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MACROMORPHOLOGICAL AND MICROMORPHOLOGICAL STUDIES OF FOUR SELECTED *PASSIFLORA* SPECIES IN PENINSULAR MALAYSIA

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Abstract

Taxonomic studies of four selected *Passiflora* species; *Passiflora edulis* Sims (Passion fruit), *Passiflora coccinea* Aubl. (Scarlet passion flower), *Passiflora foetida* L. (Stinking passion flower) and *Passiflora incarnata* L. (Fragrant passion flower) were carried out to distinguish their morphological characteristics. Macromorphological characters on the leaves, flowers and fruits of all four *Passiflora* species were observed under a binocular microscope and they varied characteristically in terms of colour, texture and shape of flowers, leaves and fruits. Jeol JSM-7500F Scanning Electron Microscope was used to observe the micromorphological characters such as stomata, trichomes, and leaf surface indumentum of each *Passiflora* species. Micromorphologically, each species varied in types and sizes of stomata, epidermal surfaces and presence of trichomes.

Key words: Macromorphology; Micromorphology; *Passiflora*; Peninsular Malaysia.

Introduction

The family Passifloraceae is represented by four genera (*Ancistrothyrsus*, *Dilkea*, *Mitostemma* and *Passiflora*) in America, of which *Passiflora* is numerically and economically the most important genus of the family (Waterhouse, 1994). The species of this genus are mostly originated in tropical America and about 200 of these species are native to Brazil (Viana *et al*., 2010). They are distributed in warm temperate and tropical regions of the world. *Passiflora* species are much rarer in Asia, Australia, and tropical Africa (Patel, 2009). Only less than eight species of *Passiflora* were recorded in Malaysia out of more than 500 species worldwide (Viana *et al*., 2010 & Waterhouse, 1994).

The genus *Passiflora* is the largest in family Passifloraceae (Ulmer & MacDougal 2004). Patel (2009) mentioned that the plants of genus *Passiflora* are shrubs, entire lobed or palmate, sometimes compound, imparipinnate; stipules grow at the base of petioles, rarely absent; tendril axillary, arising from sterile pedicels. Several have edible fruits and attractive flowers. There are about 40 cultivated species, but only fewer than six are fruit crops in the neotropics and only one, *Passiflora edulis* Sims (passion fruit or purple granadilla) and its varieties, such as the yellow flavicarpa, is economically important (Waterhouse, 1994 & McGuire, 1999). Many others are grown outdoors in the warmer parts of the world or in the glasshouses for their exotic flowers (Dhawan *et al*., 2004).

The wild species in the genus *Passiflora* have important characters for breeding programs, especially for their ornamental values. *Passiflora* has wide morphological (Crochemore *et al*., 2003a) and genetic variability (Crochemore *et al*., 2003b & Viana *et al.,* 2010). Most taxonomic studies on *Passiflora* are based on the morphological and agronomic characterization and the taxonomy of the *Passifloraceae* family has yet to be clarified (Viana *et al*., 2010). Phyto-constituents like alkaloids, phenols, glycosyl flavonoids and cyanogenic compounds are known in the genus of *Passiflora* (Patel, 2009 & Patil, 2010). It has also been discovered that a chemical component of passion flower, namely passicol, has antimicrobial activity (Nicolls *et al*., 1973 & Mohanasundari *et al*., 2007). *P. edulis* var. *flavicarpa* Deg., the yellow passion fruit and *P. edulis* Sims, the purple passion fruit, are the most economically important species in the Americas, Australia and Africa. Classical methods of selection are being used in *Passiflora* breeding programmes. The germplasm of wild species is being used to transfer desirable traits into the cultivated species in Brazil (Dornelas & Vieira, 1994).

Although various economic importances have been reported in *Passiflora* species, taxonomic information on some species are still lacking. Hence, it is the aim of the present study to add more taxonomic information particularly on the micromorphological aspects of the economically important *Passiflora* species in Peninsular Malaysia.

Materials and Methods

Macromorphological studies: *Passiflora* specimens were collected from various locations around the states of Selangor and Federal Territory of Kuala Lumpur and observed under a binocular microscope. Vegetative and reproductive morphological characters were recorded and photographs were taken.

Scanning electron microscopy techniques: Micromorphological studies were conducted by observing the abaxial and adaxial surfaces of the leaves under the scanning electron microscope. The four selected *Passiflora* leaf specimens were cut in square shape with measurement of approximately 3 mm x 3 mm and soaked in a mixed solution of 8% Glutaraldehyde and Sorencen's Buffered Phosphate solution with a ratio of 1:1 for one hour. The leaf specimens were washed with Sorencen's Buffered Phosphate solution and distilled water in a ratio of 1:1 for 5 minutes. Specimens were then soaked in a mixed solution of 4% Osmium and distilled water with a ratio of 1:1. After about 14 hours at low temperature, the dehydration process was conducted in vessel fumes. The concentration of ethanol was increased for every 15 minutes, from 10% until it reaches 100%. Specimens were soaked in a mixture of 100% ethanol and 100%

acetone with ratios of 3:1, 1:1, and 1:3 for 20 minutes respectively. The leaf specimens were then soaked in 100% acetone solution for 20 minutes, repeating them for four times. Next, the method of Critical Point Drying (CPD) was conducted using Bal-Tec CPD 030 Critical Point Dryer. Specimens were mounted on aluminium with diameter of 12.5 mm using Conducting Carbon Cement (LEIT-C), kept in a drying jar, and coated with a thin layer of gold (40-60 nm) by using BIO-Rod SEM Coating System. Stomata and the abaxial and adaxial surfaces structure of the leaves were observed under the Jeol JSM-7500F scanning electron microscope and recorded.

Results and Discussions

A summary of the morphological characteristics is given in Tables 1 and 2. Baranova (1992), Jones (1986) and Li *et al.* (2007) stated that leaf epidermal characteristics are of prospective taxonomical significance and the leaf is more sensitized and flexible to environmental change compared to other organs. This is because environment change typically results in anatomical and morphological responses on leaf, including morphology (thickness, length, width), surface (epidermis, stomata, attachment) and mesophyll (palisade, spongy, sclerified, intercellular space, vein) (Xunling & Jing, 1989 & Li *et al.*, 2007). Li *et al*. (2007) studied the structure of leaf epidermis in Wild honeysuckle and Japanese honeysuckle for classification and documented their ecological habits. They also stated that leaf morphology allows comprehension of its ecology adaptation on the leaf-structure such as well-developed epidermal hair, conducting tissue and mechanical tissue. For example, some plants adopt protective measures against drought and possess well-developed epidermal hair and wax-like water-repellent cutin covering the epidermis, which reduces the rate of transpiration and irradiation. However, in this study, we have only focused on the morphological characters to distinguish each *Passiflora* species.

Leaf: *Passiflora edulis* Sims var. *edulis* leaves are ovate, trilobed, where the middle lobe is longer than the other two side ones (Fig. 1a). The middle lobe measures approximately 3.5-8 x 2.3-5 cm. The leaf apex is cuspidate while the leaf base is cordate with serrate margin. The abaxial surfaces of the leaf are shiny, smooth and bright green. Fig. 1b shows the distribution of paracytic stomata on the leaf abaxial surface of *Passiflora edulis* Sims var. *edulis* which was observed under the scanning electron microscope. Papillose epidermal surface with undulating anticlinal wall was observed. The stomata on the abaxial surface measures 13.4 x 10.1 µm (Fig. 1c). Fig. 1d shows the absence of stomata and irregularly shaped polygonal epidermal cells on the adaxial surface. Epicuticular wax is sparsely distributed on the surface. Trichomes were absent on both abaxial and adaxial surfaces of the leaves.

Passiflora coccinea Aubl. leaves are oblong shaped (Fig. 2a). A mature leaf measures 9.5-12.5 x 6.5-8 cm. Leaves are alternate. The leaf lamina of both abaxial and adaxial surfaces are green, however, the adaxial surface has a darker shade of green compared to the abaxial surface. The leaf margins are doubly serrate, with an acute apex, and have rounded base. Scanning electron microscopy observation shows that the abaxial surface of *Passiflora coccinea* Aubl. leaf has papillose epidermal surface which is covered by a very thick epicuticular wax. Stomata and trichomes were also observed on the abaxial surface (Figs. 2b and 2c). Stomata are anomocytic and they measure 8.58 x 3.85 µm (Fig. 2d). The trichomes are simple unicellular; highly dense on the leaf midrib (Fig. 2e). The adaxial surface of *Passiflora coccinea* leaf shows absence of stomata and less trichome distribution compared to the abaxial surface (Fig. 2f).

Passiflora foetida L. leaves are pubescent on both abaxial and adaxial surfaces, including the serrate ends of the leaves. Leaves are thin and bright light green coloured (Fig. 3a). They are divided into three lobes. The middle lobe measures 6-11 x 4-4.5 cm. Leaf shape is ovate whereby the base of the leaf is cordate. Based on the scanning electron microscopy observation of a *Passiflora foetida* leaf, the abaxial surface has a rugose epidermal surface which is not covered by a very thick layer of epicuticular wax (Fig. 3b). Stomata are amphiparacytic, about 11.6 x 6.75 µm (Fig. 3c). The trichomes on a *Passiflora foetida* leaf are glandular and simple unicellular (Figs. 3d and 3e). The adaxial surface has lower density of amphiparacytic stomata compared to the abaxial surface (Fig. 3f).

Table 1. Macromorphological comparison between four *Passiflora* species.

Species	Leaf		Flower			Fruit		
	Shape	Colour/Texture	Colour	Petals	Corona	Shape	Colour	Bract
Passiflora edulis Sims var. *edulis*	Ovate, trilobe	Bright green/ smooth	White	5	White – purple base	Oval/ round	Green to purple	Absent
Passiflora coccinea Aubl.	oblong	Green/ pubescent	Red	5	Dark purple – white tip	Round	Green – whitish spots – dark green stripes	Absent
Passiflora foetida Linn.	Ovate, trilobe	Bright green/ pubescent	White	5	White – pinkish base	Oval/ small	Green to yellow	Present
Passiflora incarnata Linn.	Palmate, trilobe	Dark green/ smooth	Bluish white	5	White & purple	Oval	Green to orange	Absent

Table 2. Micromorphological comparison between four *Passiflora* species.

Species	Epidermal	Stomata	Trichomes	Epicuticular wax	Anticlinal wall
Passiflora edulis Sims var. *edulis*	Papillose	Paracytic	Absent	Sparse	Undulating
Passiflora coccinea Aubl.	Papillose	Anomocytic	Simple unicellular	Thick	Undulating
Passiflora foetida Linn.	Rugose	Amphiparacytic	Simple unicellular & glandular	Thick	Sinuous
Passiflora incarnata Linn.	Papillose	Amphipericytic	Simple unicellular	Sparse	Undulating

Fig. 1a. The habit of *Passiflora edulis* Sims var. *edulis*.

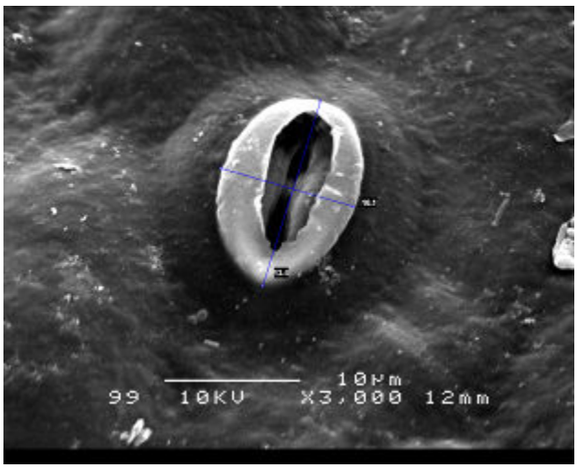

Fig. 1c. Paracytic stoma of *Passiflora edulis* measuring 13.4 µm by length and 10.1 µm by width (Magnification: 3000x).

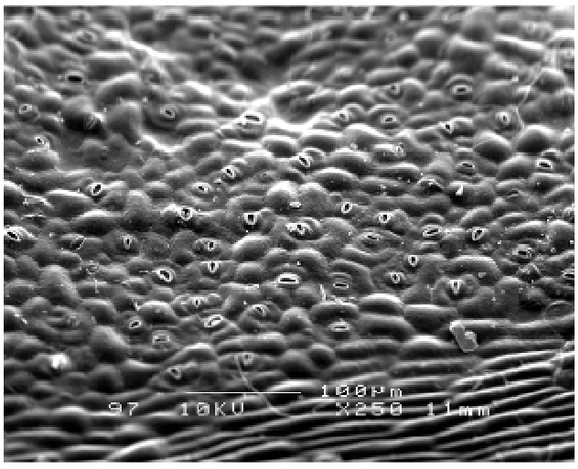

Fig. 1b. Abaxial surface of *Passiflora edulis* leaf (Magnification: 250x).

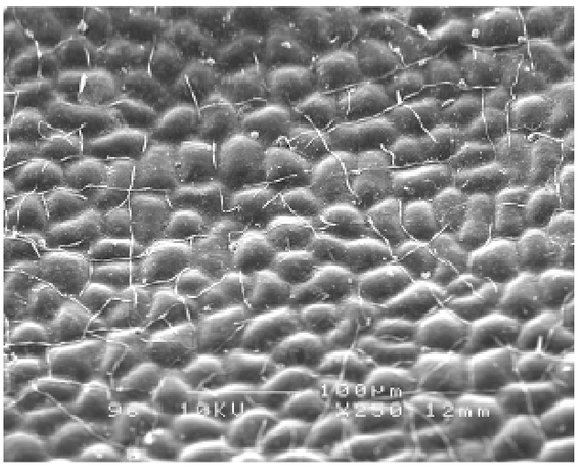

Fig. 1d. Adaxial surface of *Passiflora edulis* which shows absence of stomata (Magnification: 250x).

Passiflora incarnata L. leaves are alternate and palmately tri-lobed measuring 7-14.5 cm long (Fig. 4a). Leaf apex is cuspidate while the leaf base is cordate with serrate margin. The adaxial surface of the leaves is dark green whereas the abaxial surface is dull green. The scanning electron microscopy view of a *Passiflora incarnata* leaf shows papillose epidermal surface with amphipericytic stomata distributed on the abaxial surface (Fig. 4b). The stomata measures 12.1 x 7.15 µm (Fig. 4c).Trichomes are simple, unicellular and sparsely distributed throughout the abaxial and adaxial surfaces of the leaf (Figs. 4d and 4e). Both stomata and trichomes distribution are more dense on the abaxial surface compared to the adaxial surface.

The leaves of *Passiflora* species do not only vary in shapes and sizes, but also show multiplicity in their epidermal structures when viewed under the scanning electron microscope. The anticlinal cell walls are undulating or sinuous on both epidermis. However, abaxial epidermis reveals more wavy nature of anticlinal walls in *Passiflora coccinea* whereas the adaxial epidermis reveals more sinuous nature of anticlinal walls in *Passiflora foetida*. The stomata are paracytic, anomocytic, amphiparacytic and amphipericytic. Both *Passiflora foetida* and *Passiflora incarnata* have amphistomatic leaves, where stomata are present on both abaxial and adaxial surfaces. Trichomes are simple, unicellular in *Passiflora coccinea*, *Passiflora foetida* and *Passiflora incarnata*. However, *Passiflora foetida* has both simple unicellular and glandular trichomes, whereas *Passiflora edulis* var. *edulis* leaf shows total absence of trichome.

The micromorphological characters of leaf epidermis have been scrutinized in several plant groups over the last four decades. These characteristics have been useful at numerous taxonomic levels and valuable to distinguish among groups of existing taxa with acknowledged relatives available in the fossil record (Li *et al*., 2007). Watson & Dallwitz (1992) stated detailed descriptions of the leaf epidermis in various taxa, highlighting the importance of these characters in the systematic of Poaceae. Previous studies by Davila & Clark (1990) and Watson & Dallwitz (1992) found that the abaxial surface of the leaf blade was taxonomically informative.

Fig. 2a. The habit of *Passiflora coccinea* Aubl.

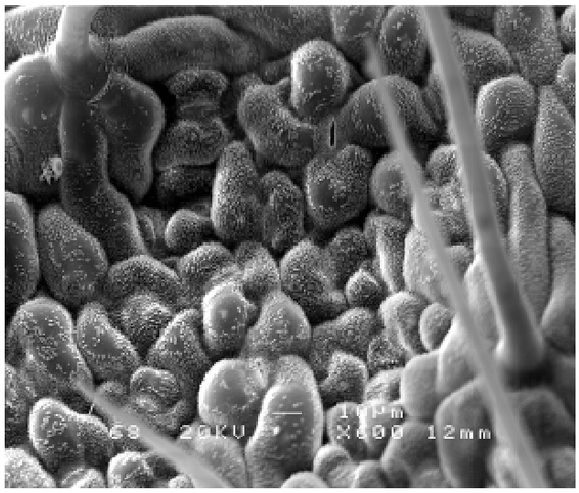

Fig. 2b. Abaxial surface of *Passiflora coccinea* leaf (Magnification: 600x).

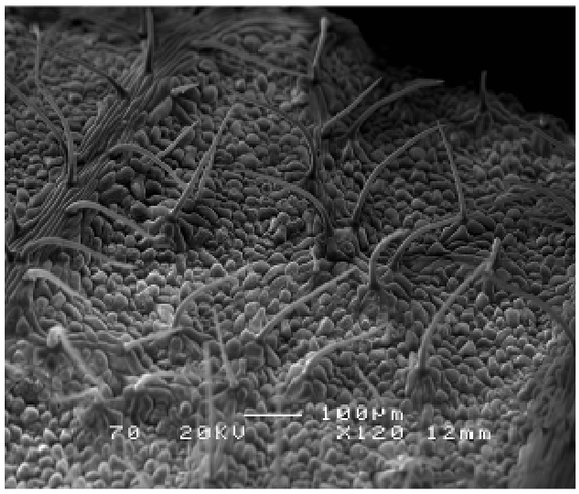

Fig. 2c. Trichomes of *Passiflora coccinea* are simple unicellular and the distribution on the abaxial surface is more than on the adaxial surface (Magnification: 120x).

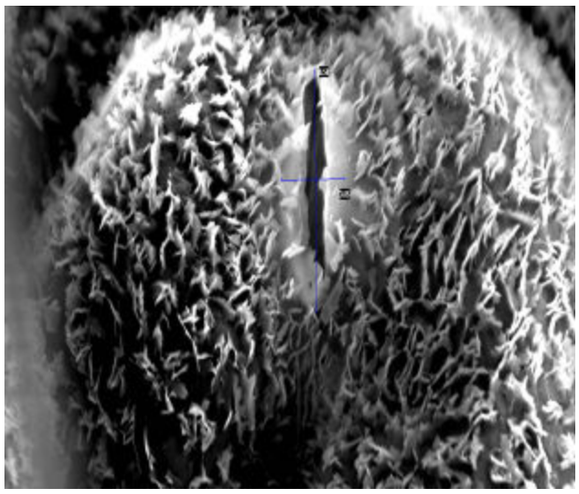

Fig. 2d. Anomocytic stoma of *Passiflora coccinea* measuring 8.58 µm by length and 3.85 µm by width (Magnification: 2500x).

Fig. 2e. *Passiflora coccinea* midrib with highly dense simple unicellular trichomes (Magnification: 150x).

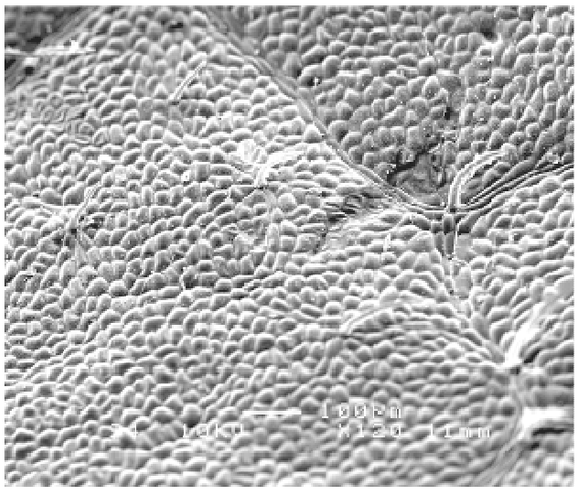

Fig. 2f. Adaxial surface of *Passiflora coccinea* leaf which shows absence of stoma and less trichome distribution compared to the abaxial surface (Magnification: 120x).

Fig. 3a. The habit of *Passiflora foetida* Linn.

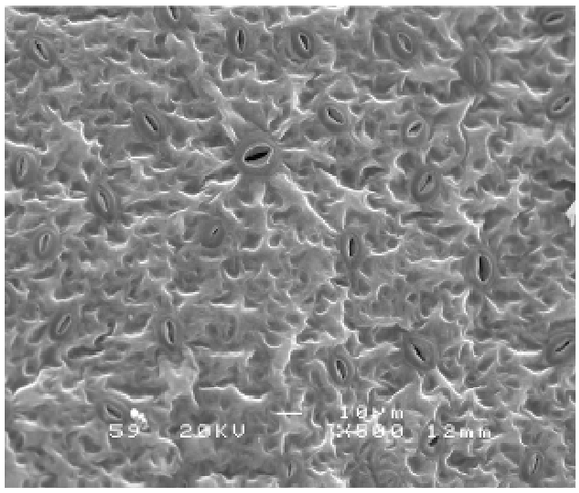

Fig. 3b. Abaxial surface of *Passiflora foetida* leaf. (Magnification: 500x).

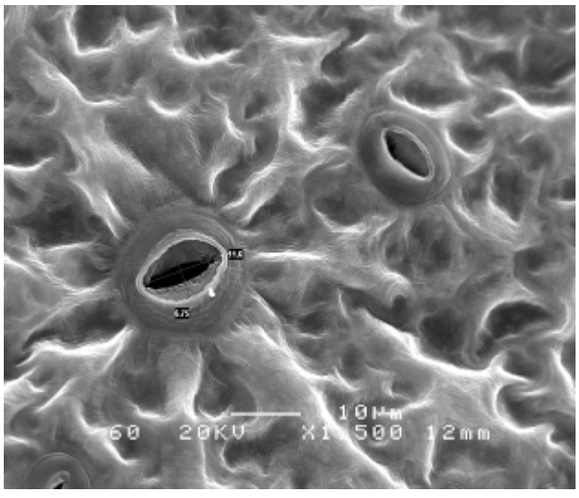

Fig. 3c. Amphiparacytic stoma of *Passiflora foetida* measuring 11.6 µm by length and 6.75 µm by width (Magnification: 1500x).

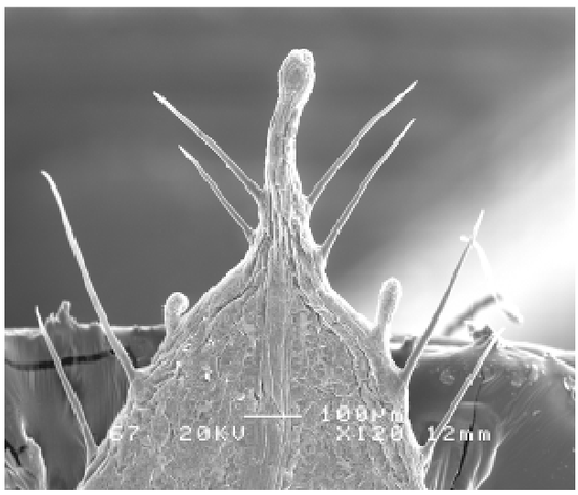

Fig. 3d. Trichomes on a *Passiflora foetida* leaf apex. One of them is glandular and the other is simple unicellular trichome (Magnification: 120x).

Fig. 3e. Glandular and simple unicellular trichomes on *Passiflora foetida* leaf margin (Magnification: 300x).

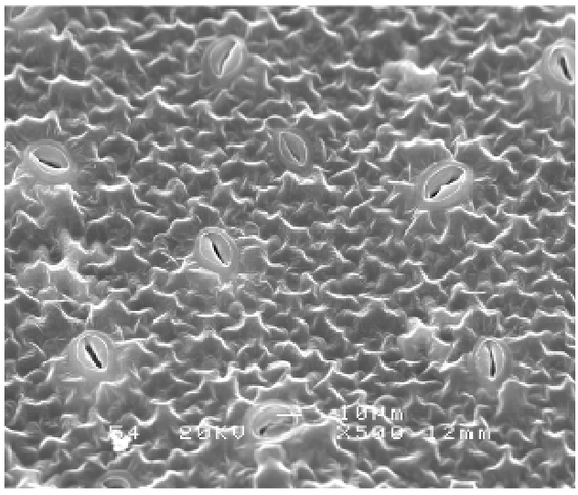

Fig. 3f. Adaxial surface of *Passiflora foetida* leaf which shows lower density of amphiparacytic stomata compared to the abaxial surface (Magnification: 500x).

Fig. 4a. The habit of *Passiflora incarnata* Linn.

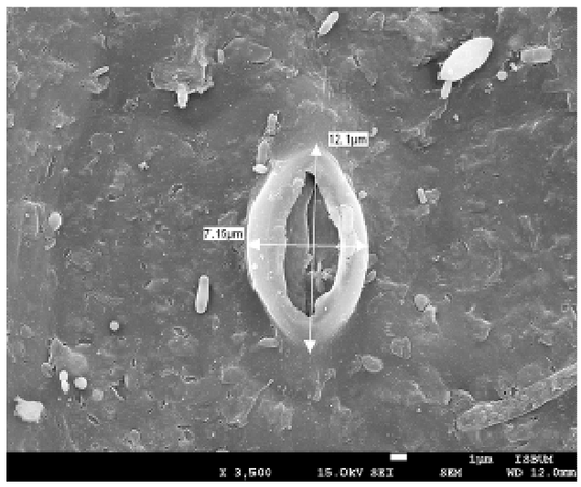

Fig. 4c. Amphipericytic stoma of *Passiflora incarnata* measuring 12.1 µm by length and 7.15 µm by width (Magnification: 3500x)

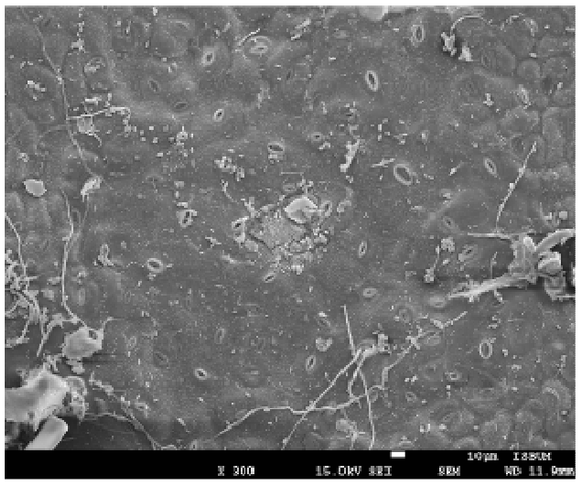

Fig. 4b. Abaxial surface of *Passiflora incarnata* leaf (Magnification: 300x).

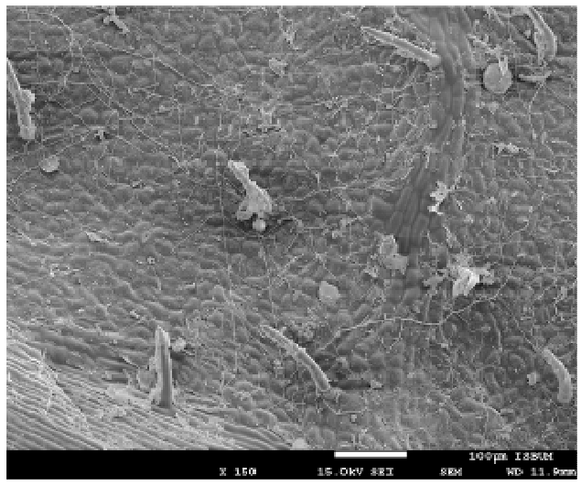

Fig. 4d. Trichomes of *Passiflora incarnata* are simple unicellular and the distribution on the abaxial surface is more than on the adaxial surface (Magnification: 150x).

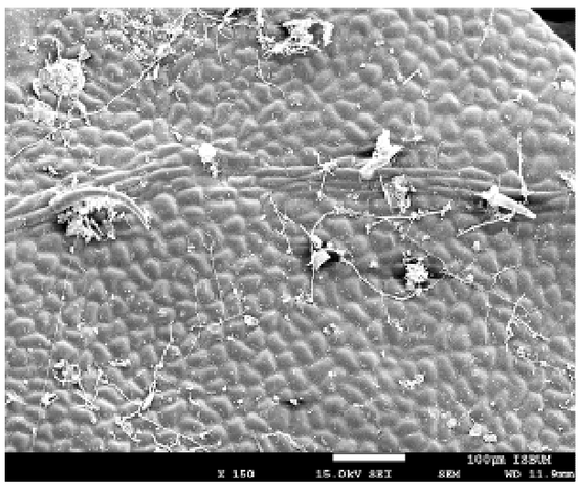

Fig. 4e. Adaxial surface of *Passiflora incarnata* leaf which shows absence of stomata and less trichome distribution compared to the abaxial surface (Magnification: 150x).

Fig. 5. The flower of *Passiflora edulis* Sims var. *edulis*. (a) petal, (b) sepal, (c) corona filaments, (d) stigma, (e) style, (f) stamen, (g) ovary, (h) anther.

Flower: The flowers of four *Passiflora* species studied are highly distinguishable by the colours of their petals and corona series (Table 1).

The flower of *Passiflora edulis* Sims var. *edulis* (Fig. 5) is white with five ovate petals and sepals. There are four series of purplish corona. The inner corona series are whitish half way up and dark purple half way down the base with slightly wavy structures. The outer corona series are longer than the inner corona series.

However, the flower of *Passiflora coccinea* Aubl. is generally striking red in colour with dull-red bracts located under the sepals (Fig. 6); constituted of five petals and five sepals. The flower also has three outer corona series that are dark purple, except for the edge of the coronas which are white.The outer coronas are longer than the other two inner series of coronas.

The flower of *Passiflora foetida* L. consists of five whitish petals and five whitish sepals (Fig. 7). There are three pinnatifid shaped bracts with hairy-like gland ended structures underneath the sepal. There are also four corona series which are divided into the outer corona series and the inner corona series. The pinkish inner corona series are shorter compared to the white-pinkish outer corona series.

Passiflora incarnata L. flowers have five bluish-white petals and exhibit a series of white and purple corona (Fig. 8). There are several corona series which are divided into the outer corona series and the inner corona series. The two violet outer corona series are wavy on the upper half and longer compared to the white-purple striped inner corona series.

Fruit: Most species have elongated or round edible fruits which are distinguishable by colours and textures, shapes, and the presence of bracts (Table 1).

The fruit of *Passiflora edulis* Sims var. *edulis* is an oval to round shaped berry with approximately 95 to 125 seeds in each locule. Seeds are flat, oblong shaped, brown blackish in colour and shining. The fruit pericarp is thick, with smooth and shiny light green outer surface, which is accompanied by white spots. The fruit colour transitions to purple when it matures.

On the other hand, the fruit of *Passiflora coccinea* Aubl. is a round berry which encloses 30 – 35 flat and brown seeds in every locule. The berry has a thick pericarp and is pubescent. The outer pericarp is light yellowish-green in colour, accompanied by green spots and six green vertical stripes. The bract transitions from pale red to green colour when the fruit is formed.

The fruit of *Passiflora foetida* L. is a berry with thin pericarp covered by bracts. Each fruit has black and shiny small seeds, approximately 20 to 25 seeds in each locule. The outer layer of the fruit is smooth, green when immature and transitions to orange in colour as it matures. The mature fruit produces a sweet smell compared to the immature one. There are three stripes of bracts which cover the fruit, where it declines and shrinks as the fruit matures.

The fruit of *Passiflora incarnata* L. is an oval berry with thick pericarp which is green at immature stage and transitions to orange in colour as it matures. The fruit consists of 80 to 100 dark brown seeds in each locule.

Fig. 6. The flower of *Passiflora coccinea* Aubl.

Fig. 7. The flower of *Passiflora foetida* Linn.

Fig. 8. The flower of *Passiflora incarnata* Linn.

Conclusions

The present study shows that the *Passiflora* species in Peninsular Malaysia can be distinguished by their leaf, flower and fruit characteristics where they varied in sizes, shapes and colours. Whereas micromorphologically, SEM observation shows that each *Passiflora* species varied in types and sizes of stomata, epidermal surfaces, and the presence and types of trichomes. This study has added new taxonomic information especially on the micromorphological aspects of four economically important *Passiflora* species from Peninsular Malaysia which has never been studied by previous researchers.

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