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EFFECTS OF NAPHTHALENE ACETIC ACID AND GIBBERELLIC ACID ON PLANT PHYSIOLOGICAL CHARACTERISTICS OF WAX APPLE (VAR. JAMBU MADU)

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Abstract

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The study was conducted to investigate the effects of napthalene acetic acid (NAA) and gibeberellic acid (GA₃) on plant physiological characteristics of Syzygium samarangense (wax apple) var. jambu madu. Different concentration was used in NAA and GA₃ treatments where NAA at 10, 20 and 40 mg/L and GA₃ at 20, 40 and 80 mg/L. In GA₃ treatment, the result shown that application of 40 mg/L concentration gives the best result while, 10 mg/L and 20 mg/L treatments were the best concentration for NAA application to improve the plant physiological characteristics of *Syzygium samarangense* leaves. In addition, GA₃ treatment had shown significant effect on new leaf length, petiole length, chlorophyll b, carotenoid content and stomatal conductance. NAA treatments had shown significant effects on petiole length, chlorophyll content (SPAD), chlorophyll b, carotenoid content and photosynthetic rate of leaf. It can be concluded that 40 mg/L GA₃ and 10 and 20 mg/L NAA treatments significantly improved the physiological characteristics wax apple plants under field conditions.

Key words: wax apple; gibberellic acid; naphthalene acetic acid; physiological characteristics

Introduction

General function of auxins, one of the growth regulators, is to control root formation and growth. They are known for having ability to increase cell enlargement, thus enhancing fruit growth in citrus (Agusti et al., 1995), peach (Agusti et al., 1999) and tomato (Serrani et al., 2008). Auxins and calcium also alter fruit ripening and preventing the normal degradation of cell wall during cold storage (Figueroa et al., 2012). Naphthalene acetic acid (NAA), one of auxins used in this study, that applied as foliar treatment combined with calcium has no significant affects on firmness, acidity and soluble solid content (SSC) of strawberry in cold storage (Figueroa et al., 2012) but NAA alone has significantly increase fruit yield, total soluble solids (TSS), total sugar content and fruit color in Bing cherry and vitamin C in guava fruits (Iqbal et al., 2009). Another growth regulator

used in this study is gibberellic acid (GA₃) that has role to regulate protein synthesis and stem elongation. GA₃ is actually recovered as a metabolic by product of the fungus *Gibberella fujikuroi*, which cause stems elongation. It is very potent hormone that naturally occurrence in plants control of their development. The plants that were treated with GA₃ usually can overcome dormancy, premature flowering by apply to young plants, increasing fruit set, hybridizing, increase growth, frost protection and for root formation. It also can increase fruit firmness, colour, yield, and soluble solid content (Basak et al., 1998).

Recently, it has been reported that application of NAA and GA₃ on wax apple showed positive effect of fruit development, reduced fruit drop as well as fruit crack and improved fruit quality of wax apple (Tuan and Chung-Ruey, 2013). However, current information on the effects of NAA and GA₃ especially on plant physiological characteristics of

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wax apple leaves is not yet available in the literature as wax apple leaves has great potential benefits for human health. In Chinese medical science (CMS), wax apple fruit, leaves and seeds are antifebrile while its roots are duiretic (APAARI, 2014). In Taiwan, wax apple or 'jambu madu' (Syzygium samarangense) is importantly competitive tropical fruit (Wang, 1983) while in Southeast Asia such as Indonesia, Thailand and Malaysia, it is economically important fruit crop (Shu et al., 2001). From three famous cultivars planted in Malaysia, namely 'Giant Green', 'Masam Manis Pink' and 'Jambu Madu Red', they have very low sugar content and watery taste. This is due to lower photosynthates supply to the developing fruits from leaves (Moneruzzaman et al., 2012a, 2012b) and these growth regulators, NAA and GA, is considered as an important practice responsible for improving photosynthesis capacity of plants. Therefore, the objectives of this study are to measure the effect of NAA and GA, on physiologycal characteristics of wax apple leaves and investigate its response with different concentration of NAA and GA₃ applications.

Materials and Methods

Plant material and experimental site

There were thirty two (32) wax apple plants (Syzygium samarangense) from Jambu madu red cultivar with uniform in size and height were used in this experiment. The plants were planted in polybag and arranged at the research plot of Faculty of Biotechnology and Food Industry, UniSZA, Besut Campus, Besut, Terengganu in Completely Randomized Design (CRD) with four replicates for each treatment of different concentration of NAA: 0, 10, 20 and 40 mg/L and GA₂: 0, 20, 40 and 80 mg/L. The treatments had been applied three days interval by spraying on the whole plant. A total of twelve (12) spraying times were carried at the vegetative stage before flowering of the plants. For each treatment 250 ml of NAA and GA, hormone solution was used and spraying was carried out with a hand held sprayer. All the spraying were applied between 12 to 1 pm for maximize the absorption of hormone solution.

Preparation of hormone

The NAA and GA₃ hormones were weighed by using electronic balance and then put in the conical flask. Few drop of acetone was added to dissolve the NAA hormone but not to GA₃. After that, distilled water was added and was shaked gently to make the hormone solution. Aluminium foil was used to wrap the mouth of the conical flask so that it does not expose to the sunlight. This solution was stored in the chiller at 4°C.

Measurement of new leaf length, petiole length and chlorophyll content

The measurements for the length of new leaf and petiole were taken every week for seven weeks. These were measured by using ruler for each treatment. The average of the result was calculated and the data was recorded. Chlorophyll content of the leaves was measured by using a SPAD-502 meter (Minolta Japan). This SPAD meter was hand-held device that widely used for rapid, accurate and non-destructive measurement of leaf chlorophyll content by means of absorbance or transmittance measurements. Before use the meter, it was calibrated about 15 minutes so that readings can take accurately. The measurement was taken by simply clamp over leafy tissue. After that, meter showed an indexed chlorophyll content reading in less than 2 seconds. The reading has scale from -9.9 to 199.9 then readings were recorded (Adel et al., 2011).

Determination of pigments content

Carotenoid content was measured by using spectrophotometer. This measurement was taken to measure the photosynthetic pigments of the leaf. This method was followed using Lichtentaller method (Lichtenthaler, 1987) with slightly modification. The leaves samples were collected for each treatment. Then, samples were cleaned and air dried. The veins of the leaf were removed and the leaf was cut into small pieces. Then, samples were weighed about one g by using electronic balance for each treatment. The weighed samples were crushed by using mortar and pestle and were homogenized in 10 ml of 80% acetone for each 0.25 g samples. Then, the homogenate was filtered through mounted in glass funnel. The filtrate was poured in 3 ml cuvette and its absorbance was measured in wavelengths of 663 nm, 645 nm and 480 nm for measurement of chlorophyll a, chlorophyll b and carotenoid, respectively. These readings were taken by spectrophotometer devices. The concentrations of the chlorophyll and carotenoids were calculated by using formula of Arnon (1949). Formula to calculate the pigments concentration as shown below:

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 (μ g/g) = [A₄₈₀ + (0.114 x A₆₆₃) – (0.638 x A₆₄₅)] / 112.5

Measurement of chlorophyll fluorescence, stomatal conductance and photosynthesis rate

The measurement of chlorophyll fluorescence of treated and untreated leaves of wax apple plants was taken by using Handy Plant Efficiency Analyzer (PEA) (Hansatech Instrument Ltd., England). A single leaf wax apple plants was attached to the leaf clip and kept in dark place for 30-45 minutes to maintain dark adaptation for correct measurement. The fluorescence signal was measured for 3 seconds

and fluorescence yield observed where, Fo = Lower fluorescence, Fm = maximum fluorescence, Fv = relative variable fluorescence (Fm- Fo). Temperature = $27 \times C$, Time range = $10 \mu s$ - 3 sec. The reading was observed on the meter screen and the result was recorded. The data was taken by three replicate for each treatment.

Stomatal conductance of leaf was measured by using a portable Porometer (Leaf Porometer, Model SC-1, USA). Before measurement was taken, the leaf chamber was kept at an ambient temperature for 10 to 15 minutes to maintain sunlight adaptation. After that, a leaf chamber was attached to one leaf and the readings were showed on the screen of porometer. This measurement was taken by three replicates at different spot of a single leaf. The average of three replicates was calculated and the results were recorded. The photosynthetic rate (Pn) was determined by using portable photosynthesis system CI-340 Handheld Photosynthesis System. All the necessary setup and calibaration were followed according to manual C1-340 Handled Photosynthesis System (CID Bio-Science, USA) given. The measurement was taken after treatment application at 9.00 a.m to 1.00 p.m. The clip of this system was clamp over the leaf for each treatment. The reading was taken by three replicate for each treatment. The reading was observed and recorded.

Determination of total soluble solids (TSS)

The TSS content of leaf was evaluated by using Atago 8469 hand-held refractometer (Atago Co. LTD., Tokyo, Japan) and was expressed as percentage Brix (% Brix). The leaves samples were collected for each treatment. The samples then were cleaned and air dried. After that, veins of leaf were removed and leaves were cut into small pieces. Then, it was weighed about 1 g for each treatment by using electronic balance. The weighed leaf samples were crushed in mortar and pestle and 1 ml of distilled was added to produce leaf

juice. Two drops of leaf juice were placed to the refractometer sensor. The readings showed in percentage and the data was recorded.

Statistical analysis

The experimental design was a completely randomized design (CRD) with four replications. The data obtained was analyzed using SPSS Statistics software version 20. One way analysis of variance (ANOVA) was used to evaluate significant difference in parameters study in different treatments. Means comparisons were performed with Tukey's test at significant value $\rho \le 0.05$.

Results

New leaf and petiole length

Based on the result in Table 1, new leaf length for NAA treatment shows significantly difference at $\rho{\le}0.05$ level with the highest value in control followed by 10 mg/L and 20 mg/L treatment, while the lowest value was shown in 40 mg/L treatment. The trend for NAA treatment showed that without application of NAA treatment to wax apple, the new leaf can grow better. However, GA $_3$ treatment at 80 mg/L was the best concentration for new leaf length with GA $_3$ treatment and it was significantly different compared to the control treatment. This followed by the reading in control, 20 mg/L treatments and 40 mg/L treatment. There was statistically different at $\rho \leq 0.05$ level for both NAA and GA $_3$ treatment.

The highest reading of petiole length for NAA treatment was 10 mg/L, followed by the control, 20 mg/L and 40 mg/L, while for GA_3 treatment, the highest value was shown in concentration 40 mg/L, followed by 20 mg/L, 80 mg/L GA_3 and the lowest value in the control. Therefore, the best concentration of petiole length for NAA treatment is 10 mg/L and 40 mg/L for GA_3 treatment (Table 1).

Table 1
The effects of NAA and GA, treatments on physiological characteristics of wax apple plants

Treatments (mg/L)	Control	NAA 10	NAA 20	NAA 40	GA ₃ 20	GA ₃ 40	GA, 80
Physiological charac.						-	
New leaf length (cm)	$6.53\pm2^{\rm a}$	4.99 ± 3^{ab}	$1.93\pm1^{\rm b}$	$0.00\pm0^{\rm c}$	5.21 ± 1^{ab}	$2.40\pm1^{\text{b}}$	7.15 ± 1^{a}
Petiole length (cm)	$1.40\pm0^{\text{bc}}$	$2.07\pm1^{\rm a}$	$0.88\pm1^{\rm b}$	$0.00\pm0^{\rm c}$	$5.03\pm2^{\rm b}$	$5.67\pm2^{\rm a}$	4.45 ± 1^{b}
Chlorophyll content (µg/mg)	54.7 ± 1^{ab}	$53.3\pm1^{\text{b}}$	54.9 ± 1^{ab}	$58.34\pm1^{\rm a}$	$54.51\pm2^{\rm a}$	$57.50\pm2^{\rm a}$	57.04 ± 2^{a}
Chlorophyll fluorescence							
Lower fluo. (F ₀)	$19.0\pm2^{\rm a}$	$19.7\pm2^{\rm a}$	$15.3\pm0^{\rm a}$	$17.7\pm2^{\rm a}$	$15.7\pm2^{\rm a}$	$16.7\pm2^{\rm a}$	$15.6 \pm 1^{\mathrm{a}}$
Higher fluo. (Fm)	$78.8 \pm 1^{\rm b}$	$83.7\pm2^{\rm a}$	$83.0\pm1^{\rm a}$	$99.3\pm2^{\rm a}$	$76.3\pm0^{\rm b}$	$90.6\pm1^{\rm a}$	77.3 ± 1^{b}
Relative var. fluo. (Fv)	$59.8\pm1^{\rm b}$	$64.0\pm4^{\rm a}$	$67.7\pm1^{\rm a}$	$81.6\pm0^{\rm a}$	$60.6\pm2^{\rm a}$	$73.9 \pm 1^{\rm a}$	61.7 ± 1^{a}
Ratio (Fv/Fm)	$0.76\pm0^{\text{b}}$	$0.77\pm0^{\rm a}$	$0.81\pm0^{\rm a}$	$0.82\pm0^{\rm a}$	$0.79 \pm 0^{\rm a}$	$0.82\pm0^{\rm a}$	$0.80\pm0^{\rm a}$

Means (\pm SE) within the same column followed by the same letter, do not differ significantly according to Tukey's test at $\rho \le 0.05$. Lower fluorescence (F₀), higher fluorescence (Fm), relative variable fluorescence (Fv), photosynthetic yield (Fv/Fm)

Chlorophyll content (SPAD)

For leaf chlorophyll content, there was significantly difference at $\rho \leq 0.05$ level of NAA treatment but no significantly difference for GA $_3$ treatment (Table 1). The highest value of chlorophyll content (SPAD) for NAA treatment was shown in concentration 40 mg/L, followed by 20 mg/L and control, while 10 mg/L was the lowest. Meanwhile, the highest reading of chlorophyll content (SPAD) for GA $_3$ treatment was shown in concentration 40 mg/L, followed by 80 mg/L and control, while the lowest was in 20 mg/L. In this present study, leaf chlorophyll content of wax apple plants was significantly affected by NAA treatments and 40 mg/L was the best concentration of NAA treatment for wax apple tree.

Chlorophyll fluorescence

The best concentration in chlorophyll fluorescence for both NAA and GA, treatment were in 40 mg/L with significant difference for both treatments at $\rho \le 0.05$ level compared to control (Table 1). The result of lower fluorescence (F₀), higher fluorescence (Fm) and relative variable fluorescence (Fv) for NAA treatment was shown the highest value in 40 mg/L. Meanwhile, the result of lower fluorescence (F₀) for GA₃ treatment was shown in control, but for higher fluorescence (Fm), the highest reading was in 40 mg/L, as well as for relative variable fluorescence (Fv). The mean differences of both NAA and GA₃ treatments for F₀, Fm and Fv was significantly different at $\rho \le 0.05$ level. The result of photosynthetic yield (Fv/Fm) for both NAA and GA₃ treatments shown the highest reading in 40 mg/L with a value of 0.82. The means differences were significantly different at $\rho \le 0.05$ level for both NAA and GA, treatment on photosynthetic yield of wax apple leaves.

Chlorophyll and Carotene content

The means differences of chlorophyll a and b have shown significantly different at $\rho \le 0.05$ level for NAA treatment with the highest reading of chlorophyll a and chlorophyll b was shown in concentration 20 mg/L and 10 mg/L, respectively (Figure 1). Meanwhile, for GA₃ treatment, the mean for both chlorophyll a and b were not significantly different at ρ ≤ 0.05 level. The concentation 20 mg/L was shown the higher reading of chlorophyll a and for chlorophyll b (Figure 1). The means differences of carotenoid for both NAA and GA3 treatment shows significantly different at $\rho \le 0.05$ level. The highest reading for NAA treatment is in concentration 40 mg/L, followed by 10 mg/L and 20 mg/L while the control gave the lowest result (Figure 2). Therefore, 40 mg/L showed the best concentration in carotenoid content for NAA treatment. Same goes for GA₃ treatment where concentration 40 mg/L showed the highest value. This followed by 80 mg/L and 20 mg/L, while control showed the lowest reading of carotenoid.

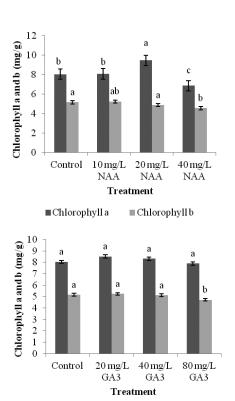


Fig. 1. The effects of NAA and GA₃ treatment on chlorophyll *a* and *b* of wax apple. The significant value indicated by a different letter

■Chlorophylla ■Chlorophyllb

Stomatal conductance

The best concentration of stomatal conductance for NAA treatment shown in 20 mg/L treatment, while GA_3 treatment was observed in 40 mg/L (Figure 3). Whereas, the means differences of stomatal conductance for both NAA and GA_3 treatments were significantly different at $\rho \leq 0.05$ level.

Photosynthesis rate of leaf

The means differences of photosynthetic rate of wax apple leaf was significantly different at $\rho \leq 0.05$ level for both NAA and GA $_3$ treatment with the highest photosynthesis rate shown in concentration of 10 mg/L for NAA treatment and 40 mg/L treatment for GA $_3$ treatment. This showed that both values 10 mg/L NAA and 40 mg/L GA $_3$ were the optimum concentrations to increase photosynthetic rate of wax apple (Figure 4).

Total soluble solids (TSS)

Total soluble solids measurement is considered to be an important parameter of quality of wax apple fruits but also

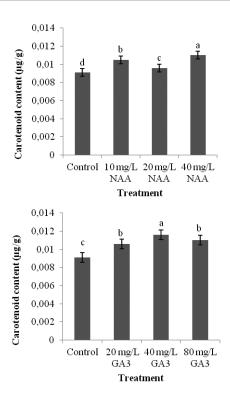


Fig. 2. The effects of NAA and GA₃ treatment on carotenoid content of wax apple. The significant value indicated by a different letter

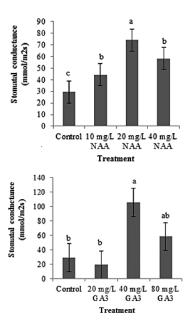


Fig. 3. The effects of NAA and GA₃ treatment on stomatal conductance of wax apple leaf. The significant value indicated by a different letter

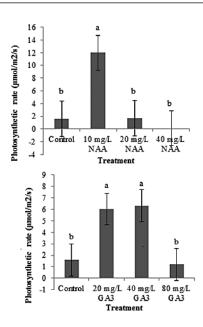


Fig. 4. The effects of NAA and GA₃ treatment on photosynthetic rate of wax apple leaf. The significant value indicated by a different letter

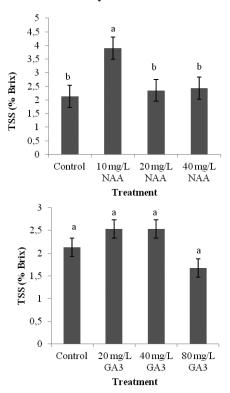


Fig. 5. The effects of NAA and GA₃ treatment on total soluble solids of wax apple leaf. The significant value indicated by a different letter

important for leaves. For wax apple leaf, the highest TSS value was recorded in 10 mg/L of NAA treatment followed by 40 mg/L and 20 mg/L, while the lowest was in control treatment (Figure 5).

The means differences were significantly different at $\rho \leq 0.05$ level. Meanwhile, GA₃ treatment showed the highest TSS value of wax apple leaf in 20 mg/L and 40 mg/L treatments with same value (2.5% Brix) followed by control treatment (2.1%) and the lowest one in 80 mg/L treatment (1.7%) (Figure 5). The mean differences showed that there was no significance different at $\rho \leq 0.05$ level.

Discussion

The leaf area of any plant is an important determinant of light interception and consequently of photosynthesis, transpiration, stomatal conductance and plant productivity. Our results showed that application of growth regulators significantly increased the new leaf and petiole length as well as leaf area of wax apple plants. Similar findings was reported by Lakshmipathi et al. (2014), who stated that application of GA, ethrel and NAA significantly increased the leaf area of cashew variety Bhaskara. This increased in leaf area with GA₃ and NAA might be related to the fact that GA and NAA promote leaf area through the increase of cell division and cell expansion in higher plant. In this present study, the leaf chlorophyll content of wax apple plants were significantly affected by NAA treatment. This result was supported by Moneruzzaman et al. (2015) and Gutam et al. (2009) whose reported similar positive effects of NAA on chlorophyll content of wax apple and Bell pepper. However, at lower GA, concentrations, chlorophyll synthesis would be enhanced while at higher concentrations of GA₃, chlorophyll synthesis would be showed a negative effect. Majidian et al. (2012) suggested that the application of GA₃ and BA increased rate of chlorophyll leaves of Zantedeschia aethiopiea plant. Lim et al. (2003) and Moneruzzaman et al. (2011) found that mepiquat chloride and GA₃ alone or combined, increased leaf area and chlorophyll content in apple and wax apple cultivars, while Janowsk et al. (2003) reported that GA₃ has structural role in membrane of chloroplast and causes to stimulate photosynthesis. It was observed that the chlorophyll a was higher than chlorophyll b for both treatments. This was supported by Farabee (2010) where chlorophyll a absorbs its energy from the violet blue and reddish orangered wavelength and little from green-yellow-orange wavelengths, while chlorophyll b absorbs energy that chlorophyll a does not absorb. Our results showed that growth regulators increase the chlorophyll content of wax apple leaves. According to Czerpak et al. (2002), synthetic auxin stimulated

the chlorophyll synthesis as well as increased the chlorophyll fluorescence.

Chlorophyll fluorescence is an indication of the fate of excitation energy in the leaf photosynthetic apparatus. It has been used to provide a rapid and nondestructive diagnostic system of detecting and quantifying physiologic injury in tree leaves and needles (photosynthetic organs) under low temperatures, salinity and water stress conditions (Sestak and Stiffel, 1997; Percival and Fraser, 2001; Percival and Sheriffs, 2002; Percival and Henderson, 2003). The best concentration in chlorophyll fluorescence for both NAA and GA, treatment were in 40 mg/L with no significant difference for both treatments at $\rho \leq 0.05$ level. Comparison between lower fluorescence (F₀), higher fluorescence (Fm) and relative variable fluorescence (Fv) showed that there was no significant difference for both NAA and GA₃ treatments at ρ ≤ 0.05 level. The photosynthetic yield ratio (Fv/Fm) is positively correlated to the PSII quantum yield and an indirect measurement of plant physiologic status (Kitajima and Butler, 1975; Maxwell and Johnson, 2001) for which values of 0.8±0.05 correspond to highly efficient use of the excitation energy in photochemical processes (Björkman and Demmig, 1987; Mohammed et al., 1995; Percival, 2005). Previous research by Percival (2004) and Maki and Colombo (2001) indicated Fv/Fm values of 0.6 below which trees were affected in terms of reduced survival, height growth and foliar necrosis. Biber et al. (2004) reported that if photosynthetic yield or quantum yield ranged from 0.65 to 0.78 then the plant is healthy but when the Fv/Fm value below 0.5 then the plant under severe stress or dying. In this study, we also recorded the photosynthetic yield ranged from 0.76 to 0.82. Moneruzzaman et al. (2010) stated that chlorophyll fluorescence and quantum yield of Bougainvillea plant increased significantly by removal of young leaf and cytokinin application.

Carotenoids play an important role in the light harvesting complex and in the photoprotection of the photosystems. Several studies have shown that these compounds are very important in protecting the photosynthesis apparatus against photodamage, by interconversions among the xanthophyll molecules (Young et al., 1997; Ort, 2001). In the xanthophyll cycle, violaxanthin goes through de-epoxidation to give rise to anteroxanthin and finally zeaxanthin (Havaux, 1988). Zeaxanthin participates intensely in the regulation of heat dissipation of PSII energy, when this has an energetic overload (Ramalho et al., 2000; Ort, 2001). The variation in total chlorophyll/carotenoids ratio has been used as a useful indicator of stress in plants because a rapid increase in total leaf carotenoid content is a recognized plant stress response (Hendry and Price, 1993). An increase in total leaf carotenoid content is a widely recognized plant stress response (Peñuelas and Filella, 1998), quantification of total leaf carotenoid content can provide indicators of plant responsiveness to stresses frequently encountered in urban and landscape environments (Strauss-Debenedetti and Bazzaz, 1991; Hendry and Price, 1993; Vieira, 1996). From the results it can be seen that GA₃ treatments significantly increased the leaf carotene of wax apple. This findings are supported by the results of our previus study that application gibbrellic acid significantly increased the carotenoids content in wax apple fruits (Moneruzzaman et al., 2011)

Stomata occupy a central position in the pathway for the transport of water vapour, CO2 and O2. The regulation of stomatal conductance (g_s) is the main mechanism by which plants control gas exchange and leaf temperature (Jones, 1998; Salleo et al., 2000). The best concentration of stomatal conductance for NAA treatment shown in 20 mg/L treatment, while GA, treatment was observed in 40 mg/L with significantly difference for both NAA and GA, treatments at $\rho \le 0.05$ level. From the result, GA, treatment has higher reading compared to NAA treatment because NAA inhibit the stomatal opening as mentioned by Davies and Mansfield (1987). Treatment of stomata with NAA induced stomatal closure (Snaith and Mansfield, 1984). According to Santakumari and Fletcher (1987), GA, increased stomatal aperture and reversed triazole-induced stomatal closure in Commelina benghalensis. It has been also reported that application of triacontanol-1 in Bougainvillea plants significantly increased the stomatal apperture (Moneruzzaman et al., 2013). Application of gibberellic acid (GA₃) to peanut increased g_s in control plants and partially relieved the effect of waterlogging, which induced a decrease of g_s (Bishnoi et al., 1992). A retardation of stomatal closure in water-stressed leaves following treatment with GA₃ was observed in lettuce (Aharoni et al., 1977).

Photosynthesis is a process used by plants and other organisms to convert light energy, normally from the Sun, into chemical energy that can be later released to fuel the organism's activities. The means differences of photosynthetic rate of wax apple leaf was significantly different at $\rho \le 0.05$ level for both NAA and GA, treatment with the highest photosynthesis rate shown in concentration 10 mg/L, followed by 20 mg/L and control, while the lowest was observed in 40 mg/L. This showed that 10 mg/L is the optimum concentration of NAA treatment to increase photosynthetic rate of wax apple. This supported by Schneider (1978) who reported that NAA reduced phloem transport of photoassimilates in apple leaves. Meanwhile, the highest reading of photosynthetic rate for GA, treatment was shown in 40 mg/L treatment, followed by 20 mg/L and control. The lowest reading was observed in 80 mg/L treatment.

Total soluble solids measurement is considered to be an important parameter of quality of wax apple fruits. For wax apple leaf, the highest TSS value of wax apple leaf was shown in 10 mg/L of NAA treatment and 20 mg/L and 40 mg/L of GA3 treatment. The result showed that there was significant difference at $\rho \leq 0.05$ level for NAA treatment. This result was supported by Huang and Huang (2005) who reported that by application of plant growth regulators significantly increase the TSS of fruit in citrus species. As the TSS value increase, it can initiate early flowering to produce the fruit. Meanwhile, GA3 treatment showed that there was no significant difference at $\rho \leq 0.05$ level of the mean differences. This is supported by Moneruzzaman et al. (2011) where GA3 increased TSS, proteins, ascorbic acid, and carotene in the wax apple fruits.

Conclusion

As conclusion, the physiological activitiy of Syzygium samarangense can improve by the application of GA_3 and NAA hormones. For the application of GA_3 , it shows significant effect on new leaf length, petiole length, chlorophyll b, carotenoid content, stomatal conductance and photosynthetic rate of leaf. NAA treatments had significant effects on petiole length, chlorophyll content (SPAD), chlorophyll b, carotenoid content, stomatal conductance, photosynthetic rate of leaf and total soluble solids (TSS). From this study, it can be concluded that $40 \text{ mg/L } GA_3$ and 10 and 20 mg/L NAA were the best treatments to improve the plant physiological activities in wax apple plant under field condition.

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