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Comparative physiological response of Three Wax Apple (*Syzygium samarangense*) Tree Cultivars at Flower Bud Development Stage

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Some species of fruit are distinct in their physiological characteristic for different cultivars. Wax apple is an edible tropical fruit in *Syzygium Samarangense* species belong to Myrtaceae family and have economically valuable in Malaysia, Thailand, Indonesia and Taiwan. The main objective of this study was to evaluate the physiological activities of three of wax apple (*Syzygium samarangense*) tree cultivars in Kampung Olek Lempit, Banting, and Selangor. Various parameters were analyzed during bud development stage for "Masam Manis Pink", "Giant Green" and "Jambu Madu Red" cultivars of *S. Samarangense.* Results showed that "Masam Manis Pink" had the highest stomatal conductance (0.14 mol $m^{-2}s^{-1}$), net photosynthesis rate (9.58 µmol $m^{-2}s^{-1}$),chlorophyll content (114.73), leaf area (522 cm^2), chlorophyll a and b (8.29 mg/L and 4.35 mg/L, respectively) and carotenoid content (3.65 µg/g) compare to "Giant Green " and "Jambu Madu Red" cultivars. The highest, the minimum fluorescence (Fo), maximum fluorescence (Fm) and variable fluorescence (Fv) were recorded in "Giant Green" cultivar with values 569, 3074 and 2505, respectively. However, the same value (0.82) of the photosynthetic yield (Fv/Fm) was shown in both "Masam Manis Pink" and "Giant Green" cultivars. Based on the results, it can be concluded that "Masam Manis Pink" is the best cultivar of *Syzygium Samarangense* compare to "Giant Green" and "Jambu Madu Red" cultivars. Indirectly, the planting of "Masam Manis Pink" cultivar is the good to increase the country's income.

Keywords: *Syzygium Samarangense*, cultivars, chlorophyll, photosynthesis yield, carotenoid

INTRODUCTION

Syzygium is a genus of flowering plant that belongs to the family Myrtaceae. Little and co-workers (1989) was reported that the Syzygium genus consists of 1100 species. In Malaysia, *Syzygium* comprises three species namely wax apple (Syzygium samarangense), water apple (Syzygium aquem) and Malay apple (Syzygium malaccense) (Morton, 1987). The wax apple (Syzygium samarangense) is non-climacteric tropical fruit also known as wax jambu, java apple and rose apple. The fruit widely grown in Malaysia and neighbouring countries like Thailand, Indonesia and Taiwan. The total planted area of wax apple plant in Malaysia was about 2000 ha in 2005 with smallholding areas ranging 1 to 5 ha (Shu et al., 2006). The colours of fruit are red, light-red, pink, green, greenish-white and cream colour (Morton, 1987). On the contrary, in Malaysia and others countries of South East Asian, there are only three famous colours of wax apple was founded including red, pink and green cultivars (Moneruzzaman et al., 2011). The edible fruit has pear and bell shape, crowned by the flesh calyx with incurved lobes, 3.5-5.5x 4.4-5.5 cm, broadly pyriform; fleshy fruit are juicy, sweet-sour taste, white spongy and aromatic. The seed is 0 to 2 and usually suppressed globose up to 8 mm in diameter (Morton, 1987). The fruits also highly perishable and have low respiration rate about 10 to 20 mg CO_2/kg h at 20 °C (Akamine and Goo, 1979). Furthermore, they also rich with nutrition that essential for our bodies such as protein, carbohydrates, vitamin A, vitamin B1 and B2, vitamin C and fibre (Wills et al., 1986). Besides fruits, the others part of wax apple tree (leaves, bark, root and stem) can be used for a treat some diseases like cough diabetes, dysentery, inflammation and ringworm (Peter et al., 2011). Galan et al., (1989) reported that even though the same species, the distinct characteristics of the fruit in the shape, size and colour was detected for different cultivars. The variation in fruit quality closest related to genetic factor but unrelated to the environment or edaphic factors (Felker et al., 2002). The variation colour of wax apple related to temperature, light, growing stage, position on the tree and leaf: fruit ratio (Shu et al., 2001). Al-Saif and co-workers (2011) found that the morphological and physiological characteristics of different cultivars of wax apple unlike among each other depending on their genetic behaviour, location and climatic conditions. The information about the differences among cultivars of wax apple ("Masam Manis Pink", "Giant Green" and " Jambu madu Red") are very important, especially for the commercial purpose. In this view, present work was conducted with the aim to evaluate the physiological activities of three cultivars of *S. samarangense* for determining their quality.

MATERIALS AND METHODS

Experimental Site and Plant Material

The experiments were conducted at the commercial farm of Kampung Olek Lempit, Banting, Selangor, Malaysia. The location of study at Banting is 2° 30 N, 112° 30 E and 1° 28 N, 112° 20 E with 45 m elevation from sea level and had the hot and humid climate regions. The present study was carried out in July 2016. Three cultivars of wax apple, *Syzygium samarangense* namely 'Masam Manis Pink', 'Jambu Madu Red' and 'Giant Green' with same horticultural management were investigated.

Measurement of the parameters

In this study, the stomatal conductance, net photosynthetic rate, chlorophyll content, chlorophyll fluorescence and leaf area were measured on the leaves selected uniform branched. The measurement was done at 11.00 am until 3.00 pm for several days under the hot and dry day during bud development. A portable Leaf Porometer (model SC-1, USA) was used to determine stomatal conductance with 0 % moisture. The leaf was clip by using the leaf chamber and kept it in ambient temperature for 10 to 15 min to maintain sunlight adaption To determine the net photosynthesis rate (Pn), the portable photosynthesis system (LI 6400, Li-COR, U. S. A) equipped with a 6400-02 LED light source was used. The selected leaf was attached on the leaf chamber and the reading was taken about 5 seconds. Measurement of total chlorophyll content was done by using CCM-200 plus Chlorophyll Content Meter. Chlorophyll reading was taken by attached a leaf on the CCM meter after it was calibrated to make sure the reading taken is accurate and precise. Chlorophyll fluorescence was recorded by a Plant Efficiency Analyzer, PEA (Hansatech Instruments Ltd., England). A clip with the closer shutter plate was attached to the selected leaf. After 10 minutes, the light imposed to the entire leaf immediately after the shutter plate was opened. The minimum fluorescence (Fo), maximum fluorescence (Fm), relative variable fluorescence (Fv=Fm-Fo) and quantum yield or photosynthetic yield determined (Fv/Fm) was display after 3 seconds on the PEA instrument. Leaves area was measured by CI-202 Portable Laser Leaf Area Meter. The data collected when the leaf that place on the palette was slid over the scanner. This meter non-destructive the plant sample and can measure the leaf area precisely.

Furthermore, the leaves of each three cultivars also were taken to analyse of their chlorophyll a, b and carotenoid content in the laboratory. About 0.25 g of leaf samples was pour with 10 ml of 80 % acetone and then pulverized with mortal and paste. The mixture was centrifuged for 10 min at 2500rpm. The supernatant was filtered by using Whatman No. 1 filter paper and then was read on Shimadzu UV-260 Spectrophotometer. To calculate the chlorophyll a, b and carotenoid content, the absorbance of the filtrate was read at wavelengths: 663 nm, 645 nm and 480 nm. 80 % acetone was used as a blank. These pigments were calculated according to Hendry and Price (1993). The formula below represents the concentration of sample chlorophyll.

Chlorophyll a (mg/L) = 12.7 x A663 - 2.69 x A645
Chlorophyll b (mg/L) = 22.9 x A645 - 4.68 x A663
Carotenoid content (µg/g) = [A480 + (0.114 x A663) - (0.638 x A645)] / 112.5

Whereas,
A663 = values for absorbance at wavelengths 663 nm
A645 = values for absorbance at wavelengths 645 nm

Statistical analysis

Completely randomized designs (CRD) have been used in this experiment. The one-way ANOVA was applied to evaluate significant differences in the parameter studied. The least significant difference (LSD) and F-test (at P = 0.05) were calculated.

RESULTS

Stomatal conductance

Stomata located at epidermis layer which is responsible for reduced water loss and control gas exchange from plant and atmosphere (Ariff et al., 2011). Jarvis and Davies (1997) were reported that stomatal conductance regulates the CO_2 concentration in leaf mesophyll tissue and it directly affects the photosynthesis rate of the plant.

Based on the results, the stomatal conductance of all *S. samarangense* cultivars did not showed the significance differences among each other's. However, "Masam Manis Pink" cultivar had highest stomatal conductance with value 0.14 mol $m^{-2}s^{-1}$. "Giant Green" and "Jambu Madu Red" cultivars were recorded the same value of stomatal conductance which is 0.13 mol $m^{-2}s^{-1}$ (Table 1). Khandaker and co-workers (2013) stated that stomatal conductance related to the cultural condition of the plant. Based on their research, they found that the application of naphthalene acetic acid (NAA) in 10 mg/L to wax apple plant increase the stomatal conductance of the leaves. Odd (2003) and Iqbal et al., (2011) also were observed that the uses of plant hormone like abscisis acid (ABA), auxins, ethylene and cytokinins affected the stomatal behaviour.

Net photosynthesis rate, *Pn*

Photosynthesis is physiochemical process occur in all plant. As we knew, this process influences by light, carbon dioxide concentration and temperature factors. So, the leaf photosynthesis activity is difference among the cultivar of the plant.

From the experiment, the results showed the significance differences among cultivar of wax apple. The highest (9.58 µmol $m^{-2}s^{-1}$) *Pn* value was observed in "Masam Manis Pink" cuitivar followed by "Giant Green" cultivar which is 4.04 µmol $m^{-2}s^{-1}$ whereas "Jambu Madu Red" cultivar produced the lowest (3.78 µmol $m^{-2}s^{-1}$) *Pn* value (Table 1).Nitrogen content and nutrient minerals in the leaf play a vital role to maintain photosynthetic activity (Zheng and Shangguan, 2007). Peri et al., (2009) reported that net photosynthesis rate vary between *Nothofagus pumilio* and *Nothofagus Antarctica* species affected by factors of light intensity, water stress and waterlogging. Similarly, Coa and co-workers (2011) also stated that the growth rate of *Illlicium lanceolatum* plant showed different reactions when exposed to certain light intensities condition.

Chlorophyll content

Chlorophyll is the green pigment present in chloroplast in addition to absorbing light from sunlight. The pigment is closely related to the photosynthetic activity (Guo and Li, 1996). Wiedemuth and co-workers (2005) stated that the rate photosynthesis of barley leaves decline response to the decrease of chlorophyll content during leaf senescence. From this study, chlorophyll content of all cultivars did not varied significantly among them. The highest chlorophyll content was recorded in "Masam Manis Pink" cultivar, which is 114.73, followed by "Giant Green" cultivar, which is 111.77, while the lowest chlorophyll content was "Jambu Madu Red" cultivar with value 103.90 (Figure 1). The finding was supported by Al-Saif et al., (2011) who reported that the chlorophyll content in new flush and mature leaf did not significantly difference among *Syzygium samarangense* cultivars. Historically, the researchers have been done evaluated that the chlorophyll content in leaf was affected by some factors like light intensities (Zhang et al, 2016), carbon (C) level (Hossain et al., 2013) and water content (Bergsten and Stewart, 2014).

Table 1. Stomatal conductance and net photosynthesis rate of three cultivars of *S. samarangense*

Cultivar	Stomatal Conductance (mol $m^{-2}s^{-1}$)	Net photosynthesis Rate, *Pn* (µmol $m^{-2}s^{-1}$)
"Masam manis Pink"	0.14±0.03a (ns)	9.58±1.28a **
"Giant Green"	0.13±0.04a(ns)	4.04±1.73b**
"Jambu Madu Red"	0.13±0.01a(ns)	3.78±1.81b**

Means (± S.E) followed by same letter in parameter do not differ by LSD test at α=0.05 probability ns = non-significant, ** significant at *p≤0.05.*

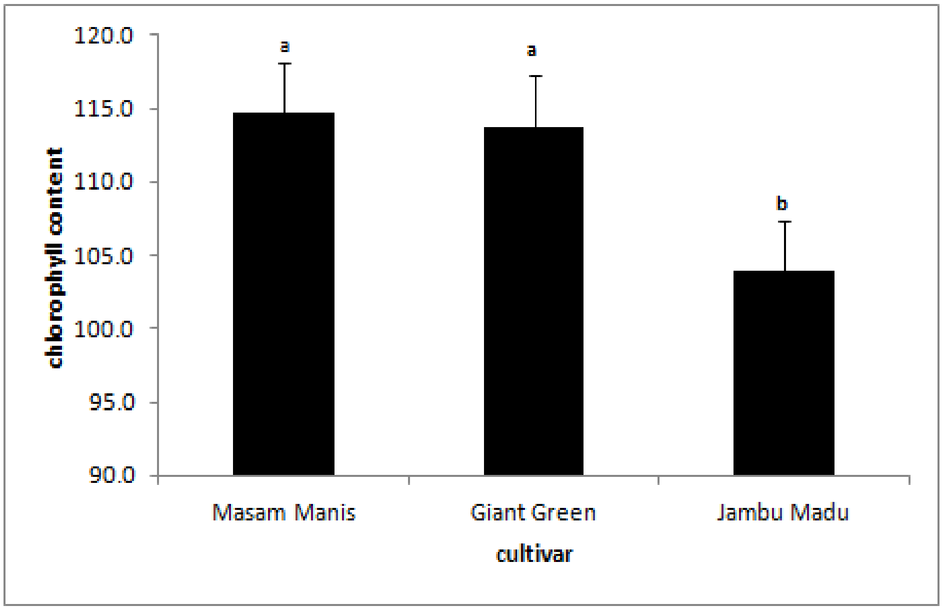

Figure 1: Chlorophyll content of three cultivars of *S. samarangense.* Means (± S.E) followed by same letter in parameter do not differ by LSD test at α=0.05. Bar indicated (± S.E).

Chlorophyll fluorescence

Nowadays, chlorophyll fluorescence application became interested among the researchers because it can give information about photosynthesis performance especially in the functionality of photosystem II, PSII (Maxwell. K and Johnson, N.G, 2000). PSII take place in the chloroplasts thylakoid membranes.

According to Moneruzzaman and co-workers (2011), chlorophyll fluorescence well documented to investigate plant tissue status in normal or stress environment conditions and it also can estimate maximum photosynthetic efficiency in certain leaves. Table 2 shown the chlorophyll fluorescence was not significance difference among each cultivar of wax apple. The highest value of minimum fluorescence (Fo) was recorded in “Giant Green” cultivar followed by “Jambu madu Red” and “Masam Manis Pink” cultivar with value 569, 534 and 527, respectively. For the maximum fluorescence (Fm), “Giant Green” cultivar was observed have the highest value compared to another cultivars which is 3074 followed by “Masam Manis Pink” cultivar (2957) and “Jambu Madu Red” cultivar (2923). The results were similar for variable fluorescence (Fv) whereas “Giant Green had the highest Fv value followed by “Masam Manis Pink” and lowest was “Jambu Madu Red” cultivar (Table 2). Results showed that the photosynthesis yield or optimum quantum yield (Fv/Fm) of “Masam Manis Pink” and “Jambu Madu Red” cultivar have a similar value which is 0.82. “Giant Green” cultivar produced only 0.81 Fv/Fm value. Based on the result Fv/Fm, we can conclude that all of the cultivars tree in good condition. Our finding was supported by Biber and co-workers (2004) who reported that the plant had Fv/Fm value below 0.5 is under unhealthy condition or stress.

Table 2. Chlorophyll fluorescence of three cultivars of *S. samarangense*.

Cultivar	Chlorophyll Fluorescence			
	Fo	Fm	Fv	Fv/Fm
"Masam manis Pink"	527±31.32	2957±111.03	2430±100.02	0.82±0.01
"Giant Green"	569±14.71	3074±150.14	2505±136.38	0.81±0.00
"Jambu Madu Red"	534±5.67	2923±119.11	2389±114.52	0.82±0.01
	ns	ns	ns	ns

Means (± S.E) followed by same letter in parameter do not differ by LSD test at α=0.05 probability ns = non-significant. , ** significant at *p≤0.05*

Leaf area

Next parameter studied was leaf area (cm²). Leaf area was analyzed by using Portable Laser Leaf Area Meter. As shown in Figure 2, the average of leaf area among the cultivar is not significance differences among them. “Masam Manis Pink” cultivar had the highest leaf area (522 cm²) followed by “Jambu Madu Red” cultivar (475 cm²) and lowest leaf area is “Giant Green” cultivar was recorded 448 cm². Capacities of certain leaf to absorb light affected the result of leaf area among the cultivars. Coa et al., (2011) found that the leaf area of *I. lanceolatum* leaves increase when the plant exposed to strong light. It has been also reported that 50 mg/L kinetin with defoliation increased the leaf area of Bougainvillea (Moneruzzaman et al., 2010). From the analysis, leaf area of each cultivar shows a positive association with stomatal conductance, chlorophyll fluorescence and chlorophyll content. The result was agreement with Khakwani and co-workers (2013) who reported that the leaf area of wheat cultivar increase when stomatal conductance, chlorophyll fluorescence and chlorophyll content of leaf readings was an increase. However, under stress condition, leaf area will decrease because limited assimilated of CO_2 (Xu and Zhou, 2008).

Chlorophyll a and b

Chlorophyll is the main pigment in photosynthesis system that is involved in absorbing the light and converts it to chemical energy (Scheer, 2006). Commonly, chlorophyll pigments for the higher plant have two types that are chlorophyll a and chlorophyll b (Palta, 1990). Chlorophyll a is bluish green and act as reaction center of optical energy and also light absorber whereas chlorophyll b is yellowish green that helps in photosynthesis by function on absorbing of optical energy (Zhang et al., 2013). Moneruzzaman et al., (2012) reported that chlorophyll content also depends on genetic properties of cultivars.

In this study, “Masam Manis pink” showed the highest chlorophyll a (8.29 mg/L) and chlorophyll b (4.35 mg/L) contents than others. Secondly, the chlorophyll a (6.96 mg/L) and b (3.57 mg/L) contents of “Giant Green” were recorded higher than “Jambu Madu Red”. Chlorophyll a and b contents for “Jambu Madu Red” are 6.46 mg/L and 2.58 mg/L, respectively (Figure 3).

Carotenoid Content

Like chlorophyll, Carotenoid also the pigment responsible in the leaves. When the quantity of Carotenoid present abundantly in leaf, so the colour of leaf change from green to yellow or orange. Carotenoid is vitamin A precursors (Goodwin, 1986) and consists of α and β carotenes and xanthophylls: violaxanthin, lutein, neoxanthin, zeaxanthin and antheraxanthin (Biswall, 1995; Palett et al., 1993 and Lichtenthaler, 1987). Colour is extremely important quality character which differ with stages of maturity of plant parts (Moneruzzaman et al., 2008). It also had a function in absorbing the light in the spectrum of blue region. Interestingly, Carotenoid have specific functions in physiochemical and photochemical like energy transfer and light harvesting (Young and Frank, 1996; Peterman et al., 1997).

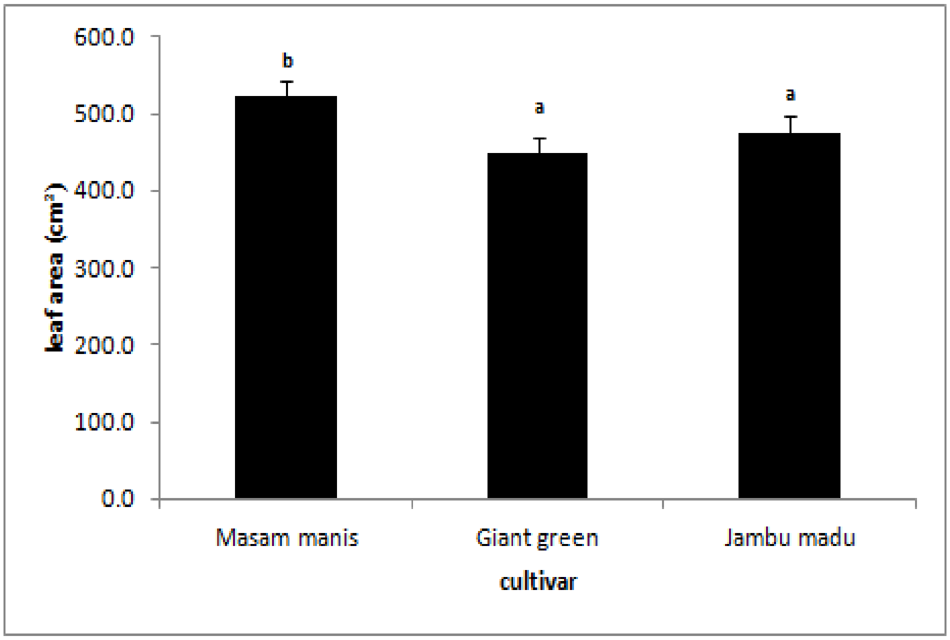

Figure 2: Leaf area of three cultivars of S. *samarangense.* Means (± S.E) followed by same letter in parameter do not differ by LSD test at α=0.05. Bar indicated (± S.E)

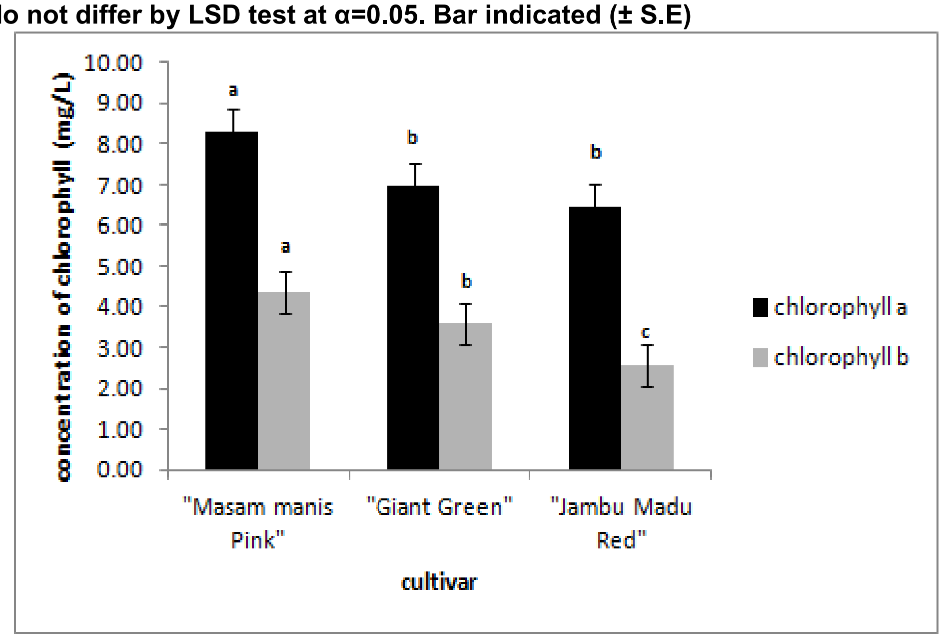

Figure 3. Concentration of chlorophyll of three cultivars of *S. samarangense.* Means (± S.E) followed by same letter in parameter do not differ by LSD test at α=0.05. Bar indicated (± S.E).

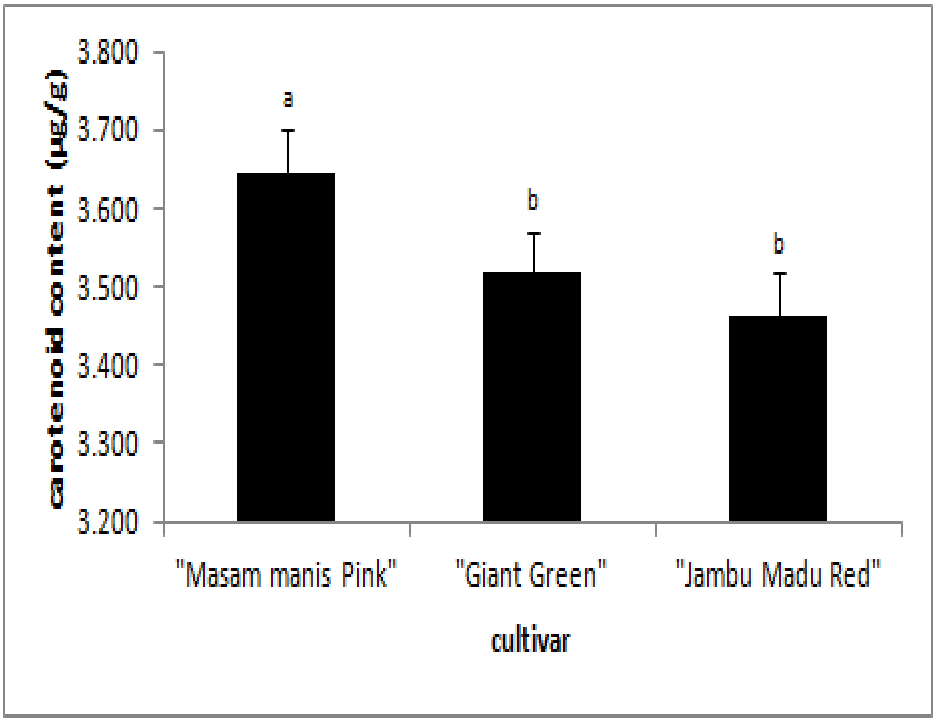

Figure 4. Carotenoid content of three cultivars of *S. samarangense.* Means (± S.E) followed by same letter in parameter do not differ by LSD test at α=0.05. Bar indicated (± S.E).

The results from this study were reported that all of the cultivars of *S. samarangense* did not show the significance differences of carotenoid content among them. As shown in Figure 4, we observed that carotenoid content of "Masam Manis' cultivar was highest (3.65 µg/g) followed by "Giant Green" cultivar and the lowest "Jambu Madu Red" cultivar produced 3.52 µg/g and 3.46 µg/g of carotenoids content, respectively. Srivastava and Shukla (2016) suggested carotenoid content varied in weed species attributed from photo-oxidation of chlorophylls.

CONCLUSION

From the results, it revealed that "Masam Manis Pink" produced the highest stomata conductance, net photosynthesis, chlorophyll content, leaf area, as well as chlorophyll a, b and carotenoid content compare others cultivar. So, it can be concluded that "Masam Manis Pink" is the best *S. samarangense* cultivar compare to "Giant Green" and "Jambu Madu Red" cultivars. In addition, Malaysia or other countries of South Asian can be recommended and encourage the cultivation of "Masam Manis Pink" to increase the nation income.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

NSI performed the experiment and wrote the manuscript. SZI reviewed the manuscript. NM provided the chemicals and equipments. MMK designed experiments and reviewed the manuscript. NSI and MMK read and approved the final version.

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