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Fruit Development, Pigmentation and Biochemical Properties of Wax Apple as Affected by Localized Application of GA_3 under Field Conditions

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ABSTRACT

This study investigated the effects of gibberellin (GA_3) on the fruit development, pigmentation and biochemical properties of wax apple. The wax apple trees were rubbing treated with 0, 20, 50 and 100 mgGA_3/l under field conditions. The localized application (rubbing) of 50 mg GA_3/l significantly increased the fruit set, fruit length and diameter, color development, weight and yield compared to the control. In addition, GA_3 treatments significantly reduced the fruit drop. With regard to the fruit quality, 50 mg/l GA_3 treatment increased the juice content, K^+, TSS, total sugar and sugar acid ratio of wax apple fruits. In addition, higher vitamin C, phenol, flavonoid, anthocyanin, carotene content, PAL and antioxidant activities were recorded in the treated fruits. There was a positive correlation between the peel colour and TSS content and between the PAL activity and anthocyanin formation in the GA_3-treated fruit. It was concluded that rubbing with 50 mg/L GA_3 at inflorescence developing point of phloem once a week from the tiny inflorescence bud until the flower opening resulted in better yield and quality of wax apple fruits and could be an effective technique to safe the environment from excessive spray.

Key words: Fruit development, pigmentation, quality, GA_3, wax apple

INTRODUCTION

The wax apple, or *jambu air madu*, as it is known in Malaysia, is a non-climacteric tropical fruit in the Myrtaceae family and is botanically known as *Syzygium samarangense* (Morton, 1987).Wax apple is widely cultivated throughout Malaysia, mainly in smallholdings ranging from 1 to 5 ha, with a total hectarage estimated at 1,500 ha in 2005 (Zen-hong et al. 2006). The pear-shaped fruits are usually pink, light red or red, but may be greenish-white or cream-colored, and are generally crisp, with a subtly sweet taste and aromatic flavor. The fruit pulp is a rich source of phenolics, flavonoids and several antioxidant compounds (Moneruzzaman et al. 2012). In Malaysia, wax apple fruits are eaten raw with salt or cooked as a sauce and almost entire fruit is edible. The fruit can be used to treat high blood pressure and several inflammatory conditions, including sore throat, and can also be used as an antimicrobial, antiscorbutic, carminative, diuretic, and astringent (Rivera and Obón 1995).

It has been well documented that the size and quality of the fruits can be affected by certain horticultural cultural practices, such as the application of plant growth hormones (Wang et al. 2004). It has been reported that spray treatment of gibberellic acid (GA_3) increases the fruit set, growth and fruit size in wax apple fruits

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(Moneruzzaman et al. 2011). Basak et al. (1998) reported that GA_3 increased the fruit firmness, fruit weight and yield and soluble solids. Wang et al. (2004) found that the application of GA_3 and other growth regulators increased the sugar contents in various mandarin and sweet orange cultivars. Xiao et al. (2005) also reported that the application of growth regulators significantly reduced the acidity and increased vitamin-C contents of the citrus fruits. GA_3 significantly promoted the biosynthesis of secondary metabolites and increased the phenol and flavonoids content in the apple (Zhou and Tan 1997). Montero et al. (1998) stated that GA_3 treatment increased the anthocyanin content in addition to the enhancement of PAL and TAL activities in *Fragaria ananossa* fruits. Currently, there are no information how the GA_3 affect the fruit quality of wax apple, an increasingly popular fruit in Asia. In this study, the effects of gibberellin, applied by rubbing, on the growth and development as well as the biochemical properties of the wax apple fruit was investigated under field conditions.

MATERIALS AND METHODS

Experimental site and Treatment application

The experiments were performed at a commercial farm in Banting at an elevation of approximately 45 m above sea level. The area under study has a hot and humid tropical climate. The soil in the orchard is peat, with a mean pH of of approximately 4.6 (Ismail et al. 1994). The experiments were conducted in three successive seasons in the same farm in Banting, Selangor, Malaysia in between 2009 to 2011. Twelve years old wax apple trees were selected for the study. The trees were planted in a 4.2 m × 4.2 m hexagonal system and received the same horticultural management. Twenty-four trees were used in the experiments for each season. One hundred twenty uniform branches (five branches/tree) of about the same length and diameter, and approximately the same number of leaves were selected for the experiments. The experiment consisted of four treatments, including control, with six replications and a single tree was taken as an experimental unit. The inflorescence developing point of bark on the selected branches was rubbed with 20, 50 and 100 mg GA_3/l and water (control) based on a Randomized Complete Block Design (RCBD) with six replications each once a week until the flower opening.

Measurement of physiological and biochemical parameters

The percentage fruit drop was calculated by dividing the total number of fruit after anthesis minus the number of fruit at harvest with the total number of after anthesis. The chlorophyll and carotene content of fruit was measured by the methods described by Hendry and Price (1993). The fruit length, fruit diameter, and fruit growth were measured weekly with the aid of a Vernier calliper. The surface colour of each tagged fruit was determined at three different points using a standard colour chart (Minolta, Osaka, Japan) and expressed as a percentage of colour cover. The yield per treatment was recorded by weighing and counting the total number of fruits per treatment at the time of harvest. The total soluble solids (TSS) was evaluated at 25°C with an Atago 8469 hand refractometer (Atago Co. ltd., Japan) and expressed as °Brix. The fruit juice was titrated with 0.1 M NaOH and the results were expressed in terms of percentage citric acid. The percentage was calculated using the Bhattarai and Gautam (2006) formula. The sugar acid ratio of the wax apple juice was given as the ratio of TSS/TA. Potassium content of fruit juice was determined by using a Cardy Potassium meter. The total soluble sugars was the determined according to the phenol-sulphuric method (Dubois et al. 1956). The total phenolic and total flavonoids content of fruits were determined by using the Folin-Ciocalteu and the aluminum chloride colorimetric assay. The total ascorbic acid (vit-C) content was determined using the method modified by Hashimoto and Yamafuji (2001). The total anthocyanin contents of the hydrophilic extracts were measured by the pH differential method (Rodriguez-Saona et al. 1999). The PAL activity in the crude enzyme extracts was assayed using the method as described by Zucker (1965) and expressed as nmol cinnamic acid yield. The DPPH free radical scavenging activity was determined as described by Yang et al. (2008), with minor modifications.

Statistical analysis

The experimental design was a Randomized Complete Block Design (RCBD) with six replications. The data obtained from three successive seasons were pooled and analyzed using MSTAT-C statistical software. One-way

ANOVA was applied to evaluate the significant difference in the parameters studied in the different treatments. Least significant difference (Fisher's protected LSD) was calculated, following significant F-test (p=0.05).

RESULTS

Fruit set, drop, weight and yield

Data in Table 1 showed that the fruit set was almost two times more in 50 mgGA_3/l treated branches compared to the control branches. The control branches showed the highest fruit drop (46%), with the least percentage of fruit dropped observed (30%) in 20 mgGA_3/l treated branches, followed by 100 and 50 mg GA_3/l treatments (Table 1). As shown in Table 1, all the GA_3 treated branches in this study yielded the fruits with higher average fruit weight than the untreated control.

The yield, on a fruit weight basis, was almost three times higher in the 50 mg GA3/l treated branches compared to the control. From the results, it could be seen that 50 mg/lGA3 treated branch produced the highest yield, followed by 100 and 20 mg/l treatment. Results were statistically significant between the treatments and control.

Table 1 - Effects of GA_3 treatments on flowering, fruit development, yield and quality of wax apple.

Treatment (mg/l)	Fruit set (%)	Fruit drop (%)	Yield (kg)	Average fruit weight (g)	Fruit juice (mg/l)	K^+ content (mg/100 g)
Control	29 ± 4.40c	46 ± 6.00b	0.31 ± 0.06d	52 ± 2.17c	71 ± 2.08a	18.5 ± 1.85b
GA_3 20	35 ± 3.84b	30 ± 4.40a	0.50 ± 0.05c	53 ± 2.57b	78 ± 1.76a	32.3 ± 7.53a
GA_3 50	60 ± 3.00a	45 ± 2.89a	0.92 ± 0.13a	67 ± 2.20a	81 ± 1.78a	25.0 ± 7.54a
GA_3 100	48 ± 7.10b	31 ± 6.42a	0.79 ± 0.05b	61 ± 2.97a	79 ± 1.75a	29.0 ± 7.23a
	**	*	**	*	ns	**

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

Fruit growth and color development

As seen in Figures 1 and 2, the GA_3 treated branches exhibited higher fruit growth rate from the first week till the 7th week, with regard to fruit length and diameter. At the 3rd week, the fruit length was 4.90 and 4.39 cm in 50 and 100 mg GA_3/l treatments, respectively, whereas it was 2.46 cm in the control. This growth trend was observed through the whole fruit developmental period until the harvesting period. The fruit growth was significant between the treatments and control. Result showed that the fruit color development was greatly enhanced by the GA_3 treatments at 50 and 100 mg GA_3/l treated where the fruits exhibited the highest peel color development from day 14 till 28 (Fig. 3).

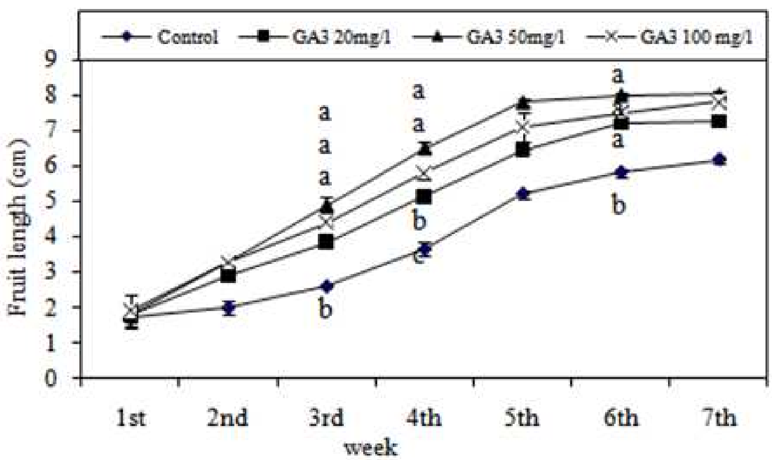

Figure 1 – Fruit growth (Length/week) as influenced by different concentrations of GA_3.

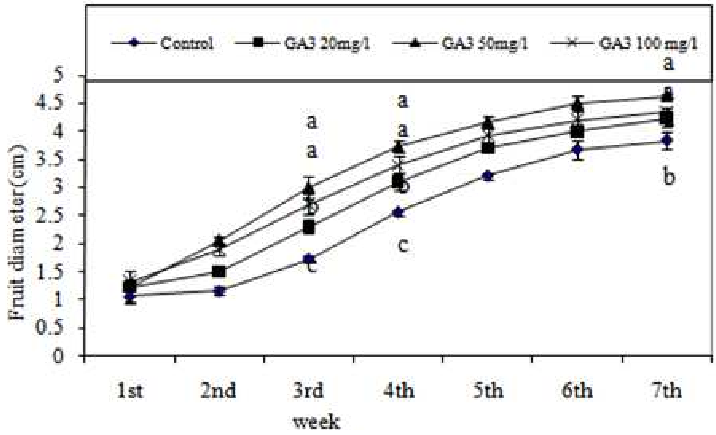

Figure 2 – Fruit growth (diameter/week) as influenced by different GA_3.concentrations.

Furthermore, it was observed that on 14 (day after anthesis) the red colour of the fruits had already started to appear in the treated branches compared to the control fruits, which only started coloring one week later. At the 28th day, the 50 mg/l treated fruits showed more or less 99% red color, whereas the control had only 35% (Fig. 3). Significant difference was observed in the peel color development between different GA_3 treatments and control.

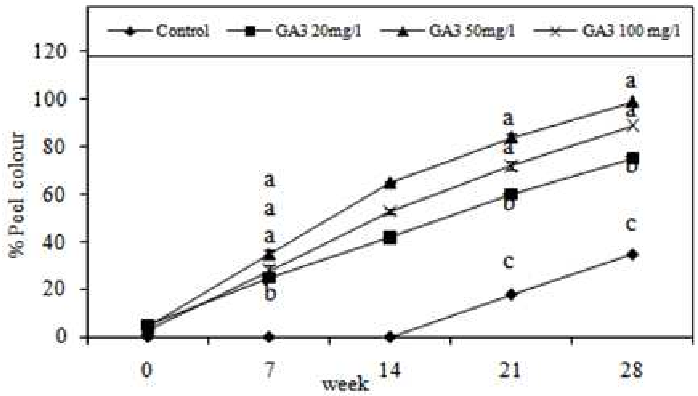

Figure 3 – The effect of different treatments of GA_3.on peel colour of wax apple fruits after anthesis.

Fruit juice and potassium content

The fruits of GA_3 treated branches produced higher amount of fruit juice, although their differences were not statistically significant (Table 1).The treatments of GA_3 produced significant effect in the case of K^+ content in the wax apple fruits (Table 1). Results showed that the highest K^+ content in the fruit juice was higher in 20 mgGA_3/l treated fruits, followed by 100 and 50 mg GA_3/l treatments; the control produced the lowest amount of K^+ content.

Titrable acidity (TA) and sugar acid ratio

The results for the TA experiments indicated that the titrable acidity was significantly affected by the GA_3 application (Table 2). The lowest amount of TA (0.64%) was observed with the 50 mgGA_3/l treatment, followed by the 10 and 20 mg GA_3/l treatments. The highest amount of the titrable acidity (0.73%) was observed in the control. The result showed that the sweetness index (sugar acid ratio) of the fruits was significantly enhanced by the GA_3 treatments (Table 2).

The highest increased sugar acid ratio (78%) was recorded in 50 mgGA_3/l treatment followed by the 100 and 20 mg GA_3/l treatments with increases of 69% and 45%, respectively, relative to the control.

Table 2 - Effect of GA_3 treatments on biochemical parameters of wax apple under field conditions.

Treatment (mg /L)	TSS (°Brix)	Titrable acidity (%)	TSS/acidity ratio	Total sugar (g /100 g pulp)	Flavonoids (mg/100 g)	Total phenols (mg GAE/100 g)
Control	6.70 ± 0.3b	0.73 ± 0.04a	9.18 ± 1.5b	3.65 ± 0.47b	18.5 ± 0.5b	0.44 ± 0.08d
GA_3 20	9.07 ± 0.3a	0.68 ± 0.03b	13.3 ± 1.7a	5.57 ± 0.25a	40.0 ± 2.0a	0.68 ± 0.15 b
GA_3 50	10.5 ± 0.6a	0.64 ± 0.04b	16.4 ± 1.9a	6.24 ± 0.23a	45.0 ± 1.2a	0.88 ± 0.05a
GA_3 100	10.1 ± 0.5a	0.65 ± 0.03b	15.5 ± 1.6a	5.82 ± 0.32a	37.9 ± 1.7a	0.74 ± 0.09a
	**	*	**	**	**	**

Mean ±S.E (n= 6) within the same column followed by the same letter, do not differ significantly according to LSD test at ά=0.01 ns, non-significant *, ** Significant at 0.05 and 0.01 level.

Total soluble solids (TSS), total sugar and correlation between peel color and TSS

Significant variations were recorded in the case of TSS content in the wax apple pulp between the fruits of different GA_3 treatments (Table 2). The highest TSS value (11.50 ºBrix) was recorded in 50 mgGA_3/l treated fruit, followed by 100 and 20 mgGA_3/l treatments, respectively, while the minimum was recorded in the control treatment. The result showed that GA_3 treatments had a significant effect on the total sugar content of the wax apple fruit. The highest amount of total sugar content (6.24g) was recorded in 50 mgGA_3/l treatment, followed by 100 and 20 mgGA_3/l treatments with 5.82 and 5.57g, respectively, whereas untreated control fruits showed the lowest sugar content of 3.65 g (Table 2). The peel color of 50 mgGA_3/l treated fruits had a strong positive correlation (R^2 = 0.97) with the TSS (ºBrix) content of fruit juice (Fig. 4). The TSS content increased simultaneously with the peel color of the fruits.

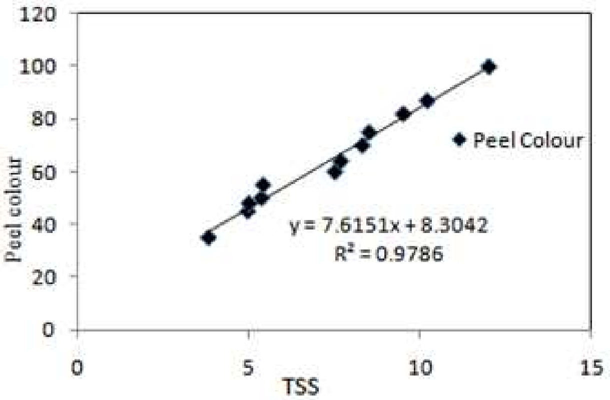

Figure 4 – Correlation between peel color and TSS content of wax apple fruits affected by 50mg/l GA_3 treatment.

Total flavonoids and phenolics

The localized application of the various concentrations of GA_3 had significant effects on the polyphenolic compounds (flavonoid and phenol) of the wax apple fruits (Table 2). The highest content of flavonoid (45.0 mg/100g) was recorded in 50 mg GA_3/l treated fruits, followed by the 20 and 100 mg GA_3/l treatment, with flavonoid contents of 40.0 and 37.9 mg/100g, respectively. The control fruits had less than the treated fruits. As shown in Table 2, the fruits from 50 mgGA_3/l treated branches had the highest amount of phenol (0.88 mgGAE /100g), followed by 100 and 20 mgGA_3/l treated fruits with 0.74 and 0.68 mgGAE /100g, respectively, while the control fruits had the lowest phenol content (0.44 mgGAE /100g).

Vitamin C, Chlorophyll, anthocyanin and carotene content

In this study, GA_3 application significantly affected the vit-C content of wax apple fruits (Table 3). The highest vit-C content (6.5 mg/100 g) was recorded in 50 mgGA_3/l treated-fruits, followed by the fruits treated with 20 and 100 mg/L GA_3, with a vit-C content of 6.2 and 5.9

mg/100 g, respectively. The lowest amount of vit-C (5.3 mg/100 g) was recorded in the control fruit. The chlorophyll content of the ripening wax apple skin was also recorded in this study. The results showed that GA_3 application significantly reduced the chlorophyll content in the fruits. The highest chlorophyll content (0.65 mg/l) was observed in the control, followed by the amounts recorded in the 20 and 100 mgGA_3/l treatments.
The lowest amount (0.32 mg/l) was recorded in 50 mgGA_3/l treated fruits (Table 3). The application of 50 mg GA_3/l resulted in the highest carotene content followed by 100 and 20 mgGA_3/l treatments. The application of GA_3 had a significant effect on the anthocyanin content of wax apple fruits (Table 3). Anthocyanin content enhanced in fruits treated with up to 50 mgGA_3/l treatment but thereafter decreased. The highest amount of anthocyanin was observed in 50 mgGA_3/l treated fruits followed by 20 and 100 mgGA_3/l treatments.

Table 3 - Effect of GA_3 treatments on vit-C, leaf chlorophyll, carotenoids and anthocyanin of wax apple.

Treatment (mg /l)	Vit-C (mg/100g)	Chlorophyll *a* fruit (mg /l)	Carotenoid (μg/g)	Anthocyanin (mg/L)	DPPH assay (% inhibition)
Control	5.3 ± 0.05c	0.65 ± 0.05a	06.54 ± 0.24 b	2.43 ± 0.20c	50 ± 2.52b
GA_3 20	6.2 ± 0.07b	0.43 ± 0.01b	09.92 ± 0.36a	4.02 ± 0.31b	75 ± 3.40a
GA_3 50	6.5 ± 0.10a	0.32 ± 0.03c	10.90 ± 0.20a	4.60 ± 0.37a	83 ± 3.50a
GA_3 100	5.9 ± 0.09b	0.34 ± 0.01b	10.01 ± 0.12a	3.60 ± 0.28b	79 ± 4.30a
	*	**	**	**	**

Mean ± S.E (n = 6) within the same column followed by the same letter, do not differ significantly according to LSD test at ά=0.01 ns, non-significant, *, ** Significant at 0.05 and 0.01 levels.

Antioxidant content and Correlation between phenolics and antioxidant activity

The DPPH radical scavenging activity measured in the wax apple fruit extracts was affected by the GA_3 (Table 3). Results showed that the IC_{50} of the DPPH radical scavenging activity increased with GA_3 application. The DPPH radical scavenging activity increased up to 66% fruit extracts from the 50 mg GA_3/l treatment, while the activity in the control was only 50% (Table 3). Figure 5 shows the relationship between antioxidant capacity and the phenolic contents of wax apple fruit. A high correlation (R^2 = 0.86) was observed between the total phenolic content and DPPH measurements in the 50 mg GA_3/l treated fruits.

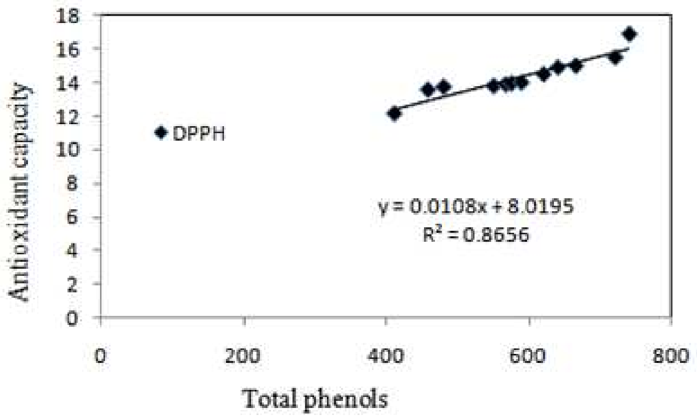

Figure 5 - Regression analysis of antioxidant capacity and total phenols of wax apple fruits.

PAL activity and correlation between PAL and anthocyanin formation

It was observed that GA_3 treatment had a significant effect on the phenylalanine ammonia lyase (PAL) activity (Fig. 6). After 30 min of incubation, the PAL activity expression of the cinnamic acid yield was the highest (36.91 nmol min^{-1} mg $protein^{-1}$), whereas the control fruits produced the lowest amount of cinnamic acid (21.37 nmol min^{-1} mg $protein^{-1}$).

A high degree of positive correlation (R2 = 0.88) was observed between the PAL activity and anthocyanin formation in the treated ripening fruits (Fig.7).

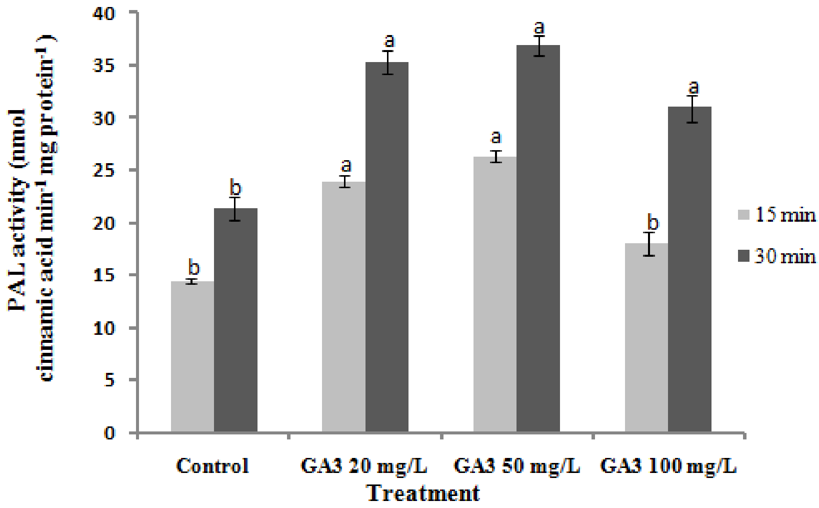

Figure 6 – The effect of GA_3 treatment on PAL activity of ripening wax apple fruits. Bars indicate (± S.E).

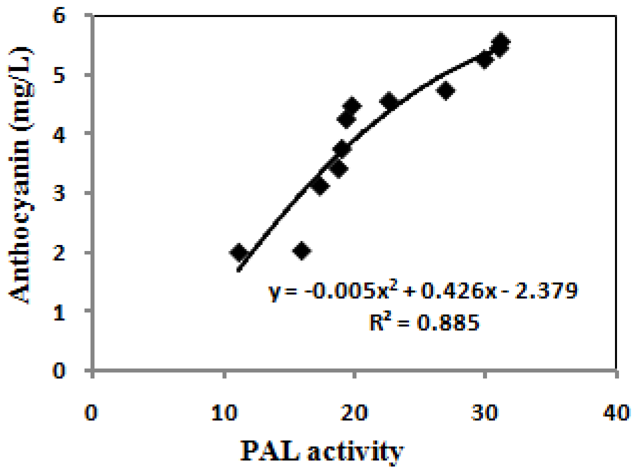

Figure 7 – Correlation between PAL activity (nmol-cinnamic acid min^{-1} mg $protein^{-1}$) and anthocyanin content in 50 mg/L GA_3 – treated wax apple fruit.

DISCUSSION

Results showed that all the GA_3 treated branch posted significantly higher fruit set values compared to the control. These results were in agreement with the findings of Moneruzzaman et al. (2011) who reported that GA_3 as a spray treatment increased the fruit set, number of fruit and growth in the wax apple. In this study, positive effects of GA_3 on the fruit drop on the wax apple fruits were also found. Similar observations in citrus fruit were also reported by Almeida et al. (2004). Several previous studies have reported that the GA_3 treatments could increase the fruit weight, peel thickness and fruit diameter of Valencia oranges (Almeida et al. 2004). Results for the fruit weight and yield were in agreement with that of

Basak et al. (1998) who observed that GA_3 significantly influenced the fruit weight as well as the yield in cherry. The treated fruits grew at a faster rate and were larger than the untreated control fruits. Singh and Lal (1980) also described a similar trend in Yu Her Pau' litchi over two years in Taiwan. They also stated that the GA_3 treatment increased the fruit and aril weight. The development of red pigmentation in the peels of maturing wax apple fruits was the result of a massive accumulation of anthocyanin content and chlorophyll degradation during the maturation period (Zhang et al. 2008). The present results for the peel color development were in agreement with those of Ganji and Mokhtarian (2006) who reported that the application of GA_3 increased the color of the fruits.

Fruit juice content, which is related to fruit size, is an important parameter in industrial processing. In this study, GA_3 treatment showed a positive effect on the juice content of wax apple fruits. These results were in agreement with that of Wang et al. (2004) who reported that the application of GA_3 significantly increased the juice percentage in various citrus species. It has been reported that potassium regulates the translocation of photosynthates, protein synthesis, ionic balance and plant stomatal opening and also known as a quality nutrient because of its important effects on fruit quality factors such as size, shape, color, taste, shelf life and fiber quality (Almeselmani et al. 2010). The present results suggested that GA_3 had positive effects on K^+ content in the fruits. In this study, GA_3 treatments produced significant effects on the TSS content of the wax apple. These findings were in agreement with the Basak et al. (1998) who reported that *a*uxin and gibberellins could significantly increase the total soluble solids contents of the fruit in sweet cherry. The TSS content of tomato fruits increased with gradual advancement of fruit maturity and the highest TSS was recorded in fully ripened tomatoes (Moneruzzaman et al. 2008). The present results for the correlation of the peel color with TSS were in agreement with Moneruzzaman et al. (2009). The argument for this is that the TSS is indicative of higher sugar content in the fruits and this in turn supplies the energy for the synthesis of the red color pigments found in these fruits, as ripening sets in.

In the present study, GA_3 application significantly reduced the titrable acidity content in the wax apple fruits. Thakur et al. (1996) similarly reported that the titrable acidity was significantly reduced with GA_3 and auxin application. Wahdan et al. (2011) reported that GA_3 treatments significantly increased the TSS, sugar acid ratio and total sugar content of mango. Similarly, in this study significant changes in the sugar acid ratio due to growth regulator application in wax apple fruits were also recorded. In fact, the sugar acid ratio could be the key factor affecting the quality of wax apple under tropical climates. Accordingly, in the current study, GA_3 treatments produced a significant effect on total sugar content of wax apple fruits. These results were similar to the results of Wang et al. (2004) who found that the application of 2, 4-D, GA_3 and some other growth regulators increased the sugar contents in various mandarin and sweet orange cultivars. In the present work, GA_3 treatments clearly had a significant effect on the total flavonoid and phenolic content of the wax apple fruits. Results also showed that the phenolic content positively correlated with the antioxidant activity in the GA_3-treated fruits. These results were in agreement with the findings of Pourmorad et al. (2006) who reported that the extracts of *M. officinalis* containing the highest amounts of flavonoid and phenolic compounds exhibited the maximum antioxidant activity.

Vit-C content in the fruits varies among the crop species and is affected by the environmental factors, time of fruit harvesting, plant vigor, the age of the plant and the use of growth regulators. In this study, GA_3 treatments showed a significant effect on the vit-C content in the wax apple. This finding was in agreement with the results of Wahdan et al. (2011) that 'Succary Abiad' mango trees sprayed with GA_3 two months after the full-bloom at 40 ppm exhibited significant increases in the vit-C contents. During the ripening, there is degradation of chlorophyll which is accompanied by the synthesis of other pigments, usually either anthocyanin or carotenoids. In this study, it was observed that chlorophyll loss gradually took place with the GA_3 application at color turning stage. Similar results were reported by Perez et al. (1993), who found that the plant growth regulator methyl jasmonate promoted the chlorophyll degradation of the skin of Golden Delicious apple fruit.

The positive effects of GA_3 on PAL and TAL of *Fragaria ananossa* fruits have also been noted previously (Montero et al. 1998). This study showed that GA_3 treatment enhanced the PAL enzyme activities in the fruits, which might stimulate the anthocyanin accumulation. Carotenoids are the precursors of vitamin A and those commonly occurring in the nature include, *α*, *β* and *γ* carotene, lycopene and cryptoxanthin (Goodwin 1986). Results showed that GA_3 treatment significantly increased the anthocyanin and carotene content in the fruits. These results concurred with the findings of Roussos et al. (2009) who observed that the anthocyanin content in the strawberry fruit increased significantly when the plants were treated with GA_3. It suggested that GA_3 could also play a role in the accumulation of pigments in the fruits. The present results showed that the fruits treated with the GA_3 exhibited higher antioxidant capacity than the control fruits. These findings were consistent with the results of Klessig and Malamy (1994) who reported that GA_3 significantly promoted the biosynthesis of secondary metabolites in the fruit with the highest antioxidant activity.

CONCLUSION

From the above results, it could be concluded that the localized application of the GA_3, particularly, 50 mg GA_3/l could stimulate the fruit growth and pigmentation as well as the biochemical properties of wax apple fruit. The treatment with 50 mg GA_3/l increased the fruit set, growth, size, juice and k^+ content; in addition, TSS, total sugar, sugar acid ratio, vit-C, anthocyanin, carotenoid, phenolics and flavonoids and antioxidant activity in the fruits were also significantly increased. The GA_3 treatments also significantly reduced the chlorophyll and titrable acidity in the fruits, which also showed positive correlations between the peel color and TSS, between the phenolic compounds and antioxidant activity and between the PAL activity and anthocyanin formation. Finally, it could be concluded that 50 mgGA_3/l treatment was promising for enhancing the fruit development, pigmentation and improving the biochemical properties of the wax apple fruits under the field conditions.

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