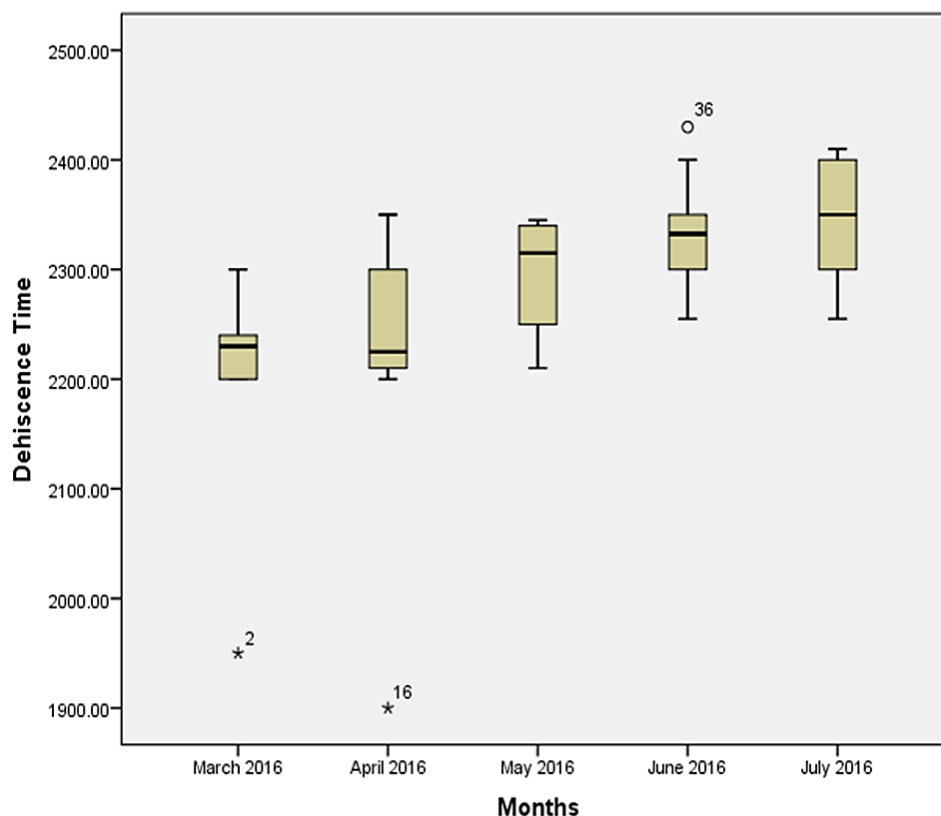


Figure 6.10: Time of anther dehiscence in cultivar ‘Semongok Aman’ between March and July 2016. Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent minimum and maximum value. Outliers represented by open circles and asterisks.

6.3.2.3 Pollen Viability Study

This study on pollen viability aimed to identify the optimum time for pollen collection for each of the ten cultivars. Assessment of pollen germination percentage and pollen tube length measurement via *in vitro* induced germination (Figure 6.11) was carried out to indicate the viability of pollen at various stages of collection.

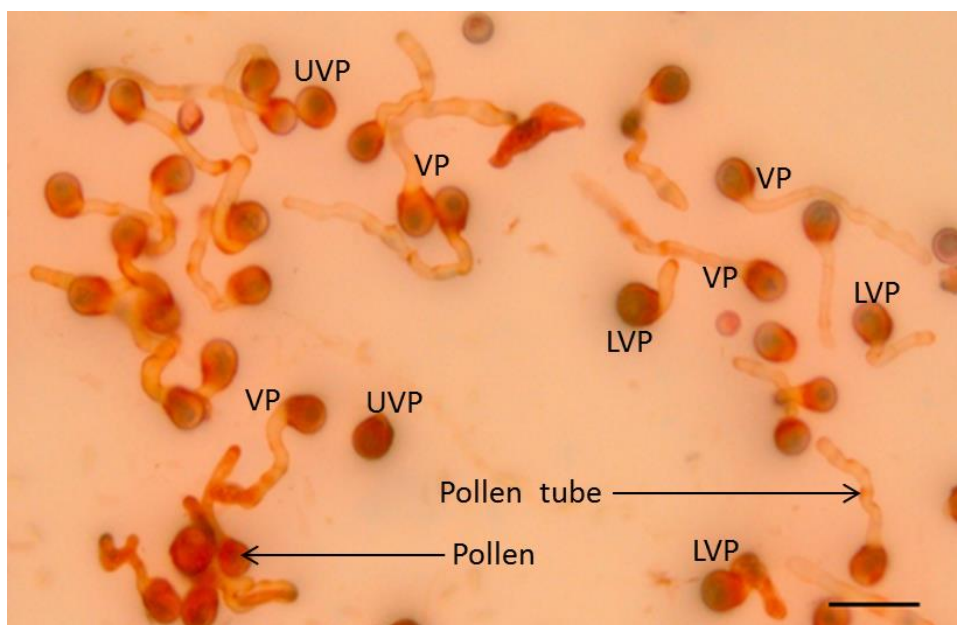


Figure 6.11: *In vitro* germinated pollen of cv. 'Semongok Aman' at different viability level. VP= Viable pollen; LVP= Less viable pollen; UVP= Unviable pollen. Scale: 10µm.

Pollen germination percentage results (Figure 6.12) proved that there were variable differences in the viability of pollen collected at the five stages of the anther dehiscence, and the trend of viability among the cultivars also showed some variations. In most of the cultivars, the percent germination showed significant differences in Stage 5 compared to Stages 2, 3, and 4. The germination of pollen in Stage 1 was not significantly different when compared to Stage 5. This was observed on cultivars 'Kuching', 'Semongok Emas', 'Semongok Perak', 'Nyerigai', and 'Sarikei'. Cultivars 'Lampung Daun Lebar' and 'Yong Petai' also showed similar viability trends; however, insignificant variation was observed at Stage 3 on cultivar 'Lampung Daun Lebar', and Stage 3 and Stage 4 for cultivar 'Yong Petai'. The viability trend of cultivar 'Semongok Aman' only showed significant differences at Stage 5 pollen, while from Stage 1 to Stage 4, the pollen germination percentages showed no significant difference. Cultivar 'Semongok 1' also exhibited

slightly different viability trends, where Stage 1 and Stage 5 showed no significant difference in germination percentage but was significantly different if compared to Stage 2, Stage 3 and Stage 4. Cultivar ‘India’ showed no substantial different in pollen germination percentage for all five distinct stages of the anther dehiscence.

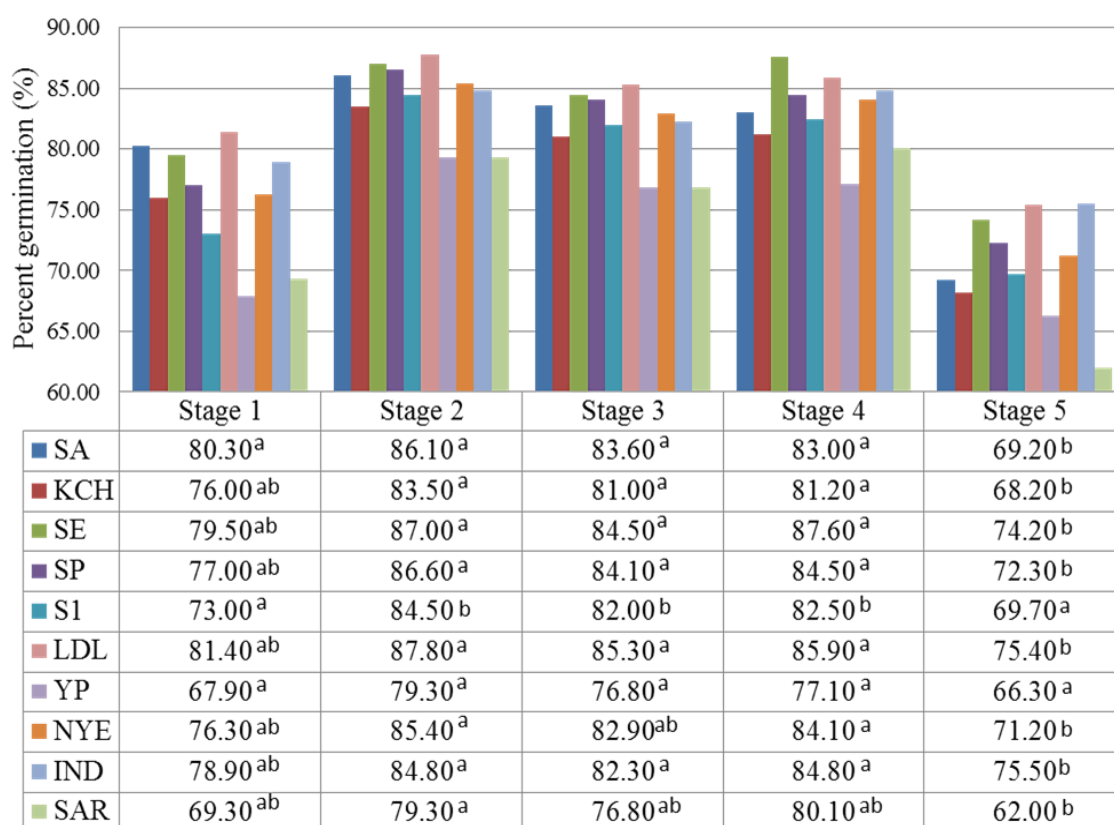


Figure 6.12: Percentage of pollen germination at various stages of pollen for the ten cultivars. SA - ‘Semongok Aman’, KCH - ‘Kuching’, SE - ‘Semongok Emas’, SP - ‘Semongok Perak’, S1 - ‘Semongok 1’, NYE - ‘Nyerigai, IND - ‘India’, LDL - ‘Lampung Daun Lebar’, SAR - ‘Sarikei’ and YP - ‘Yong Petai’. Stage 1: Right after anther dehiscid; Stage 2: Five hours after anther dehiscid; Stage 3: Ten hours after anther dehiscid; Stage 4: Fifteen hours after anther dehiscid; and Stage 5: Twenty hours after anther dehiscid. Means followed by the different superscript letter within the same column are significantly different at $P \leq 0.05$.

Pollen tube length of *in vitro* germinated pollen at various stages was also investigated (Figure 6.13). The tube length was measured using stereomicroscope with a built-in reticule. Results show cultivars ‘Semongok Aman’, ‘Kuching’, ‘Semongok Emas’, ‘Semongok Perak’, ‘Lampung Daun Lebar’, ‘Sarikei’ and ‘Yong Petai’ exhibited similar trend of viability in all the pollen stages. All cultivars only showed significant differences in pollen tube length measurements at Stage 5, pollen collected after 20 hours of anther dehiscence. Pollen collected at Stages 1, 2, 3, and 4 showed no significant difference in tube length assessment. The pollen tube length ranged from 9.2 μm (cultivar ‘Sarikei’, Stage 5 pollen) to 22 μm (cultivar ‘Kuching’, Stage 4 pollen). Cultivars ‘Semongok 1’, ‘Nyerigai’, and ‘India’ demonstrated different trends of pollen tube growth. Cultivar ‘Semongok 1’ exhibited three significantly different mean groupings among 5 stages of pollen, with Stage 1 (mean group 1) significantly greater in length compared to mean group 2 (Stages 2, 3, & 4) and mean group 3 (Stage 5). Stage 5 pollen in mean group 3 had significantly shorter length compared to pollen at mean group 2, thus is among the less viable stage of pollen, if based on the tube length indicator. Cultivar ‘Nyerigai’ also has three distinctively different mean groups: mean group 1 (Stage 1 to Stage 3), mean group 2 (Stage 4), and mean group 3 (Stage 5). Among the three mean groups, mean group 1 showed the greatest length, ranging from 23.70 μm to 24.80 μm , while mean group 3 with Stage 5 pollen only achieved an average length of 11.20 μm . Cultivar ‘India’ showed a similar trend to ‘Nyerigai’, where the greatest length recorded was 20.20 μm in the first distinct group, followed by 19.80 μm (mean group 2) and 9.70 μm (mean group 3). Pollen collected from Stages 2, 3, and 4 showed better pollen tube growth performance compared to pollen at Stage 1 and Stage 5. In Stage 5, the length of the pollen tube was among the shortest due to retarded growth.

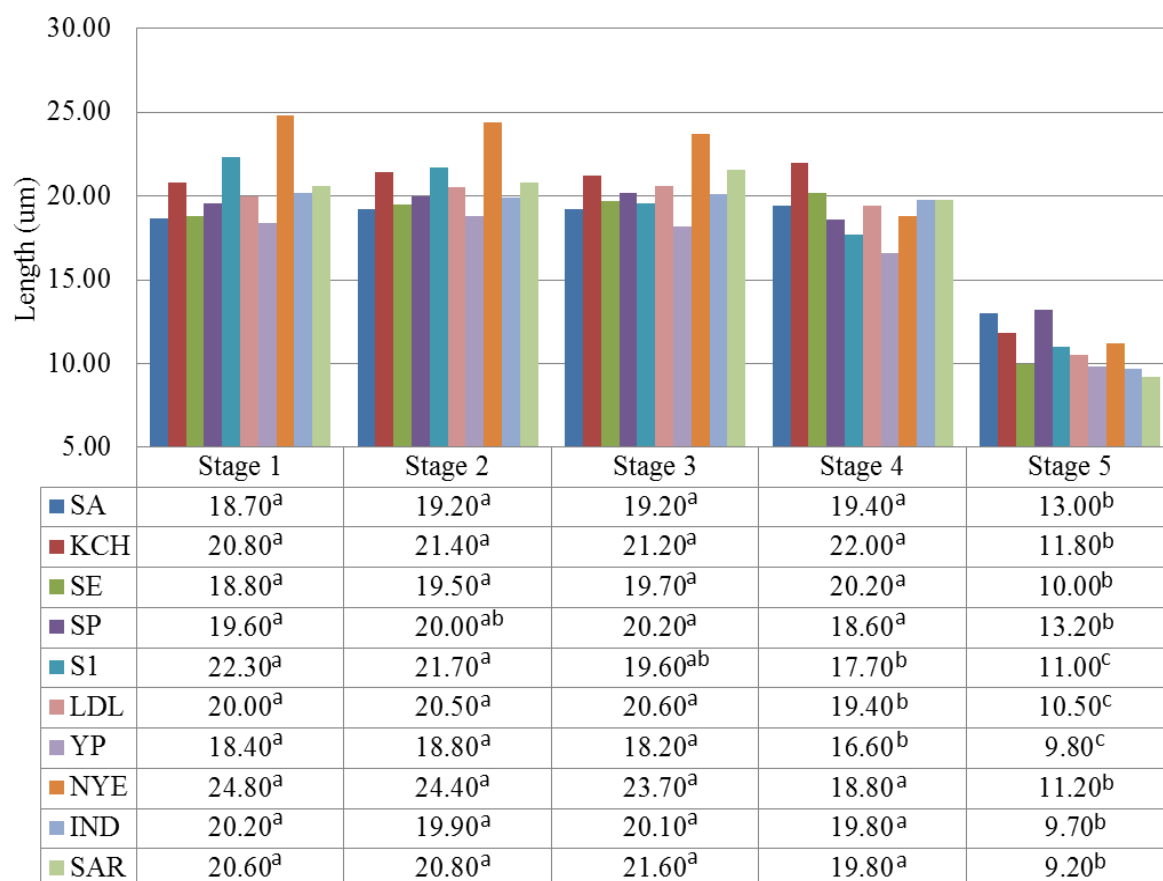


Figure 6.13: Pollen tube length at five various stages of the anther dehiscence for the ten cultivars. SA - ‘Semongok Aman’, KCH - ‘Kuching’, SE - ‘Semongok Emas’, SP - ‘Semongok Perak’, S1 - ‘Semongok 1’, NYE - ‘Nyerigai’, IND - ‘India’, LDL - ‘Lampung Daun Lebar’, SAR - ‘Sarikei’ and YP - ‘Yong Petai’. Stage 1: Right after anther dehiscence; Stage 2: Five hours after anther dehiscence; Stage 3: Ten hours after anther dehiscence; Stage 4: Fifteen hours after anther dehiscence; and Stage 5: Twenty hours after anther dehiscence. Means followed by the different superscript letter within the same column are significantly different at $P \leq 0.05$.

ANOVA tests proved that the pollen collected between 5 and 15 hours of anther dehiscence was generally more viable based on the percent of germination and pollen tube elongation. Pollen collected at Stage 1 showed a low viability of about 67.90%, as recorded for cultivar ‘Yong Petai’. However, the pollen tube elongation performance for Stage 1 pollen is comparable to Stages 2, 3, and 4 pollens. This may be due to delayed

germination for pollen collected right after anther dehiscence. In most cases, pollen grains are metabolically dormant and highly desiccated when released from the anthers (Heslop-Harrison, 1979; Buitink et al., 2000). Thus, slightly poorer performance of Stage 1 pollen in germination studies may not be due to the poor viability of pollen collected at this Stage. Kearns and Inouye (1993) reported that pollen collected immediately after dehiscence was generally most viable.

In this experiment, pollen collected after 20 hours (Stage 5) showed relatively low germination percentage and short pollen tube elongation. The pollen collected from this stage may be non-viable, even though the pollen is able to achieve satisfactory high germination percentage and pollen tube elongation. According to Heslop-Harrison (1979), non-viable pollen grains could hydrate to the same extent as living pollen grains, swell, and even develop short tubes before the tubes eventually ruptured.

6.3.2.4 Stigma Receptivity Study

In this study, three stages of stigma (Figure 6.14) were sampled to examine their receptivity level. Stages are as follows:

- Stage 1: First appearance of stigma (Day 1 of emergence);
- Stage 2: Elongation and spreading of stigma (Day 2-3 of emergence); and
- Stage 3: Complete emergence and wide spreading of stigma (Day 4-6 of emergence).

Development of a blue dot on the stigma after the treatment was recorded as a positive result, while no colour change was recorded as a negative result. The percentage

of receptive stigma was calculated for each stage, and the results are tabulated in Table 6.2. The results show nine out of ten cultivars that have been tested showed comparable results on the receptivity trend. Statistical analysis showed only Stage 1 stigma has a significantly lower receptive level, compared to stigma at Stages 2 and 3, for all nine cultivars except cultivar SA. Cultivar LDL recorded 52.60% (Stage 1) as the lowest, while cultivar IND achieved 97.70% at Stage 2, the highest percentage overall. Cultivar SA showed three stages of stigma significantly different on a receptive level, with Stage 2 recording the highest percentage at 97.70%, followed by Stage 3 at 90.10%, and Stage 1 at 80.30%.

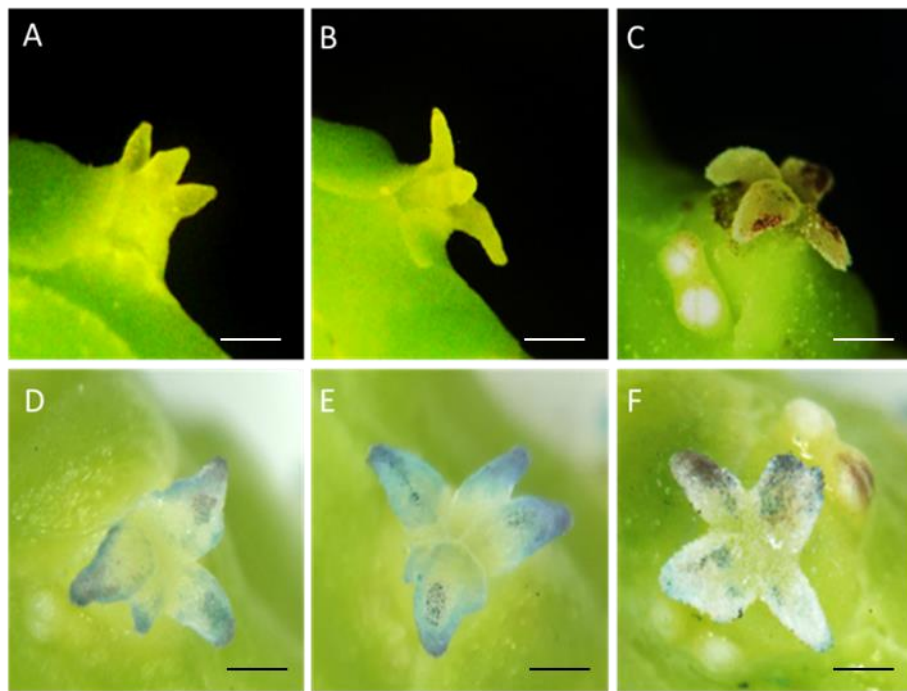


Figure 6.14: Stages of stigma. A. Stage 1: First appearance of stigma. B. Stage 2: Elongation and spreading of stigma. C. Stage 3: Complete emergence and wide spreading of stigma. D-F. Stigma after Peroxtesmo KO treatment. Scale bar: 0.5 mm.

Peroxtesmo KO test paper is a tool for quick and easy detection of peroxidase (Dafni & Maue's, 1998). Dafni (1992) and Galen and Plowright (1987) proved the reliability of Peroxtesmo KO test paper for identification of stigma receptivity. Stigma receptivity could be evaluated by the arrival of peroxidase on stigmatic surfaces of the pepper flower while the presence of peroxidase is indicated by the appearance of a blue or greenish colour (Dafni & Maue's, 1998). This method has been adopted in the reproductive biology study of other plants, including *Macleania bullata* (Luis, 2000), *Origanum syriacum* (Rodriguez-Riano & Dafni, 2007) and *Manekia naranjoana* (Tatiana & Joseph, 2008).

In most plants, the stigma is receptive to pollination over a wide range of floral developmental stages (Amy & Rosanna, 2010; Chen et al., 2013). However, the results obtained from this study via ANOVA test showed that the stigma of pepper at Stage 1 has significantly lower receptivity compared to Stages 2 and 3. Purseglove (1974) reported that peak receptivity occurred at three to five days of emergence. He added that stigma may remain receptive for up to 10 days. This is also supported by Sedgley et al. (1985) and Kalinganire et al. (2000) in their studies on silky oak and macadamia, respectively. They recorded that lower receptivity occurred at early stigma emergence stage. However, Chen (2011) revealed a study on receptivity via a Hydrogen Peroxide test, showing no significant difference at any stages of stigma, including the early emergence stage. Thus, the variation in this study, even though significant, at Stage 1 may become an indicator as a reasonably receptive stage of stigma for the ten cultivars studied. Helen and Lauren (2002) reported that stigmatic age is uncorrelated with receptivity. The stigma normally remains receptive at any stage before receiving pollen. After the stigma received pollen, the

stigmatic cells collapsed and eventually dried, once pollen hydration and germination occurred (Wetzstein & Sparks, 1989). Without pollination, stigmatic surfaces may remain receptive for a longer period (Wetzstein & Sparks, 1989).

Table 6.2: Receptivity difference at three stages of stigma for ten cultivars.

SA		KCH		SE		SP	
Stage	Mean	Stage	Mean	Stage	Mean	Stage	Mean
1	80.30 ^a	1	64.20 ^a	1	82.60 ^a	1	69.00 ^a
2	97.70 ^b	2	96.50 ^b	2	97.40 ^b	2	96.50 ^b
3	90.10 ^c	3	95.90 ^b	3	96.80 ^b	3	97.30 ^b
S1		NYE		IND		LDL	
Stage	Mean	Stage	Mean	Stage	Mean	Stage	Mean
1	69.00 ^a	1	62.60 ^a	1	63.60 ^a	1	52.60 ^a
2	97.30 ^b	2	97.80 ^b	2	97.70 ^b	2	96.90 ^b
3	97.00 ^b	3	93.80 ^b	3	96.10 ^b	3	95.40 ^b
SAR		YP					
Stage	Mean	Stage	Mean				
1	70.60 ^a	1	74.30 ^a				
2	96.90 ^b	2	96.90 ^b				
3	96.70 ^b	3	95.50 ^b				

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'. Stage 1: First appearance of stigmata; Stage 2: Elongation and spreading of stigmata and Stage 3: Complete emergence and wide spreading of stigmata. Means followed by the different superscript letter in the same column are significantly different at $P \leq 0.05$.

6.4 Conclusion

Productivity analysis on ten important black pepper cultivars based on physiological parameters has been fulfilled in this study. The physiological yield attributing parameters includes gas exchange (photosynthesis rate, stomatal conductance and transpiration rate) evaluation and leaf area index (LAI), has been evaluated in this study. Results showed significantly positive correlation among all the gas exchange parameters and between photosynthesis rate (A) and LAI. The physiological parameter based yield performance analysis suggest cv. 'Kuching' as the most productive cultivar of all by judging on the high gas exchange performance and moderately high leaf area index. Cv. 'Nyerigai' and cv. 'India' also have good performance in gas exchange and high leaf area index, thus were classified as good yield potential cultivars also. The poor gas exchange performing cultivars are cv. 'Sarikei' and cv. 'Lampung Daun Lebar', thus classified as poor yield potential group. The two also have low leaf area index compared to others cultivars. Whilst, moderate performers are cv. 'Semongok 1', cv. 'Semongok Emas', cv. 'Semongok Perak', cv. 'Semongok Aman' and cv. 'Yong Petai'. LAI assessment was found significantly ($r^2=0.978$) correlated with photosynthesis rate, thus support the yield potential suggestion that has been made.

Investigation on anther dehiscence time, pollen viability and stigma receptivity for the ten important cultivars in Malaysia has been completed in this study, with aim to improve efficiency of conventional breeding. Generally, anther dehiscence for all the cultivars ranged 2200 h - 2400 h, however variations were observed between months in this study. The climate factor was believed to have contributed to the variation. In pollen

viability study, viability is indicated by pollen germination percentage and pollen tube growth as evaluated by tube elongation. ANOVA analysis proved that pollen collected between 5 to 15 hours after anther dehiscence was significantly more viable compare to other stages of pollen, for all the cultivars. Whilst in stigma receptivity study, Stage 1 stigma (First appearance of stigmata) has a significantly lower receptive level compared to stigma at Stage 2 (Elongation and spreading of stigmata) and Stage 3 (Complete emergence and wide spreading of stigmata). On the contrary, Stage 2 and 3 stigmas were more receptive for pollen growth. However, receptivity variation was observed on cv. ‘Semongok Aman’, where this cultivar showed significant difference in the three stages of stigma. Stage 2 (Elongation and spreading of stigmata) stigma is most receptive for this cultivar.

Floral biology study provides invaluable information to improve the efficiency of artificial pollination. Based on the results, pollen collection for all ten cultivars may be done between 0400h and 1400h, since their anther dehiscence was found to occur between 2200h and 2400h. While the stigma was proven more receptive at Stages 2 and 3 for all the cultivars studied, cultivar ‘Semongok Aman’ showed that it was most receptive with stigma of Stage 3. The information generated from this study may assist breeders with dispersal of the pollen onto the stigma at the right stage and time to increase pollination efficiency and enhance success in pepper breeding programmes.

CHAPTER 7

GENERAL DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

7.1 General Discussions

Previously, there is no comprehensive documentation on important black pepper cultivars in Malaysia. The population of cultivars were also unrevealed. This has constrained varietal regulation at black pepper farm. In this study, botanical documentation for ten important black pepper cultivars in Malaysia has been achieved in this project. The ten cultivars namely 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'. Among all, cv. 'Kuching' is the most widely distributed cultivar, especially in the state of Sarawak. Cultivars like cv. 'Semongok Aman' and cv. 'Semongok Emas' are common cultivars also, with distribution reasonably diverse throughout Malaysia. Whilst, cv. 'Semongok Perak', cv. 'Semongok 1', cv. 'Nyerigai', cv. 'India', cv. 'Lampung Daun Lebar', cv. 'Sarikei', and cv. 'Yong Petai' are less widespread cultivars, with distribution mostly limited to Sarawak only. In term of population by region, Sarawak is still the major state for black pepper planting with approximately 95% of planting hectare domination. Peninsular Malaysia and state of Sabah have only less than 5% of population. The planting distribution is mainly influenced by cultivar productivity potential, and resistance against pest and diseases (Paulus et al., 2011) but not so much on soil. However, urbanization (Shaharuddin, 2005; Alam et al., 2014) and climate change (Wong et al., 2018) in Peninsular Malaysia has constrained distribution of black pepper cultivars to these region in Malaysia. Black pepper planting in Sabah less

popular mainly due to the state government agricultural policy more favourable to oil palm, rubber, paddy, coconut and cocoa (Department of Statistic Malaysia, 2019).

Due to high genetic relatedness among black pepper cultivars (Ho et al., 2005), morphological analysis has been conducted to ease varietal identification. The qualitative and quantitative data that has been analysed in this study suggests the key diagnosis for the ten cultivars, as outlined below:-

- Cv. ‘Semongok Aman’ - Mucronate shaped leaf apex; High percentage of fruit set high, *ca.* 76%.
- Cv. ‘Kuching’ - High number of inflorescence (spike) per branch per node, *ca.* 58.67; High number of node/ 30 cm of stem, *ca.* 4.73.
- Cv. ‘Semongok Emas’ - Leaf colour of green group 137 series (based on RHS code); Fruit colour of green group 141 series (based on RHS code).
- Cv. ‘Semongok Perak’ - Acute leaf apex and oblique leaf base; Low drying conversion rate (*ca.* 36.12% for black peppercorn while *ca.* 24.21% for white peppercorn)
- Cv. ‘Semongok 1’ - Cordate shaped leaf; Anthocyanin absent at shoot tip.
- Cv. ‘Nyerigai’ - Erect branching behaviour at mature vine stage.
- Cv. ‘India’ - Lanceolate shaped leaf; Light seed weight, *ca.* 0.14 g.
- Cv. ‘Lampung Daun Lebar’ - Oblique shaped leaf base; Blade length-width ratio low, *ca.* 1.52.

- Cv. ‘Sarikei’- Small leaf area, *ca.* 36.90 cm²; Short inflorescence, *ca.* 6.06 cm; Short fruit spike, *ca.* 8.07 cm; Small fruit size, *ca.* 5.78 mm; Small seed size, *ca.* 3.84 mm; Thin pericarp, *ca.* 1.73 mm.
- Cv. ‘Yong Petai’- Long inflorescence length, *ca.* 12.75 cm; Long fruit spike length, *ca.* 17.07 cm; Thin inflorescence, *ca.* 2.90 mm.

The morphological analysis also enables estimation of yield potential for the ten cultivars. The assumption on the yield potential is based on the morphological yield attributing characters that mentioned by Mathai (1983), Ibrahim et al. (1985 and 1987), Sujatha and Namboodiri (1995) and Paulus et al. (2011). The calculation showed cv. ‘Kuching’ has the highest yield potential index. This is followed by cv. ‘Semongok 1’, cv. ‘Nyerigai’, cv. ‘Sarikei’, cv. ‘India’, cv. ‘Lampung Daun Lebar’, cv. ‘Semongok Perak’, cv. ‘Semongok Emas’ and cv. ‘Semongok Aman’, that occupied second to ninth position. Whilst, cv. ‘Yong Petai’ has the lowest yield potential index. However, this assumption does not reflect actual productivity of cultivar. The actual productivity of black pepper could be varied by soil fertility factor (Mathew et al., 1995), cultivation system (Nybe & Sujatha, 2008), maintenance quality (Pillai, 1977; Kurien & Nair, 1988; Mathai & Sastry 1988; Azmil & Yau, 1993; Gunaratne & Heenkenda, 2004) and climate factor (Krishnamurthy et al., 2010).

Piperine is responsible for the pungency of black pepper while aroma is attributed to the volatile oil found in the berry (Krishnapura, 2009; Gorgani et al., 2017). In GCMS and HPLC analysis of peppercorn, both cv. ‘Semongok Aman’ and cv. ‘Lampung Daun

Lebar' were found relatively rich in phytochemical content compared to other cultivars. The content of piperine and essential oil for both cultivars are 4.25% and 2.50% for cv. 'Semongok Aman' while 4.20% and 2.24% for cv. 'Lampung Daun Lebar'. Important constituents that has been identified in peppercorn consists of β -Caryophyllene (17.42%-42.97%), β -Pinene (7.69%-21.11%), Limonene (9.48%-15.88%), δ -3-Carene (2.57%-20.58%), α -Pinene (2.82%-4.80%), α -Humulene (1.84%-3.23%) and δ -Elemene (0.29%-5.50%). An optimal aroma (pleasant) is obtained if the content of monoterpenes (except α - and β - pinene) is high and at the same time the amount of pinenes is low (Schulz et al., 2005). The pleasant aroma attributed monoterpene consists of α -Thujene, Myrcene, α -Phellandrene, δ -3-Carene, Limonene, γ -Terpinene, Terpinolene, Linalool and Terpinen-4-ol. The aroma analysis based on monoterpene constituent has revealed peppercorn of cv. 'Nyerigai', cv. 'Sarikei' and cv. 'Kuching' have more pleasant aroma (high pleasant aroma index) while cv. 'Lampung Daun Lebar', cv. 'India' and cv. 'Semongok Aman' have high unpleasant aroma index.

Productivity analysis on ten important black pepper cultivars based on physiological parameters (photosynthesis rate, stomatal conductance and transpiration rate and LAI) has also been evaluated in this study. In black pepper, the positive correlation particularly on yield has been proven by Mathai (1983) and also Khrisnamurthy and Chempakam (2009). Beside net photosynthesis rate, the leaf stomatal conductance (Ishihara & Saito, 1987; Hirasawa et al., 1988; Kusumi et al., 2012; Golam Sarwar et al., 2013; Sabagh et al., 2017) and transpiration rate (De Wit, 1958; Smeal et al., 1991; Ben-Gal & Shani, 2002; Madrid, 2013) were among the physiological yield attributing factors that related to plant productivity. The study outcome suggests cv. 'Kuching' as the most

productive cultivar of all by judging on the high gas exchange performance and moderately high leaf area index. Cv. 'Nyerigai' and cv. 'India' were classified as good yield potential cultivars also by judging on good performance in gas exchange and high leaf area index. Moderate performers include cv. 'Semongok 1', cv. 'Semongok Emas', cv. 'Semongok Perak', cv. 'Semongok Aman' and cv. 'Yong Petai', with average performance on gas exchange performance and LAI index. The other two cultivars namely cv. 'Sarikei' and cv. 'Lampung Daun Lebar' were grouped under poor yield potential group, with poor gas exchange performance at the same time leaf area index relatively low.

In floral biology study, investigation on anther dehiscence time, pollen viability and stigma receptivity for the ten important cultivars has been completed. Analysis showed trivial variation in pollen viability among the ten cultivars. Generally, this analysis suggests pollen collection for all the ten cultivars ideally between 0400 h and 1400 h, since anther dehiscence was found to occur between 2200 h and 2400 h. At the same time, the stigma was proven more receptive at Stages 2 (Elongation and spreading of stigmata) and 3 (Complete emergence and wide spreading of stigmata) for all the cultivars studied beside cultivar 'Semongok Aman'. The efficiency of artificial pollination can be improved based on these studies outcome.

All the studies that have been carried out in this project have effectively achieved all the objectives and are stated in the conclusion below. Three scientific manuscripts from this project have been successfully published in SCOPUS indexed journal, as attached at Appendix 23.

7.2 Conclusions

7.2.1 Taxonomy of *Piper nigrum* L. and Its Cultivars in Malaysia

Ten important black pepper cultivars namely 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’ has been comprehensively described through photography, vouchers (dried specimens) deposition at herbarium and botanical described. The distributions of these cultivars throughout Malaysia were also documented with assistance of *E-Pekebun* (*Sistem Maklumat Pekebun Lada*), a database owned by Malaysian Pepper Board.

7.2.2 Morphological Characteristics of Black Pepper Cultivars

Analysis showed identification of cultivars such as cv. ‘Semongok 1’ (The only cultivar with cordate shaped leaf; The only cultivar with anthocyanin absent at shoot tip), cv. ‘Yong Petai’ (Longest inflorescence length, *ca.* 12.75 cm; Longest fruit spike length, *ca.* 17.07 cm), cv. ‘Lampung Daun Lebar’ (The only cultivar with ovate leaf shape and oblique shaped leaf base), cv. ‘Semongok Aman’ (The only cultivar with mucronate shaped leaf apex) and cv. ‘India’ (The only cultivar with lanceolate shaped leaf) are easy with distinct qualitative characteristics. Whilst, identification for cv. ‘Semongok Emas’ and cv. ‘Semongok Perak’ need at least two characteristics assessment for the identification. The diagnostic characteristics include cv. ‘Semongok Emas’ (Leaf colour of green group 137 series; Fruit colour of green group 141 series) and cv. ‘Semongok Perak’ (Acute leaf apex and oblique leaf base; Lowest dying conversion rate, *ca.* 36.12% for black peppercorn while *ca.* 24.21% for white peppercorn). Identification of cv. ‘Nyerigai’ is easy

at mature stage with erect branching behaviour ($>90^\circ$) however, highly similar to cv. 'Kuching' on the rest of the morphological characteristics. Thus, identification of this cultivar could be complicated at immature stage. Analysis showed cv. 'Kuching' and cv. 'Sarikei' have high similarity (98%), at the same time do not showed distinct qualitative characters that enable morphological diagnosis. Quantitative assessment on cv. 'Kuching' (High number of inflorescence (spike) per branch per node, *ca.* 58.67; High number of node/ 30 cm of stem, *ca.* 4.73) and cv. 'Sarikei' (Small leaf area, *ca.* 36.90 cm²; Short inflorescence, *ca.* 6.06 cm; Short fruit spike, *ca.* 8.07 cm) is necessary for the diagnosis. This study also revealed yield potential comparison among cultivars based on morphological yield attributing characters. The analysis suggests cv. 'Kuching' as high yield potential cultivar (highest yield potential index) while cv. 'Yong Petai' rated as low potential cultivar (lowest yield potential index).

7.2.3 Phytochemical Characterization of Black Pepper Cultivars

This study revealed both cv. 'Semongok Aman' and cv. 'Lampung Daun Lebar' relatively rich in phytochemical content, particularly essential oil and piperine. Besides, aroma analysis based on monoterpene also revealed the peppercorn of cv. 'Nyerigai', cv. 'Sarikei' and cv. 'Kuching' have higher pleasant aroma index.

7.2.4 Physiological and Floral Biology of Black Pepper Cultivars

This analysis revealed yield potential for all the ten cultivars studied. Gas exchange performance and leaf area index analysis suggests cv. 'Kuching', cv. 'Nyerigai' and cv.

‘India’ as good yield potential cultivars. Moderate performers include cv. ‘Semongok 1’, cv. ‘Semongok Emas’, cv. ‘Semongok Perak’, cv. ‘Semongok Aman’ and cv. ‘Yong Petai’, with average performance on gas exchange performance and LAI index. Cv. ‘Sarikei’ and cv. ‘Lampung Daun Lebar’ were rated as poor yield potential group, with poor gas exchange performance at the same time leaf area index relatively low. Whilst, floral biology study suggests pollen collection for all the ten cultivars ideally between 0400 h and 1400 h, to ensure the pollen obtained for artificial pollination is highly viable. Whilst, the stigma was proven more receptive at Stages 2 (Elongation and spreading of stigmata) and 3 (Complete emergence and wide spreading of stigmata). The efficiency of artificial pollination can be improved based on this guideline.

7.3 Recommendations or Future Prospects

Previously, cv. ‘Kuching’, cv. ‘Semongok Aman’ and cv. ‘Semongok Emas’ are the three most recommended cultivars of black pepper in Malaysia. However, this study outcome only supports cv. ‘Kuching’ as recommended cultivar, judging on high yield potential and high pleasant aroma index. Cv. ‘Semongok Aman’ did not produce high yield potential index based on both morphological characters and physiological performance. At the same time, phytochemical analysis revealed this cultivar produced high unpleasant aroma index based on constituent content analysis due to high α -Pinene and β -Pinene. Whilst, cv. ‘Semongok Emas’ also did not achieve high yield potential, it was ranked 8th position (morphological yield attributing character analysis) and 5th position (photosynthetic performance) among the ten cultivar however the pleasant aroma index (index of 15.17, at 5th position) is at satisfactory level. This study has discovered the

potential of cv. 'Nyerigai' as new recommended cultivar in future. The recommendation is based on high yield potential index (3rd ranking for morphological yield attributing characters assessment; second high group on physiological assessment) and high pleasant aroma index (1st ranking). Thus, this cultivar could be proposed as one of recommended cultivar for farmer beside cv. 'Kuching'

The economic value of the ten cultivars has also been highlighted in this study. In pharmacological industry prospect, peppercorn of cv. 'Semongok Aman' and cv. 'Lampung Daun Lebar' are more promising. The two cultivars were found to have relatively higher piperine and essential oil content compared to other cultivars, thus ensuring higher ROI (Return on Investment) in this industry. For the fragrance industry, peppercorn produced by cultivars such as cv. 'Nyerigai', cv. 'Sarikei' and cv. 'Kuching' has better potential based on high pleasant aroma index. The unique monoterpene constituent of peppercorn could be valuable in perfumery industry.

In perspective of varietal improvement, the superior morphological yield attributing characteristics are valuable. The morphological analysis has revealed both cv. 'Semongok 1' and cv. 'Yong Petai' with extraordinary long inflorescence and long fruit spike length, cv. 'Semongok Aman' with high percentage of fruit set, cv. 'Semongok 1' with high number of flower per inflorescence, cv. 'Kuching' with high number of inflorescence (spike) per branch per node, cv. 'Semongok 1' and cv. 'Yong Petai' with superior peppercorn size and cv. 'Semongok Emas' with more viable conversion rate (fresh to

dried). These are superior characteristics which is important for black pepper breeding program, to develop a more elite black pepper hybrid in future.

Pests and diseases remain as one of black pepper cultivation problem. Sim (1985) has highlighted in a report, the estimated crop losses of 5-10% annually and up to 95% for individual farmers due to this problem. Important pests include pepper weevil, tinged bug, green pepper bug and mealy bug (Megir, 2011) while main diseases are *Phytophthora* foot rot, *Fusarium* wilt, white root and black berry disease (Lily, 2011). This study has not included pest and diseases evaluation due to research facility constraints. This is one of important parameter in cultivar recommendation. The resistance or tolerance index for the ten cultivars against these pests and diseases, are yet to be revealed.

Distribution of black pepper planting in Peninsular Malaysia was mainly constrained by climate factor, particularly inconsistent rainfall (Mohammad Lani et al., 2018) and high temperature (Wong et al., 2018). Moreover, temperature was expected to increase drastically (Wong et al., 2018) in few peninsular state in future due to the climate change effects (Kabiri et al., 2015) and also urbanization and industrialization (Shaharuddin, 2005; Alam et al., 2014). Thus, the drought tolerance evaluation could be an essential consideration for recommended cultivar in future. The drought tolerance index should be further verified even though some assessment has been conducted via WUE evaluation in this study. However, WUE index in this study is merely an assumption outcome based on gas exchange assessment, which is less conclusive. The *in situ*

assessment under fully controlled environment should be conducted to legitimate the drought tolerance index.

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APPENDICES

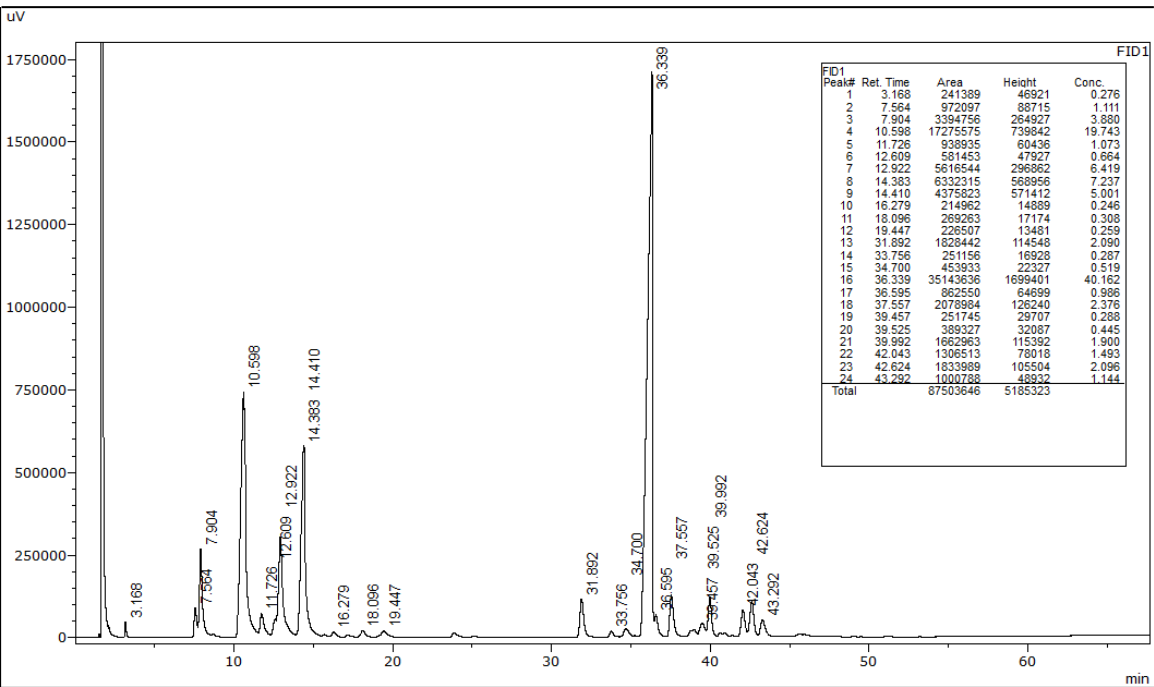
Appendix 1: Correlation matrix for morphological characteristics.

		Leaf shape	Leaf apex	Leaf base	LAI (cm2)	Leaf colour	Blade width (cm)	Blade length (cm)	Blade width-length ratio	Inflorescence length (cm)	Inflorescence colour	Inflorescence thickness (mm)	No. of flower/ Inflorescence	No. of Inflorescence/ branch	Fruit spike length (mm)
Correlation	Leaf shape	1.000	.373	.658	.495	0.000	.756	0.000	-.655	.354	0.000	.577	.394	-.214	.456
	Leaf apex	.373	1.000	.523	.344	-.201	.423	.124	-.488	.264	-.264	.215	.323	-.670	.238
	Leaf base	.658	.523	1.000	.622	.207	.829	0.000	-.670	.465	-.465	.506	.639	-.507	.480
	LA (cm2)	.495	.344	.622	1.000	.067	.810	.549	-.504	.816	-.117	.190	.948	-.678	.843
	Leaf colour	0.000	-.201	.207	.067	1.000	.127	-.449	-.147	.238	0.000	-.389	-.080	.375	.277
	Blade width (cm)	.756	.423	.829	.810	.127	1.000	.378	-.825	.535	-.134	.546	.744	-.486	.604
	Blade length (cm)	0.000	.124	0.000	.549	-.449	.378	1.000	-.073	.354	.354	.096	.525	-.571	.380
	Blade width-length ratio	-.655	-.488	-.670	-.504	-.147	-.825	-.073	1.000	-.154	.309	-.630	-.473	.234	-.199
	Inflorescence length (cm)	.354	.264	.465	.816	.238	.535	.354	-.154	1.000	0.000	-.204	.696	-.454	.968
	Inflorescence colour	0.000	-.264	-.465	-.117	0.000	-.134	.354	.309	0.000	1.000	-.204	-.278	.151	.161
	Inflorescence thickness (mm)	.577	.215	.506	.190	-.389	.546	.096	-.630	-.204	-.204	1.000	.341	-.247	-.132
	No. of flower/Inflorescence	.394	.323	.639	.948	-.080	.744	.525	-.473	.696	-.278	.341	1.000	-.751	.701
	No. of Inflorescence/ branch	-.214	-.670	-.507	-.678	.375	-.486	-.571	.234	-.454	.151	-.247	-.751	1.000	-.469
	Fruit spike length (mm)	.456	.238	.480	.843	.277	.604	.380	-.199	.968	.161	-.132	.701	-.469	1.000
	Fruit size in diameter (mm)	.415	.371	.437	.767	.112	.628	.554	-.272	.881	.294	0.000	.638	-.516	.910
	Fruit weight (single) (g)	.447	.333	.392	.147	.302	.338	-.149	-.293	0.000	.316	.258	.088	-.287	.204
	Fruit colour (hard dough stage)	0.000	.156	-.123	.092	-.895	-.132	.349	.229	0.000	-.247	.202	.234	-.404	-.096
	Percent fruit set (%)	0.000	.248	.291	-.493	.168	-.157	-.554	.091	-.294	-.294	0.000	-.474	.160	-.341
	Conversion rate %(fresh to black pepper)	0.000	-.333	0.000	0.000	0.000	0.000	.149	.293	.316	0.000	-.258	-.088	.287	.204
	Conversion rate %(fresh to white pepper)	0.000	-.145	-.385	-.354	-.263	-.369	-.163	.319	0.000	0.000	-.282	-.442	.481	-.134
	Pericarp thickness (mm)	.559	.667	.294	.332	-.302	.423	.186	-.488	.395	0.000	.323	.264	-.287	.357
	Seed diameter (mm)	.373	.167	-.131	.467	-.201	.282	.497	-.163	.527	.527	0.000	.323	-.192	.578
	Seed weight (g)	-.354	0.000	-.310	-.117	-.715	-.401	.118	.309	-.250	-.250	.204	.139	-.303	-.323
	Branch column types	-.188	.618	-.099	.137	-.533	-.142	.251	.041	0.000	-.133	0.000	.245	-.670	-.034
	Internode length (cm)	0.000	-.371	.109	-.219	.168	-.157	-.554	.091	0.000	-.587	0.000	-.147	.516	-.152
	Number of node/ 1feet stem	-.745	-.111	-.784	-.639	-.201	-.845	-.248	.488	-.527	0.000	-.430	-.558	.287	-.612
	Anthocyanin	.745	.111	.784	.639	.201	.845	.248	-.488	.527	0.000	.430	.558	-.287	.612

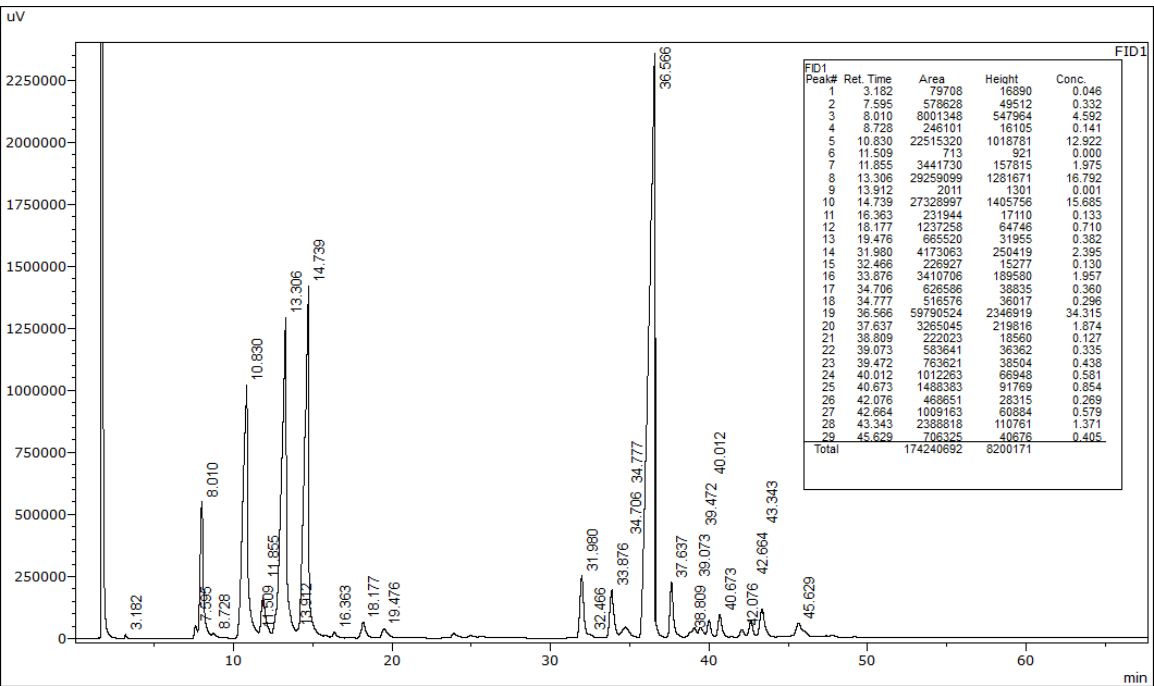
Appendix 1 continued

		Fruit size in diameter (mm)	Fruit weight (single) (g)	Fruit colour (hard dough stage)	Percent fruit set (%)	Conversion rate % (fresh to black pepper)	Conversion rate % (fresh to white pepper)	Pericarp thickness (mm)	Seed diameter (mm)	Seed weight (g)	Branch column types	Internode length (cm)	Number of node/1feet stem	Anthocya nin
Correlation	Leaf shape	.415	.447	0.000	0.000	0.000	0.000	.559	.373	-.354	-.188	0.000	-.745	.745
	Leaf apex	.371	.333	.156	.248	-.333	-.145	.667	.167	0.000	.618	-.371	-.111	.111
	Leaf base	.437	.392	-.123	.291	0.000	-.385	.294	-.131	-.310	-.099	.109	-.784	.784
	LA (cm2)	.767	.147	.092	-.493	0.000	-.354	.332	.467	-.117	.137	-.219	-.639	.639
	Leaf colour	.112	.302	-.895	.168	0.000	-.263	-.302	-.201	-.715	-.533	.168	-.201	.201
	Blade width (cm)	.628	.338	-.132	-.157	0.000	-.369	.423	.282	-.401	-.142	-.157	-.845	.845
	Blade length (cm)	.554	-.149	.349	-.554	.149	-.163	.186	.497	.118	.251	-.554	-.248	.248
	Blade width- length ratio	-.272	-.293	.229	.091	.293	.319	-.488	-.163	.309	.041	.091	.488	-.488
	Inflorescence length (cm)	.881	0.000	0.000	-.294	.316	0.000	.395	.527	-.250	0.000	0.000	-.527	.527
	Inflorescence colour	.294	.316	-.247	-.294	0.000	0.000	0.000	.527	-.250	-.133	-.587	0.000	0.000
	Inflorescence thickness (mm)	0.000	.258	.202	0.000	-.258	-.282	.323	0.000	.204	0.000	0.000	-.430	.430
	No. of flower/Infloresce nce	.638	.088	.234	-.474	-.088	-.442	.264	.323	.139	.245	-.147	-.558	.558
	No. of Inflorescence/ branch	-.516	-.287	-.404	.160	.287	.481	-.287	-.192	-.303	-.670	.516	.287	-.287
	Fruit spike length (mm)	.910	.204	-.096	-.341	.204	-.134	.357	.578	-.323	-.034	-.152	-.612	.612
	Fruit size in diameter (mm)	1.000	.186	-.087	-.310	.186	-.122	.557	.681	-.294	.047	-.310	-.557	.557
	Fruit weight (single) (g)	.186	1.000	-.469	.186	-.600	-.655	0.000	0.000	-.316	.084	-.557	-.333	.333
	Fruit colour (hard dough stage)	-.087	-.469	1.000	-.203	.156	.375	.234	.156	.741	.513	.087	.156	-.156
	Percent fruit set (%)	-.310	.186	-.203	1.000	.186	.122	-.093	-.681	-.294	-.203	.310	-.062	.062
	Conversion rate %(fresh to black pepper)	.186	-.600	.156	.186	1.000	.655	0.000	0.000	-.316	-.590	.557	-.333	.333
	Conversion rate %(fresh to white pepper)	-.122	-.655	.375	.122	.655	1.000	.327	.218	0.000	-.202	.527	.218	-.218
	Pericarp thickness (mm)	.557	0.000	.234	-.093	0.000	.327	1.000	.667	0.000	.295	-.093	-.167	.167
	Seed diameter (mm)	.681	0.000	.156	-.681	0.000	.218	.667	1.000	0.000	.197	-.371	-.111	.111
	Seed weight (g)	-.294	-.316	.741	-.294	-.316	0.000	0.000	0.000	1.000	.666	0.000	.527	-.527
	Branch column types	.047	.084	.513	-.203	-.590	-.202	.295	.197	.666	1.000	-.516	.477	-.477
	Internode length (cm)	-.310	-.557	.087	.310	.557	.527	-.093	-.371	0.000	-.516	1.000	-.062	.062
	Number of node/ 1feet stem	-.557	-.333	.156	-.062	-.333	.218	-.167	-.111	.527	.477	-.062	1.000	-1.000
	Anthocyanin	.557	.333	-.156	.062	.333	-.218	.167	.111	-.527	-.477	.062	-1.000	1.000

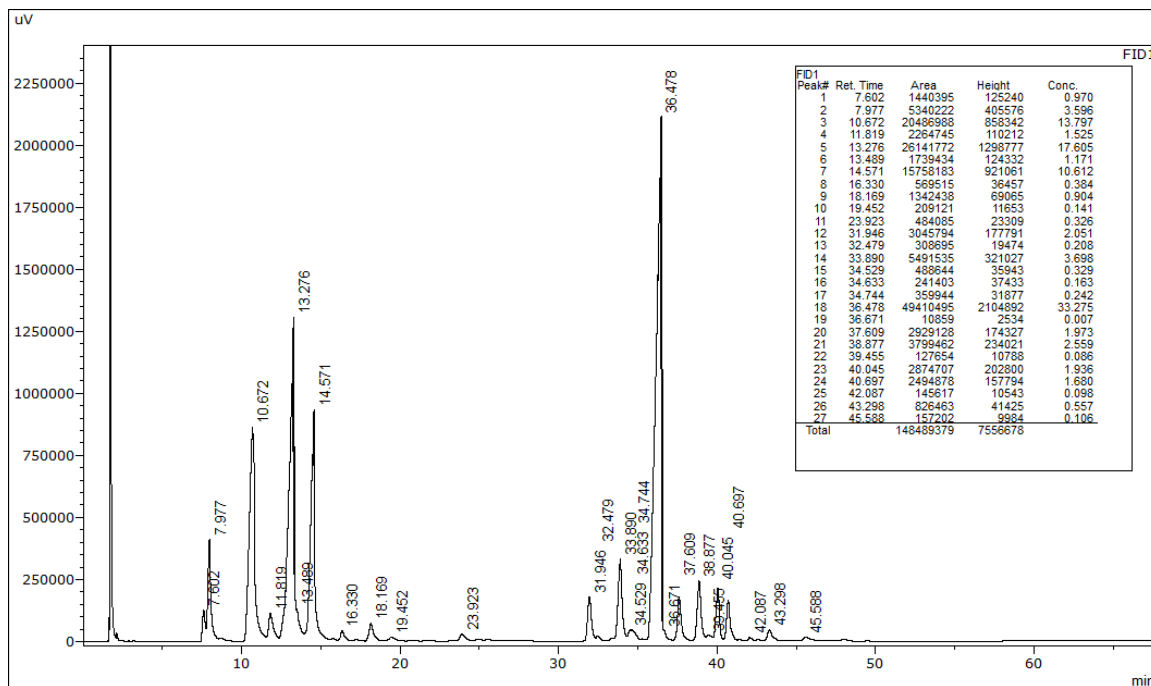
Appendix 2: HPLC chromatogram of essential oil for peppercorn from cv. ‘Semongok Aman’.



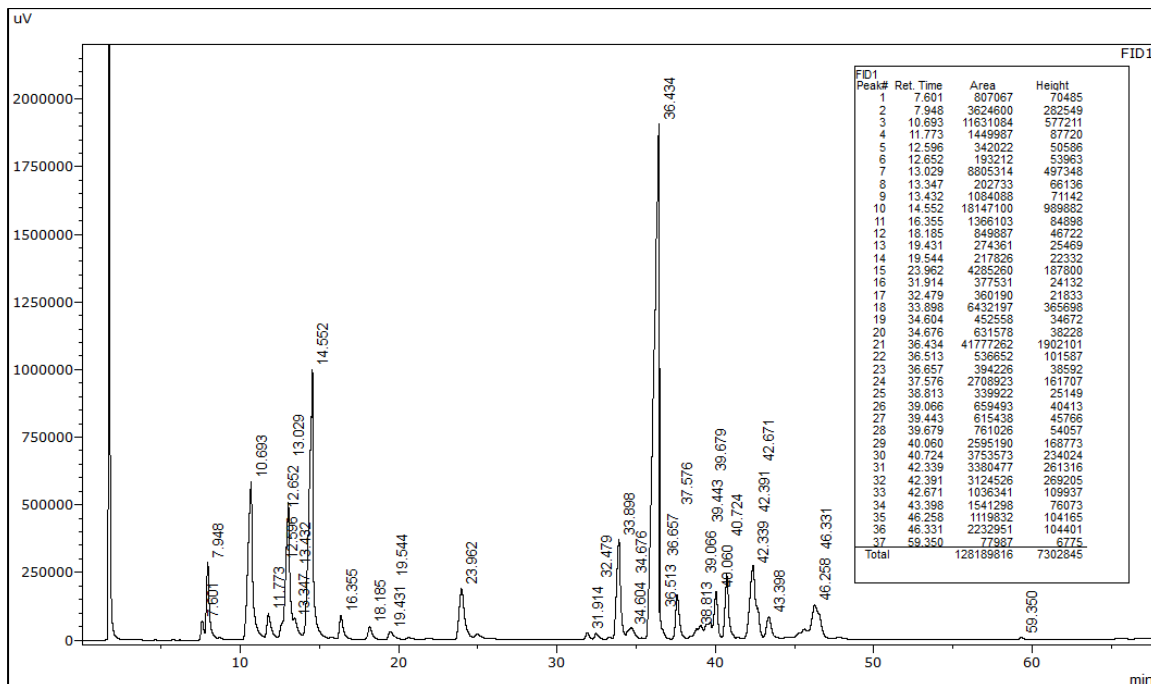
Appendix 3: HPLC chromatogram of essential oil for peppercorn from cv. ‘Kuching’.



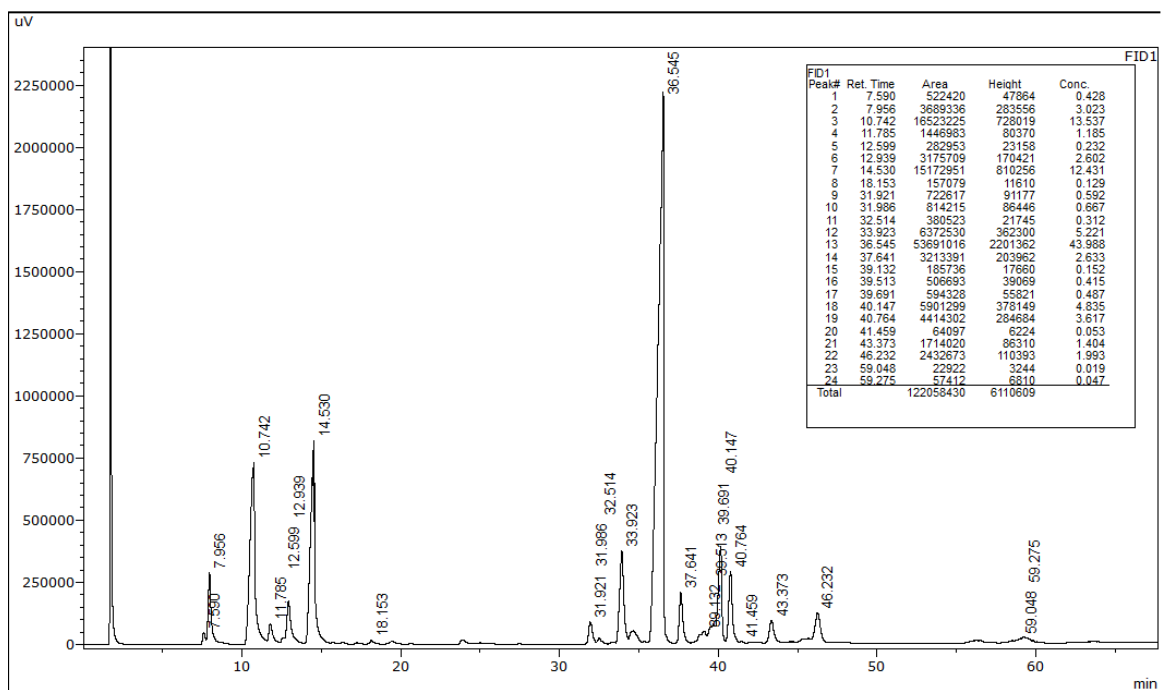
Appendix 4: HPLC chromatogram of essential oil for peppercorn from cv. ‘Semongok Emas’.



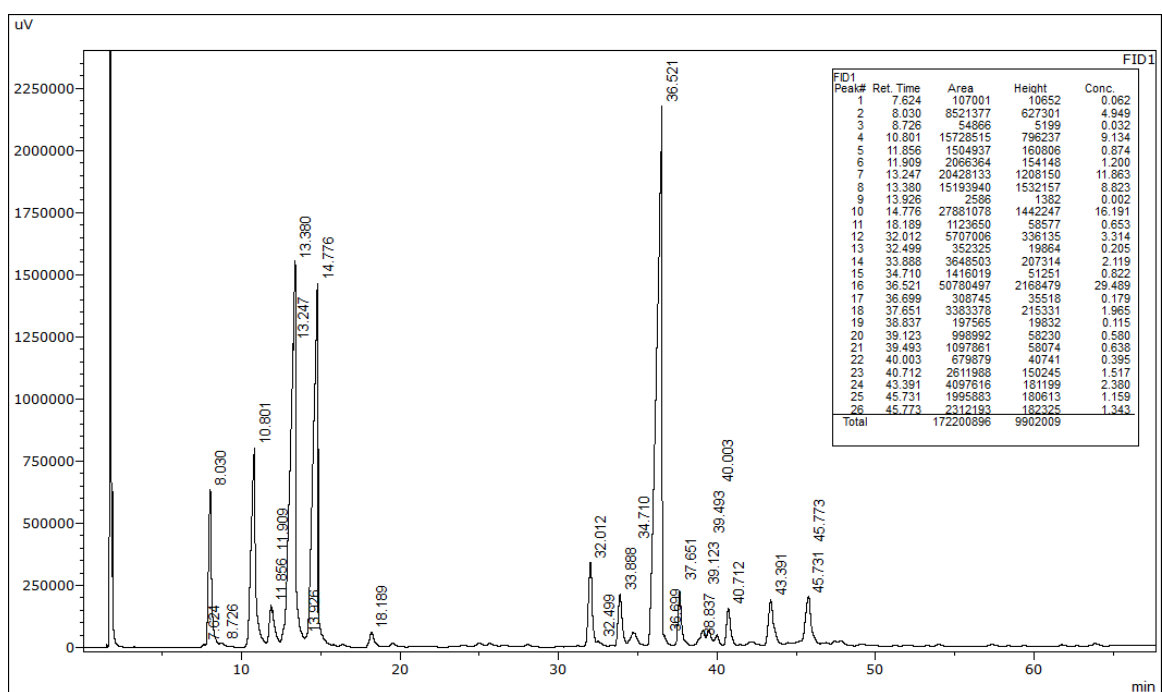
Appendix 5: HPLC chromatogram of essential oil for peppercorn from cv. ‘Semongok Perak’.



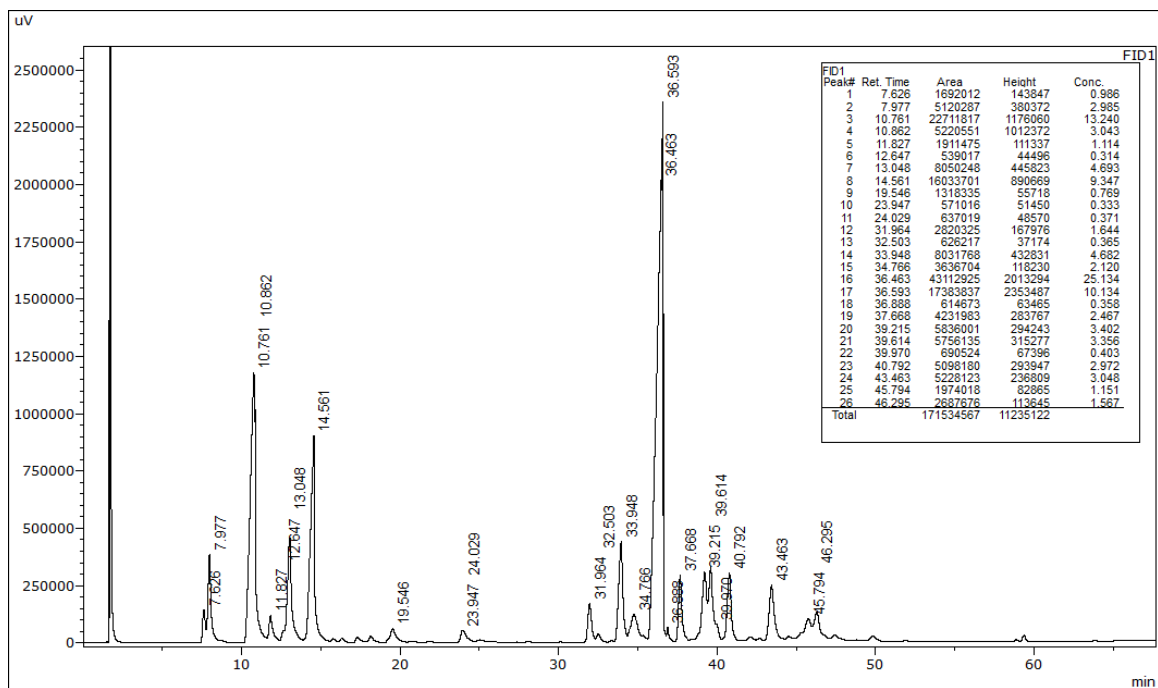
Appendix 6: HPLC chromatogram of essential oil for peppercorn from cv. ‘Semongok 1’.



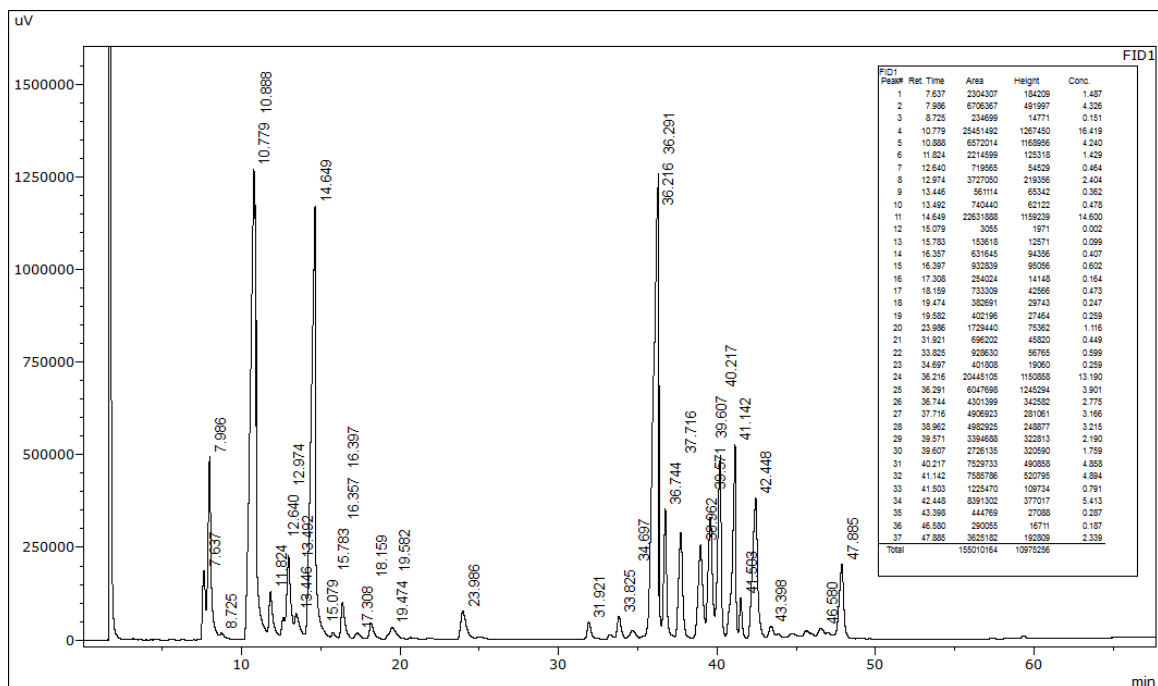
Appendix 7: HPLC chromatogram of essential oil for peppercorn from cv. ‘Nyerigai’.



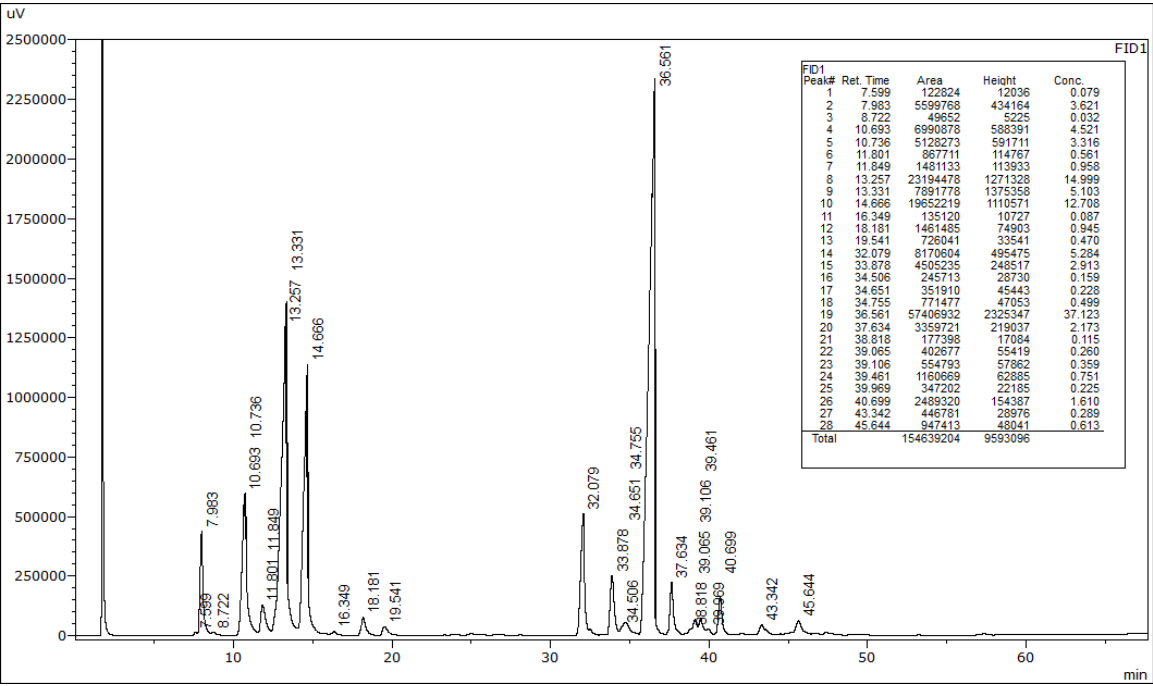
Appendix 8: HPLC chromatogram of essential oil for peppercorn from cv. 'India'.



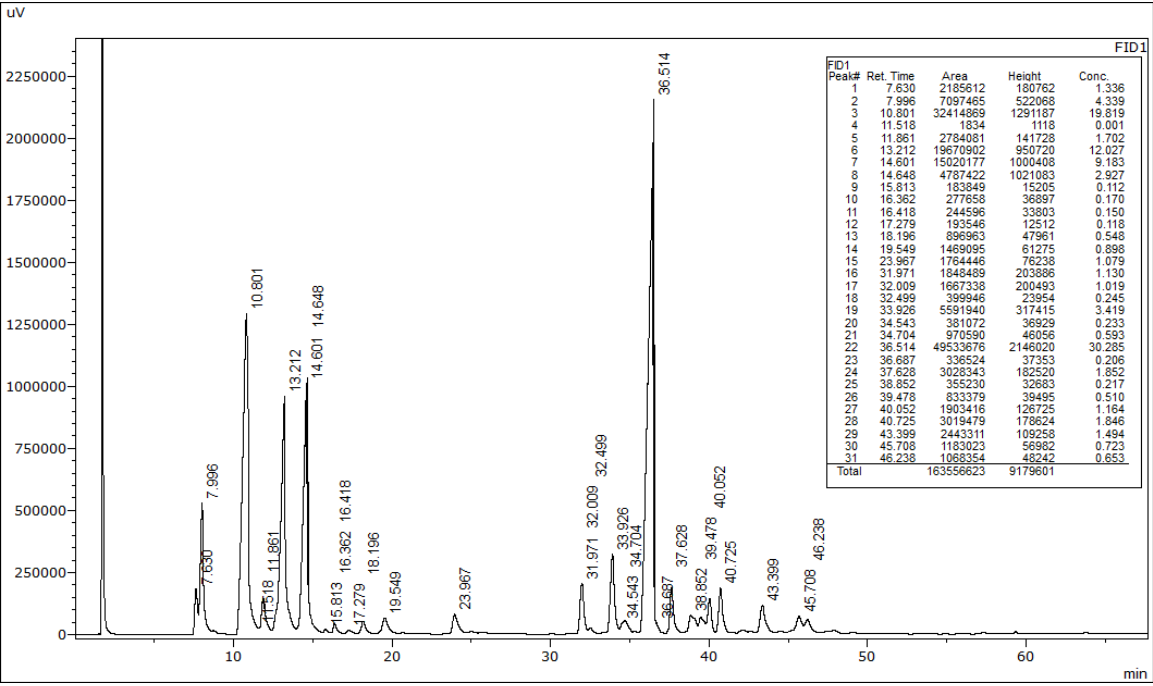
Appendix 9: HPLC chromatogram of essential oil for peppercorn from cv. 'Lampung Daun Lebar'.



Appendix 10: HPLC chromatogram of essential oil for peppercorn from cv. ‘Sarikei’.



Appendix 11: HPLC chromatogram of essential oil for peppercorn from cv. ‘Yong Petai’.



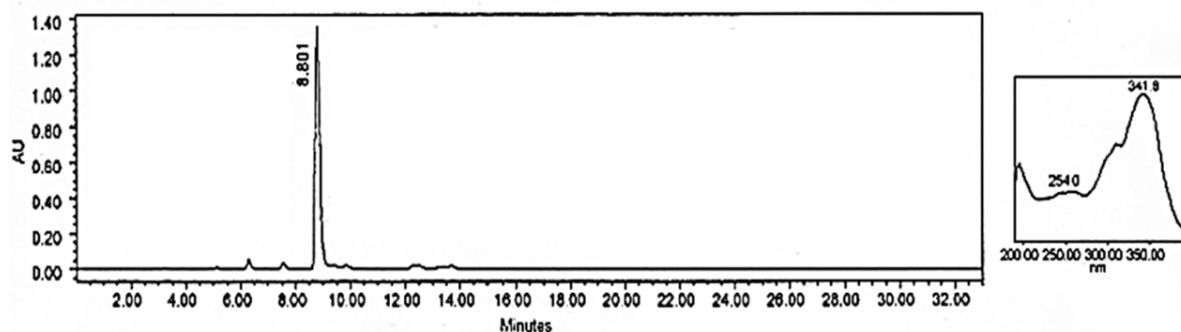
Appendix 12: Correlation matrix for essential oil constituents.

		α - Thujene	β - Pinene	α - Phellandrene	δ - 3- Carene	γ - Terpinene	Terpinolene	Linalool	Terpinen-4-ol	δ - Elemene	α - Cubebene	α - Copaene	β - Elemene	γ - Elemene	α - Humulene
Correlation	α - Thujene	1.000	.111	.040	-.441	.620	-.167	.048	.481	-.364	.681	-.555	-.201	-.207	.364
	β - Pinene	.111	1.000	-.441	-.361	.207	-.250	-.048	.259	-.677	.557	.128	-.804	.207	.677
	α - Phellandrene	.040	-.441	1.000	-.275	.224	-.241	-.189	.094	-.056	-.022	-.262	.254	.672	.056
	δ - 3- Carene	-.441	-.361	-.275	1.000	-.224	.843	-.155	-.495	.808	-.648	-.046	.290	-.224	-.808
	γ - Terpinene	.620	.207	.224	-.224	1.000	.155	-.089	.620	-.484	.576	-.079	-.374	.231	.097
	Terpinolene	-.167	-.250	-.241	.843	.155	1.000	.071	.028	.625	-.371	-.032	.075	-.155	-.820
	Linalool	.048	-.048	-.189	-.155	-.089	.071	1.000	.587	-.067	-.027	.238	.302	-.089	-.156
	Terpinen-4-ol	.481	.259	.094	-.495	.620	.028	.587	1.000	-.503	.557	.128	-.302	.207	.156
	δ - Elemene	-.364	-.677	-.056	.808	-.484	.625	-.067	-.503	1.000	-.667	-.220	.518	-.291	-.756
	α - Cubebene	.681	.557	-.022	-.648	.576	-.371	-.027	.557	-.667	1.000	-.166	-.448	.115	.667
	α - Copaene	-.555	.128	-.262	-.046	-.079	-.032	.238	.128	-.220	-.166	1.000	-.039	-.079	-.180
	β - Elemene	-.201	-.804	.254	.290	-.374	.075	.302	-.302	.518	-.448	-.039	1.000	-.187	-.518
	γ - Elemene	-.207	.207	.672	-.224	.231	-.155	-.089	.207	-.291	.115	-.079	-.187	1.000	.291
	α - Humulene	.364	.677	.056	-.808	.097	-.820	-.156	.156	-.756	.667	-.180	-.518	.291	1.000
	(E) - β - Farnesene	-.111	-1.000	.441	.361	-.207	.250	.048	-.259	.677	-.557	-.128	.804	-.207	-.677
	Germacrene D	.667	.167	-.241	-.060	.620	.063	-.286	.167	-.156	.557	-.192	-.302	-.310	.156
	β - Selinene	-.111	.111	.040	-.441	-.207	-.583	.524	.111	-.364	.062	.299	.302	.207	.364
	α - Selinene	-.111	.111	.040	-.441	-.207	-.583	.524	.111	-.364	.062	.299	.302	.207	.364
	β - Bisabolene	.535	.356	.129	-.611	.497	-.367	-.420	.356	-.543	.695	-.068	-.645	0.000	.543
	α - Cadinene	-.430	0.000	.155	-.311	0.000	-.323	.184	.143	-.403	0.000	.826	.195	.160	0.000
	Germacrene B	-.111	.111	.441	-.040	.620	.250	.048	.481	-.364	.062	.299	-.201	.620	-.156
	(E) - Nerolidol	-.156	.156	.620	-.056	.097	-.039	-.379	-.017	-.024	.087	-.380	-.283	.872	.268
	Caryophyllene oxide	.429	.524	-.155	-.533	.089	-.429	.429	.365	-.602	.557	-.238	-.086	.089	.602
	α - Zingiberene	1.000	.111	.040	-.441	.620	-.167	.048	.481	-.364	.681	-.555	-.201	-.207	.364
	(E) - γ - Bisabolene	1.000	.111	.040	-.441	.620	-.167	.048	.481	-.364	.681	-.555	-.201	-.207	.364
	Herdicaryol	1.000	.111	.040	-.441	.620	-.167	.048	.481	-.364	.681	-.555	-.201	-.207	.364
	α - Bisabolol	1.000	.111	.040	-.441	.620	-.167	.048	.481	-.364	.681	-.555	-.201	-.207	.364

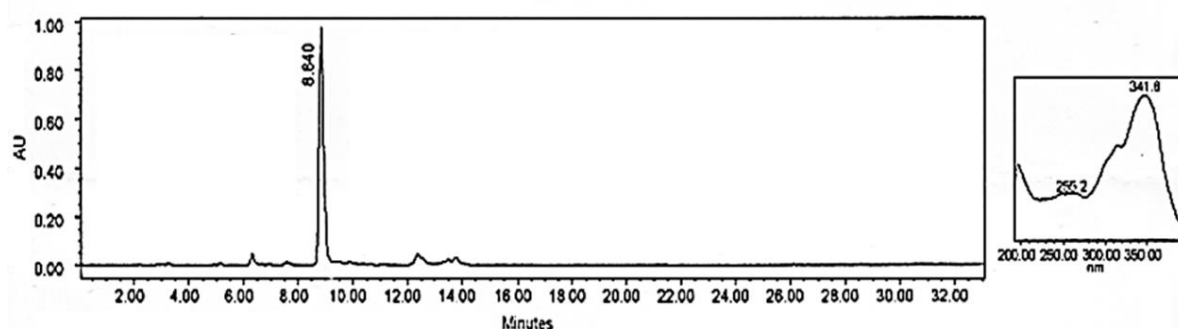
Appendix 12 continued

		(E) - β - Farnesene	Germacrene D	β - Selinene	α - Selinene	β - Bisabolene	α - Cadinene	Germacrene B	(E) - Nerolidol	Caryophyllen e oxide	α - Zingiberene	(E) - γ - Bisabolene	Herdicaryol	α - Bisabolol
Correlation	α - Thujene	-.111	.667	-.111	-.111	.535	-.430	-.111	-.156	.429	1.000	1.000	1.000	1.000
	β - Pinene	-1.000	.167	.111	.111	.356	0.000	.111	.156	.524	.111	.111	.111	.111
	α - Phellandrene	.441	-.241	.040	.040	.129	.155	.441	.620	-.155	.040	.040	.040	.040
	δ - 3- Carene	.361	-.060	-.441	-.441	-.611	-.311	-.040	-.056	-.533	-.441	-.441	-.441	-.441
	γ - Terpinene	-.207	.620	-.207	-.207	.497	0.000	.620	.097	.089	.620	.620	.620	.620
	Terpinolene	.250	.063	-.583	-.583	-.367	-.323	.250	-.039	-.429	-.167	-.167	-.167	-.167
	Linalool	.048	-.286	.524	.524	-.420	.184	.048	-.379	.429	.048	.048	.048	.048
	Terpinen-4-ol	-.259	.167	.111	.111	.356	.143	.481	-.017	.365	.481	.481	.481	.481
	δ - Elemene	.677	-.156	-.364	-.364	-.543	-.403	-.364	-.024	-.602	-.364	-.364	-.364	-.364
	α - Cubebene	-.557	.557	.062	.062	.695	0.000	.062	.087	.557	.681	.681	.681	.681
	α - Copaene	-.128	-.192	.299	.299	-.068	.826	.299	-.380	-.238	-.555	-.555	-.555	-.555
	β - Elemene	.804	-.302	.302	.302	-.645	.195	-.201	-.283	-.086	-.201	-.201	-.201	-.201
	γ - Elemene	-.207	-.310	.207	.207	0.000	.160	.620	.872	.089	-.207	-.207	-.207	-.207
	α - Humulene	-.677	.156	.364	.364	.543	0.000	-.156	.268	.602	.364	.364	.364	.364
	(E) - β - Farnesene	1.000	-.167	-.111	-.111	-.356	0.000	-.111	-.156	-.524	-.111	-.111	-.111	-.111
	Germacrene D	-.167	1.000	-.167	-.167	.468	-.323	-.167	-.234	-.071	.667	.667	.667	.667
	β - Selinene	-.111	-.167	1.000	1.000	-.356	.430	-.111	-.156	.429	-.111	-.111	-.111	-.111
	α - Selinene	-.111	-.167	1.000	1.000	-.356	.430	-.111	-.156	.429	-.111	-.111	-.111	-.111
	β - Bisabolene	-.356	.468	-.356	-.356	1.000	0.000	.089	.125	.038	.535	.535	.535	.535
	α - Cadinene	0.000	-.323	.430	.430	0.000	1.000	.430	-.202	0.000	-.430	-.430	-.430	-.430
	Germacrene B	-.111	-.167	-.111	-.111	.089	.430	1.000	.364	-.048	-.111	-.111	-.111	-.111
	(E) - Nerolidol	-.156	-.234	-.156	-.156	.125	-.202	.364	1.000	-.067	-.156	-.156	-.156	-.156
	Caryophyllene oxide	-.524	-.071	.429	.429	.038	0.000	-.048	-.067	1.000	.429	.429	.429	.429
	α - Zingiberene	-.111	.667	-.111	-.111	.535	-.430	-.111	-.156	.429	1.000	1.000	1.000	1.000
	(E) - γ - Bisabolene	-.111	.667	-.111	-.111	.535	-.430	-.111	-.156	.429	1.000	1.000	1.000	1.000
	Herdicaryol	-.111	.667	-.111	-.111	.535	-.430	-.111	-.156	.429	1.000	1.000	1.000	1.000
	α - Bisabolol	-.111	.667	-.111	-.111	.535	-.430	-.111	-.156	.429	1.000	1.000	1.000	1.000

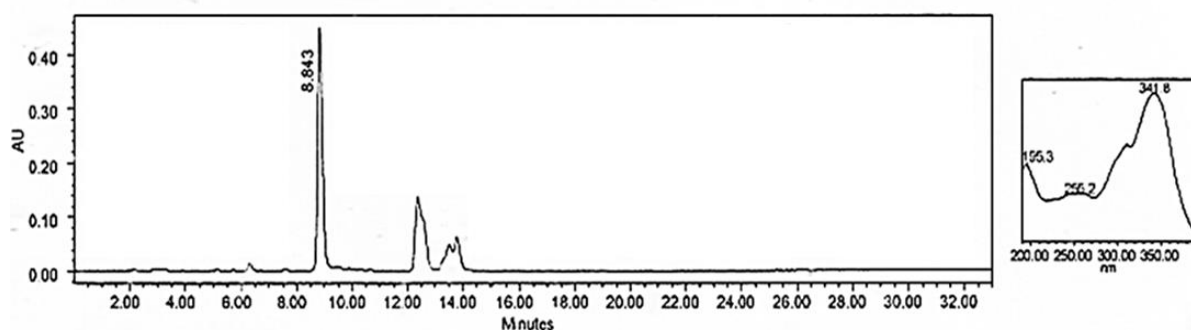
Appendix 13: HPLC chromatogram of piperine extracted from peppercorn of cv. 'Semongok Aman'.



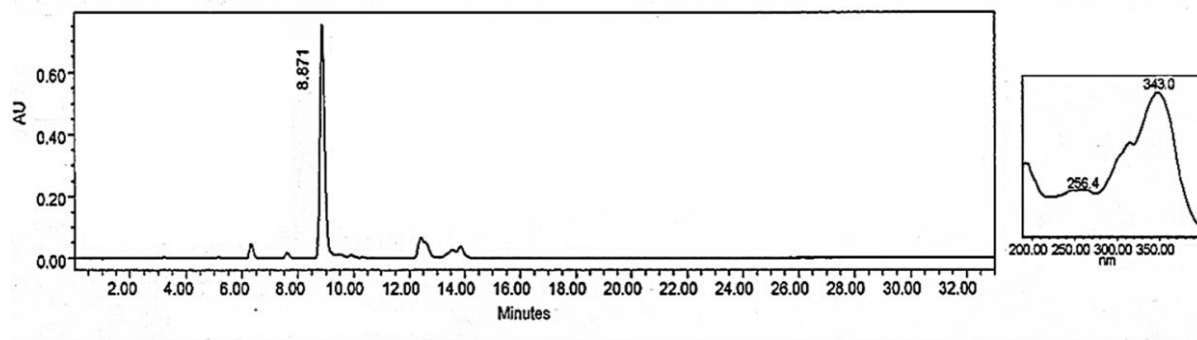
Appendix 14: HPLC chromatogram of piperine extracted from peppercorn of cv. 'Kuching'.



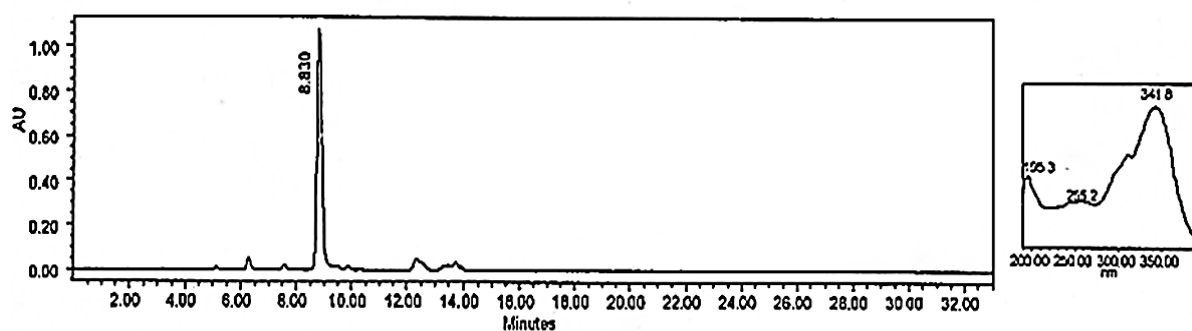
Appendix 15: HPLC chromatogram of piperine extracted from peppercorn of cv. 'Semongok Emas'.



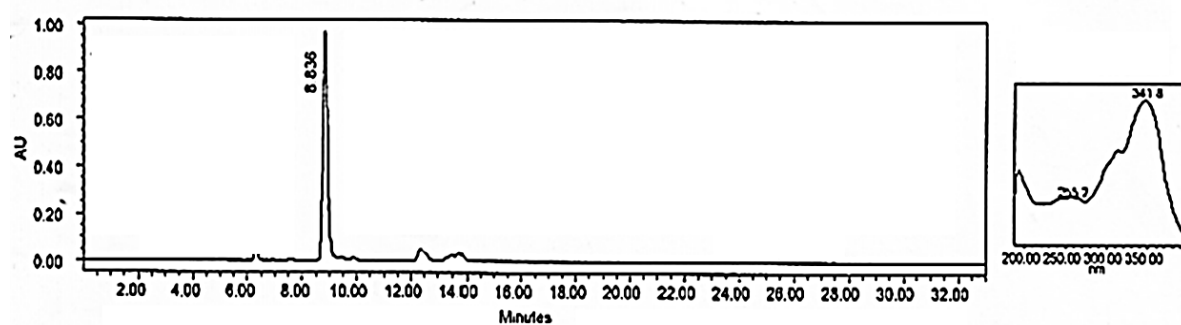
Appendix 16: HPLC chromatogram of piperine extracted from peppercorn of cv. 'Semongok Perak'.



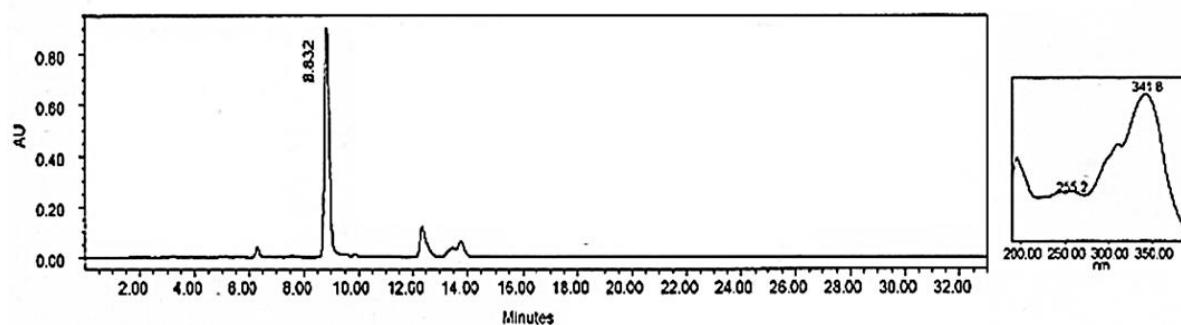
Appendix 17: HPLC chromatogram of piperine extracted from peppercorn of cv. 'Semongok 1'.



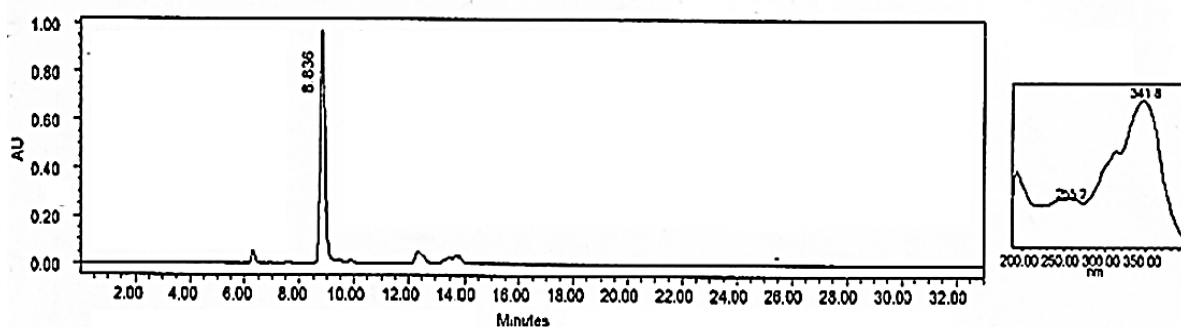
Appendix 18: HPLC chromatogram of piperine extracted from peppercorn of cv. 'Nyerigai'.



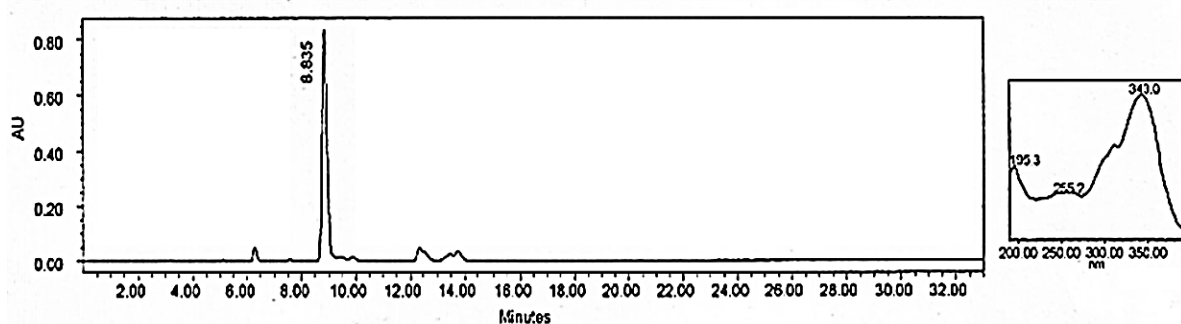
Appendix 19: HPLC chromatogram of piperine extracted from peppercorn of cv. 'India'.



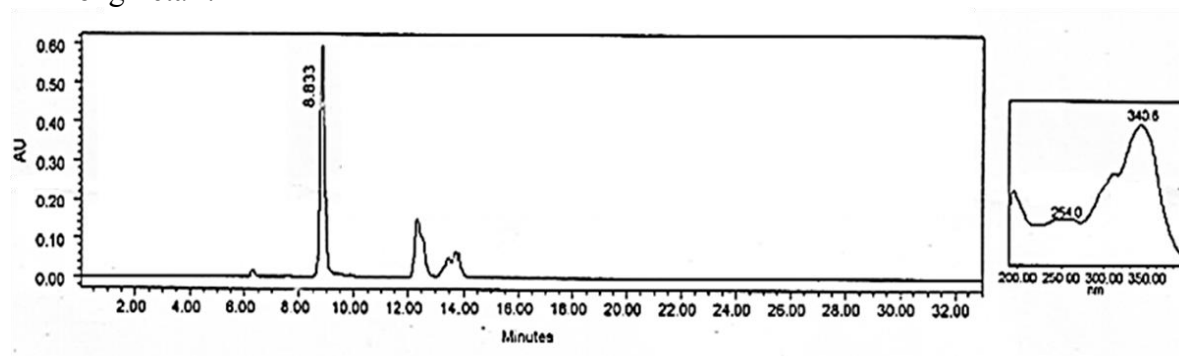
Appendix 20: HPLC chromatogram of piperine extracted from peppercorn of cv. 'Lampung Daun Lebar'.



Appendix 21: HPLC chromatogram of piperine extracted from peppercorn of cv. 'Sarikei'.



Appendix 22: HPLC chromatogram of piperine extracted from peppercorn of cv. 'Yong Petai'.



Appendix 23: Journal Publications

1. **Chen, Y. S.**, Dayod, M. & Tawan, C.S. (2018). Anther Dehiscence, Pollen Viability and Stigma Receptivity Study on Cultivars of Black Pepper (*Piper nigrum* L.), *Pertanika Journal of Tropical Agricultural Science*, 41(2): 801-814.
2. **Chen, Y. S.**, Dayod, M. & Tawan, C.S. (2018). Phenetic Analysis of Cultivated Black Pepper (*Piper nigrum* L.) in Malaysia, *International Journal of Agronomy*, Article ID 3894924, Volume 2018.
3. **Chen, Y. S.** & Tawan, C.S. (2018). Floral Biology Study on Black Pepper (*Piper nigrum* L.) to Improve Efficiency of Conventional Breeding. In *4th International Plant Breeding Conference 2018*, 13-15 November 2018, Hotel Bangi-Putrajaya, Malaysia.
4. **Chen, Y. S.** & Tawan, C.S. (2020). Botany, Diversity, and Distribution of Black Pepper (*Piper nigrum* L.) Cultivars in Malaysia. *Borneo Journal of Resource Science and Technology*, 10(1): 10-23.
5. **Chen, Y. S.**, Dayod, M. & Tawan, C.S. (2020). Analysis of Qualitative and Quantitative Trait Variability among Black Pepper (*Piper nigrum* L.) Cultivars in Malaysia. *Pertanika Journal of Tropical Agricultural Science*, 43(3): 8-19.