PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES OF THREE CULTIVARS OF WAX APPLE (Syzygium samarangense [Blume] Merrill & L.M. Perry) FRUITS

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Abstract: A study under field condition was carried out to evaluate physiological and biochemical properties of three cultivars of wax apple (*Syzygium samarangense*) namely 'Jambu Madu Red', 'Masam Manis Pink' and 'Giant Green'. Physicochemical parameters, such as stomatal conductance, fruit development, pigmentation, fruit shape, yield, total soluble solids (TSS), titratable acidity (TA), sugar acid ratio, vitamin-C (vit-C), chlorophyll, carotene and anthocyanin content, in three cultivars of wax apple were investigated. The highest stomatal conductance, color development and yield were recorded in 'Jambu Madu Red' cultivar. Lowest amount of TA, highest TSS, sugar acid ratio and carotene content were also observed in this cultivar. Earlier color development, fruit maturity, good shape, highest vit-C and anthocyanin content were found in 'Masam Manis Pink'. Meanwhile, the highest chlorophyll *a, b* and total chlorophyll (chl_{ab}) content and late maturation fruit were recorded in 'Giant Green' Cultivar. Stomatal conductance showed positive correlation with yield and fruit biomass. It is concluded that 'Jambu Madu Red' and 'Masam Manis Pink' cultivars are comparatively better yield than those of 'Giant Green' cultivar grown under tropical field conditions.

Keywords: Wax apple, yield, TSS, TA, chlorophyll, carotene, fruit development.

Introduction

The wax apple (Syzygium samarangense) is a non-climacteric tropical fruit species. It is also known as wax apple, rose apple or java apple. Its pear-shaped fruits are usually pink, light-red, red, green, sometimes greenishwhite, or cream-colored and are generally crisp, with a subtly sweet taste or aromatic flavor (Morton, 1987). The species presumably originated in Malaysia and other South-east Asian countries. It is widely cultivated and grown throughout Malaysia and in neighboring countries such as Thailand, Indonesia and Taiwan (Moneruzzaman et al..Currently in Malaysia it is cultivated mainly as a smallholding business ranging from 1 to 5 ha for each farmer. The cultivated area throughout the country is estimated at about 2000 ha in 2005 (Shu et al., 2006). In Malaysia, the species shows a great potential to develop as an export fruit industries.

There are three species of Syzygium, namely the water apple (Syzygium aquem),

Malay apple (Syzygium malaccense) and wax apple (Syzygium samaragense) bear edible fruits. Wax apple contains fruit with more round and oblong in shape and less watery compare to the other Syzygium species and the fruits are eaten raw with salt or cooked as sauce. The wax apple fruit has a very low respiration rate, with 10–20 mg CO₂/kg h at 20°C, although they are highly perishable fruits (Akamine and Goo, 1979). The composition of wax apple per 100 g edible portion is water with more than 90% portion, protein 0.7 g, fat 0.2 g, carbohydrates 4.5 g, fibre 1.9 g, vitamin A 253 IU, vitamin B1 and B2 with traces amount, vit-C 8 mg, and energy with 80 kJ/100 g (Wills et al., 1986). Fruit growth and development are associated with the morphological, anatomical and physiological changes of the plant (El-Otmani et al., 1987). Fruit maturation is associated with changes in rind texture, juice composition and taste (El-Otmani et al., 1987). Felker et al., (2002) reported that the major variation in fruit quality is not related to the environment

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or edaphic factors but rather ascribed to genetic factors. Color is probably the most important quality factor depending on light, temperature, position on the tree, growing stage, leaf: fruit ratio number (Shu et al., 2001). Chang et al., (2003) stated that sucrose, glucose and fructose are important quality parameters that influence the anthocyanin biosynthesis in wax apple fruits. The flowers, which contain tannins, desmethoxymatteucinol, 5-O-methyl-40-desmethoxymatteucinol, oleanic acid. and b-sitosterol, are used in Taiwan to treat fever and halt diarrhea (Morton, 1987). Ethanolic leaf extract of wax apple exhibited immunostimulant activity (Srivastava al., 1995), the hexane extract was found to relax the hyper motility of the gut (Ghayur et al., 2006), while the alcoholic extract of the stem bark showed antibacterial activity (Chattopadhayay et al., 1998). The fruit of wax apple can also be used to treat high blood pressure and several inflammatory conditions, including sore throat. It was also reported as useful fruits antimicrobial, antiscorbutic, carminative, diuretic, and astringent (Rivera and Obón, 1995). The fruit pulp of Masam Manis Pink cultivar of wax apple is a rich sources of phenolics content, flavonoids and several antioxidant compounds (Simirgiotis et al., 2007). It was also reported that edible fruits of wax apple may have potential benefits for human health because the presence of polyphenolic antioxidants in it.

For the commercial purpose, the difference in fruit quality among the cultivars is important in order to grade the fruit. Since there are no report that has been published on the physiological and biochemical quality on the cultivars of wax apple, this project was conducted with the aims to evaluate the fruit development, pigmentation and quality on different cultivars of wax apple based on the physiological and biochemical measurements of the three cultivars Jambu Madu Red, Masam Manis Pink and Giant Green of wax apple.

Methodology

Experimental Site

The experiments were carried out in an orchard located at a commercial farm in Banting, Selangor (2° 30N, 112° 30E and 1°28 N, 111° 20E) at an elevation of about 45 m above sea level. The area was covered by hot and humid tropical climatic condition. The soil in the orchards is peat with pH 4.6 (Ismail *et al.*, 1995). The experiments were conducted in between the year of 2009 to 2010. The experiments were carried out in the first season from October 2009 to February 2010, and in the second seasons from April to August 2010.

Plant Material

Twelve years old wax apple plants in the field were selected for the study. The planted trees were in a 4.5 ft \times 4.5 ft hexagonal arrangement and received the same intercultural operation: fertilization. insecticide irrigation and application. Three wax apple cultivars namely; 'Giant Green', 'Masam Manis Pink' and 'Jambu Madu Red' were used in the study. Three trees per cultivar were selected and used for fruit sampling for each season. Thirty six uniform branches (four branches per tree) of about the same length, and diameter, and approximately the same number of leaves from nine trees were selected for fruit harvesting. The experiments consist of 3 treatments (cultivar), with twelve replications and four uniform branch was taken as an experimental unit. The selected uniform branches were tagged properly at the beginning of flower opening until fruit maturation. The experiments were arranged in a complete randomized design.

Measurement of Physiological Parameters (Stomatal Conductance, Fruit Development, Fruit Biomass and Color Development).

Leaves of selected uniformed branches were used for stomatal conductance measurement that was done at 11.00 am under fully sunshine condition during fruit developmental stage. Stomatal conductance was measured by using a

portable Leaf Porometer, (Modal SC-1, USA). Fruit shape (length and diameter ratio) is an important component of the visual fruit quality and can be influenced by several factors. Fruit shape was classified by visual inspection as round, round-flattened, oblate [spheroid; length: diameter ratio (L: D) >1.5], oblong (blossom end larger than stem-end; L: D 1.5-2), pyriform (having a secondary constriction; L: D 1.5-2), elliptical (blossom- and stemend equal size; L: D 2-3), and elongate (L: D> 3) (Miriam et al., 2008). Fruit length, fruit diameter and fruit growth was measured with the Vernier caliper. Total yield were determined by total number and weighing mature fruits per individual plant. Fruit dry biomass was determined by weighing the pulp mass at 0% moister content. The skin color of the fruits was measured by using a Minolta colorimeter, CR-300, Konica, (Japan). Color parameters such as "L" (lightness), "a" (greenness to redness) and "b" (blueness to yellowness) were determined at three different spots at the top, middle and end of the fruits. The changing in color of the tagging fruits was measured at the stage of fruit development from pit stage until harvest. Fruit sample colors were calculated and expressed in L*, a*, b* Hunter parameter, by using the formula $(L^* \times a^*) / b^*$. Fruit length and diameter ratio was measured immediately after harvested from the tree.

Measurement of Biochemical Parameters

Fruits of different cultivars were randomly harvested from the selected outside branches at fully ripening stage during the first and second fruiting seasons of the trees. Fruit maturity was measured by observing the skin color of wax apple cultivars. Harvesting was carried out manually in the early morning with a minimum mechanical injury. Fully ripened fruits were kept in a refrigerator at 4°C with 80-90% RH for used in biochemical analysis. A total of 24 fruits were taken randomly from each cultivar for use in the analysis.

Titrable Acidity (TA)

The fruit juice was extracted by using a Phillips HR1833 Juicer Extractor. The fruit juice was titrated with 0.1 M NaOH and the results were expressed in terms of percentage of citric acid which was calculated by using the Bhattarai and Gautam (2006) formula. Thus TA percentage can be calculated as following,

$$TA \ (\%) = \frac{N_b \times V_b \times E_a \times df \times 100}{V_s}$$

Where N_b = normality of the base, V_b = volume of the base, E_a = mill equivalent weight of citric acid, V_s = volume of sample and df = dilution factor.

Total Soluble Solids (TSS) and Acidity Ratio

Total soluble solids (TSS) of fruit is one of the parameters that strongly affect consumer acceptability of a variety. The total soluble solids (TSS) value was determined at 25°C by using portable hand refractometer, 8469 (Atago Co. LTD., Tokyo, Japan) and expressed the value as °Brix. The pulp of the fruit was homogenized by using a blender. A small fraction of the homogenous fruit pulp was centrifuged at 4000 ×g for 10 min, and the clear supernatant was analyzed chemically for determinate the TSS. The sugar acid ratio of the fruit juice is given as the ratio of TSS/TA.

Total Ascorbic Acid (Vitamin-C) Content

Total ascorbic acid (vit-C) content was determined by adopting the method modified by Hashimoto and Yamafuji (2006). Five g of fruit pulp was homogenized with cold 5% metaphosphoric acid and then filtered through the cloth sheet. A 0.8 mL of filtrate was then reacted with the mixture of 0.4 mL of 2% diindophenol, 0.4 mL of 2% thiourea and 0.4 mL of 1% dinitrophenol hydrazine. After that the mixture was incubated at 37 °C for 3 hours and then 2 mL of 85% sulphuric acid was added. The solution was again left at room temperature for 30 minutes and the absorbance at 540 nm was then recorded using spectrophotometer.

Total vit-C content was expressed as mg vit-C per 100 g fresh weight.

Chlorophyll, Carotene and Anthocyanin Content

In this study, the chlorophyll in the fruits skin was measured at the fully ripening stage. The chlorophyll of fruits was determined by methods described by Hendry and Price (1993). The total carotene and anthocyanin contents of the hydrophilic extracts were measured by using the pH-differential method with cyanidin-3-glucoside used as a standard (Rodriguez-Saona *et al.*, 2001).

Statistical Analysis

The experimental design was a completely randomized design (CRD) with twelve replicates. The data from the two seasons were pooled and analyzed using MSTAT-C statistical software. One way ANOVA was applied to evaluate the significant difference between cultivars for each parameters studied. Least significant difference (Fisher's protected LSD) was calculated, and F-test at (p = 0.05) was determined as the significant level.

Results and Discussion

Physiological Measurement

Stomatal Conductance

Stomatal conductance affects the photosynthesis rate by regulating CO₂ fixation in leaf mesophyll tissue. Accumulation of dry matter content in the plants depends on the stomatal conductance.

With regard to the stomatal conductance of the leaves, 'Jambu Madu Red' cultivar produced a significantly difference from the 'Giant Green' and 'Masam Manis Pink' cultivars. The result of this study indicated that, stomatal conductance measured in a sunny day at 11.00 am was highest (0.039 mol H₂O m⁻²s⁻¹) in 'Jambu Madu Red', followed by conductance of 0.036 mol H₂O m⁻²s⁻¹ in 'Masam Manis Pink'. The lowest stomatal conductance 0.031 mol H₂O m⁻²s⁻¹ was recorded in 'Giant Green' cultivar (Table1). Stomatal conductance also depends on cultural conditions. Nahar and Takeshi (2002) observed that the using of synthetic auxin (figaron) with a lower concentration had increased the stomatal conductance but with a higher concentration had decreased the conductance in soybean.

Fruit Development (Number of Day Flowering Opening to Maturity)

The variations for bloom to maturity time of apple cultivars have been reported by Westwood (1978) and it was 135 and 150 days in 'McIntosh' and 'Golden Delicious' apple cultivars respectively. In this study, for bud development, 'Masam Manis Pink' cultivar requires 18 ± 4 days small tiny bud to open and 'Jambu Madu Red' requires 20 ± 5 days, whereas, 'Giant Green' cultivars requires 24 ± 5 days (Figure 1). There were no differences among the cultivars from flower opening to anthesis periods. It takes more or less 3 days. The fruit developmental period after anthesis varied significantly with different cultivars of *Syzygium samarangense*. Results showed that

Table 1: Fruit developmen	t of different cultivars	s of Syzygium	samarangense
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Cultivar	Stomatal Conductance (Gs)	Bud development	Flower opening & anthesis	Fruit development
	mol H ₂ Om ⁻² s ⁻¹	Fruit set, Cell div	ision, Cell expansion	1 & Maturation
'Giant Green'	$0.031 \pm 05b$	24±5 day	3±2 day	50±5DAA
'Masam Manis Pink'	$0.036 \pm 11b$	18±4 day	3±2 day	38±4DAA
'Jambu Madu Red'	0.039±13a	20±5 day	3±2 day	45±5DAA
	**	ns	ns	**

Means (\pm S.E) within the same column followed by the same letter, do not differ significantly according to LSD test at α =0.01 ns, non-significant * Significant at 0.05 levels, ** Significant at 0.01 levels

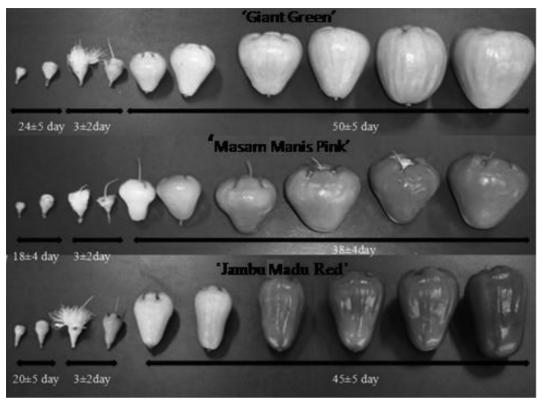


Figure 1: Photograph showing fruit development and ripening of different cultivars of Syzygium samarangense

'Masam Manis Pink' cultivar had the earliest fruit development and maturity approximately 38 days after anthesis followed by 'Jambu Madu Red' cultivar with nearly 45 days (Figure 1). On the other hand, 'Giant Green' cultivar had late maturity which about 50 days to reach harvest stage from anthesis.

Our findings supported the results of Morton (1987) who reported that the average period from anthesis to berry maturity in wax apple cultivars is about 35 to 50 days.

Fruit Development (Length Diameter Ratio of the Fruit)

Length and diameter ratio of the wax apple varied significantly among the three cultivars. The highest length diameter ratio (1.78) was recorded in "Jambu Madu Red" cultivar, followed by 'Giant Green' with a L/D ratio of 1.21, whereas, the lowest (1.0) length diameter

ratio was recorded in 'Masam Manis pink' cultivar (Figure 1 & Table 2).

Color Development

Color is an important aspect of both fresh and processed fruits particularly for commercial reasons. Colors in the fruits reflect the presence of certain biologically active phytochemical compounds and antioxidants that reportedly can promote good health. The development of red pigmentation in the skin of maturing wax apple fruits is the result of a massive accumulation of anthocyanin content and chlorophyll degradation during the maturation period (Zhang *et al.*, 2008). Positive values of a* and b*, as observed in this work, are attributed to carotenoids or anthocyanins present in the skin.

Fruits of wax apple produced the significance difference of skin color among the three cultivars. Figure 2 shows that fruit

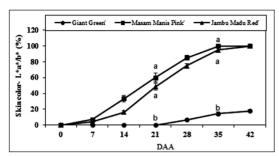


Figure 2: Colour development in the fruit skin of three cultivars of *Syzygium samarangense*. DAA = Day After Anthesis

color development was drastically change in the fruits of 'Masam Manis Pink' cultivar by exhibiting the greatest percentage of skin color between 14 and 35 DAA. It was also observed that on day seven the pink and red color of the fruits of the 'Masam Manis Pink' and 'Jambu Madu Red' cultivar; beginning to appear, but in 'Giant Green' cultivar, the color begins to develop at three weeks after anthesis. At the 35th day of observation, the 'Masam Manis Pink' cultivar fruits displayed at most 99% pink color and fruits of 'Jambu Madu Red' cultivar showed about 95% color development, whereas, 'Giant Green' cultivar was only 14% (Figure 2). Figure 1, showed that 'Masam Manis Pink' and 'Jambu Madu Red' cultivar fruits produced significantly different in skin color development from the fruit of 'Giant Green' cultivar.

Fruit Biomass Development (Yield)

The results of this study showed that 'Masam Manis Pink' and 'Jambu Madu Red' cultivar produced the highest number of fruits per plants than the 'Giant Green' cultivar (data not shown). The total yield (kg/tree) was highest (76.66 kg) in the 'Jambu Madu Red' cultivar followed by 'Masam Manis Pink' cultivar with a yield of 74.67 kg/tree, whereas minimum yield (58 kg/ tree) was recorded in 'Giant Green' cultivar (Figure 3). These differences were found to be statistically significant (P>0.05) among the different cultivars of wax apple.

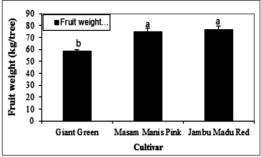


Figure 3: Fruit weight (kg/tree) of three cultivars of *Syzygium samarangense* under field conditions

The results were in agreement with that of Shu *et al.*, (1998) who observed that trees of *S. samarangense* yielded about 700 fruits per plant with the fruit weight varies among cultivars. Chiu (2003) reported that wax apple (pink) is a heavy producer plant on well fertilized good soils that can produce more than 200 fruit clusters per trees, with 4-5 fruits per cluster when reach maturity. They also reported that average fruit weight of 'Masam Manis Pink' variety is about 100 g per fruit.

Correlation between Stomatal Conductance and Biomass

Stomatal conductance affects the photosynthesis rate by regulating CO, fixation in leaf mesophyll tissue, that's ultimately affects the photosynthesis and yield of the crops. Accumulation of dry matter content in plants depends on stomatal conductance. The results of this study showed that stomatal conductance had a strong correlation ($R^2 = 0.88$) with the fruit biomass development of wax apple. Highest stomatal conductance (Gs) and fruit biomass yield were observed in 'Jambu Madu Red' cultivar followed by 'Giant Green' cultivar, whilst 'Masam Manis Pink' cultivar had the lowest photosynthetic and dry fruit biomass yields (Figure 4). The results of this study were in line with the result of Nahar and Takeshi (2002) which found that the stomatal conductance of leaf regulates the dry matter accumulation and yield in soybean.

Cultivar	L/D ratio	Titratable acidity (TA) (%)	TSS (% Brix)	TSS/TA ratio
'Giant Green'	1.21 ±0.01b	$0.83 \pm 0.05b$	8.56±0.23a	10.31± 1.10a
'Masam Manis Pink'	$1.0 \pm 0.040c$	$0.90 \pm 0.04a$	8.89±1.18a	$09.88 \pm 0.98b$
'Jambu Madu Red'	1.78 ±0.07b **	0.78 ± 0.04b	9.06±0.17a ns	11.61±1.15a *

Table 2: Content of different pigments in ripening fruit of three cultivars of Syzygium samarangense

Means (\pm S.E) within the same column followed by the same letter, do not differ significantly according to LSD test at α =0.01 ns, non-significant * Significant at 0.05 levels, ** Significant at 0.01 levels

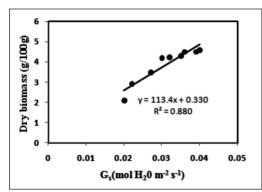


Figure 4: Correlation between the stomatal conductance and dry biomass yield of cultivars of wax apple

Biochemical Analysis

Titratable Acidity (TA)

Various organic acids have been reported present in fruits and these include citric, malic, acetic, fumaric, tartaric and lactic acids. The main acid accounting for titratable acidity in fruits is citric acid (Melgarejo et al., 2000). It was reported that the decrease in fruit acidity was coincided with an increase in sugar content of the fruits. The results for the TA analysis is shown in Table 2. Our results clearly indicated that TA was significantly affected by genetic of different cultivars. The lowest amount of TA (0.78%) was observed in the 'Jambu Madu Red' cultivar, followed by 'Giant Green' (0.83 %) and 'Masam Manis Pink' (0.90%). Our results are in agreement with the results of Supapvanich et al., (2011) which reported that the range of TA in fresh cut wax apple was 0.75 -0.80 % of citric acid.

Total Soluble Solids (TSS) and TSS/TA Ratio

TSS content of fruit was not statistically different among the cultivars of wax apple (Table 2). It can be seen that the soluble solids content in 'Jambu Madu Red' was wideranging from 5.63 to 12.5% Brix. From this study, it was observed that TSS content of the fruits did not varied significantly among the fruits within the same cultivar (Table 2).

The significant changes of sugar acid ratio is the key factor affecting quality of the wax apple cultivars. As shown in Table 2, the sweetness index (sugar acid ratio) of fruits was significantly affected by the different cultivars of wax apple. The 'Jambu Madu Red' cultivar increased the sugar acid ratio by 18%, followed by the 'Giant Green' with increases of 5% relative to the 'Masam Manis Pink'.

Vitamin-C Content

Vit-C content in fruits varies among crop species and is affected by environmental factors, maturity, plant vigor and the age of the plant. Figure 5 shows that Vit-C content varied significantly among of different cultivars of wax apple. The highest amount of vit-C content (5.7 mg/100g) was recorded in 'Masam Manis Pink' cultivar followed by 'Jambu Madu Red' and 'Giant Green' cultivar with a vit-C content of 5.53 and 5.45 mg/100g respectively (Figure 5). Similar range of vitamin-C in fresh cut wax apple fruit was observed by Supapvanich *et al.*, (2011).

Cultivar	Chlorophyll-a (mg/L)	Chlorophyll-b (mg/L)	Total chlorophyll (mg/L)	Carotene (µg/g)	Anthocyanin (mg/100g)
'Giant Green'	2.12±0.05a	1.31±0.13 a	3.43±0.18a	3.83±0.15c	9.5±0.15b
'Masam Manis Pink'	0.13±0.04c	0.16±0.08c	0.31±0.09c	5.16±0.31b	30.5±0.20a
'Jambu Madu Red'	0.72±0.07b **	0.61±0.05b **	1.33±0.09b **	6.23±0.78a **	27.8±0.15a **

Table 3: Content of various pigments in ripening fruit of three cultivars of Syzygium samarangense

Means (\pm S.E) within the same column followed by the same letter, do not differ significantly according to LSD test at α =0.01 ns, non-significant * Significant at 0.05 levels, ** Significant at 0.01 levels

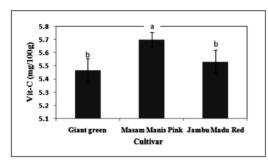


Figure 5: Vit-C content in different cultivars of Syzygium samarangense

Chlorophyll Content

It is well documented in the literature that during ripening, the skin of fruits changes from green to a different brighter color. The most obvious change which take place is the degradation of chlorophyll content and accompanied by the synthesis of other pigments usually either anthocyanin or carotenoids. It was observed that the chlorophyll content reduce loss gradually according to the color turning changes of the fruits. The results showed that 'Giant Green' cultivar had a significantly difference of chlorophyll (a, b and ab) content compared to the 'Jambu Madu Red' and 'Masam Manis Pink' cultivars. The highest (3.43 mg/L) total chlorophyll content in fruit skin was recorded in 'Giant Green' cultivar followed by 'Jambu Madu Red' and 'Masam Manis Pink' cultivar with a chlorophyll content of 1.33 mg/L and 0.31 mg/L respectively.

Carotene Content

Carotenoids are the precursors of vitamin A, and those commonly occurring in nature include α , and γ carotene, lycopene and cryptoxanthin (Goodwin, 1986). Among these, β -carotene precursors, a major proportion of vitamin A activity is analysed to represent the content of carotene in the fruits. The results of this study showed that the cultivars of S. samarangense produced the significant difference of carotene content among themselves. Table 3, showed that 'Jambu Madu Red' cultivar fruits has the highest (6.23 mg/L) carotene content as compared with 'Masam Manis pink' and 'Giant Green' cultivar with carotene content of 3.16 and 1.83 mg/L respectively.

Anthocyanin Content

Anthocyanin pigments are responsible for the red, purple, and blue colors of many fruits, vegetables, cereal grains, and flowers. As a result, research on anthocyanin pigments has intensified recently because of their possible health benefits as dietary antioxidants (Ronald, 2001). The anthocyanin content in 'Masam Manis Pink' and 'Jambu Madu Red' cultivars were significantly different from Giant Green cultivar. 'Masam Manis Pink' and 'Jambu Madu Red' cultivars produced 3.05 and 2.78 mg/L of anthocyanin respectively, meanwhile 'Giant Green' cultivar produced 0.95 mg/L which is the lowest amount of anthocyanin contents in the species. Khandaker et al., (2012) also reported similar amount of anthocyanin

content in jambu madu with hydrogen peroxide treatments. Anthocyanin content in the fruits also varies within the cultivar which is depending on the conditions of the environment like light, temperature, growth substances etc.

The observations recorded in the present investigation suggested that the three cultivars of wax apple varied markedly with respect to physiological and biochemical characteristics under field conditions. These differences appeared due to their genetic variations among the cultivars and the 'Jambu Madu Cultivar' showed the better quality.

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