Australian Journal of Crop Science
AJCS

AJCS 9(12):1221-1227 (2015) ISSN:1835-2707

The influence of gibberellic acid on the chlorophyll fluorescence, protein content and PAL activity of wax apple (*Syzygium samarangense* var. jambu madu) fruits

Mohammad Moneruzzaman Khandaker[1]*, Ali Majrashi[2], Amru Nasrulhaq Boyce[3]

[1]School of Agriculture Science & Biotechnology, Faculty of Bioresource and Food Industry, Universiti Sultan Zainal Abidin, Tembila Campus, 22200 Besut, Terengganu, Malaysia
[2]Department of Biological Science, Faculty of Science, Taif University, Taif, Saudi Arabia
[3]Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

***Corresponding author: moneruzzaman@unisza.edu.my**

Abstract

A study was carried out to investigate the effects of gibberellic acid on chlorophyll fluorescence, phenylalanine ammonia lyase (PAL) activity and anthocyanin biosynthesis in *Syzygium samarangense* var. jambu madu fruits. Selected horticultural parameters were monitored during the growth period from May 2014 to April 2015 using different concentrations of GA_3 ranging from 20 to 100 mg L^{-1}. Results revealed that 50 mg L^{-1} GA_3 treatment increased chlorophyll content, soluble protein and produced the highest chlorophyll fluorescence and quantum yield. It was observed that 50 mg L^{-1} GA_3 treatments increased the PAL activity and anthocyanin content of fruits significantly. Furthermore, a positive correlation (R2 = 0.69) between the PAL activity and anthocyanin biosynthesis was observed in treated fruit. With regard to fruit maturity, red ripen stage showed the highest PAL activity and anthocyanin content. From this study, it can be concluded that 50 mg L^{-1} GA_3 once each week from the beginning of flower opening through fruit development, exhibited highest chlorophyll fluorescence and quantum yield, stimulated PAL activity and anthocyanin biosynthesis of the jambu air madu fruits under field conditions.

Keywords: *Wax apple*, gibberellin, chlorophyll fluorescence, PAL, anthocyanin, quality.
Abbreviations: PAL_ Phenylalanine ammonia-lyase, GA3_Gibberellic acid, PSII_Photosystem II, FW_Fresh weight, F_Flourescence.

Introduction

The *jambu air* is a tropical fruit which belongs to the genus *Syzygium* in the family Myrtaceae and is fairly widely cultivated and grown throughout Malaysia mainly as smallholdings ranging from 1 to 5 ha with its hectare age estimated at 1,500 ha in 2005 (Zen-hong et al., 2006). Malaysian climate is suitable for the wax apple production and the fruit can be harvest all year-round (Khandaker et al., 2011). It has become an increasingly popular fruit in the tropical region where it can fetch a price of up to 3USD per kilogram and has the potential to bring great benefit to local farmers and the country's economy. There is a great scope to develop wax apple industry in Malaysia and other tropical countries. It has been well documented that the quality of the fruits can be affected by certain horticultural cultural practices, such as the application of plant growth hormones (Guardiola, 1992). PGRs enhance the rapid changes in physiological and biochemical characters and improve crop productivity. GA_3 has been found to increase fruit firmness, color, yield, and soluble solid content (Basak et al., 1988). Lim et al. (2004) reported that mepiquat chloride and GA_3 alone or combined, increased leaf area and chlorophyll content in grape. Color is an extremely important quality factor and indicator of the eating quality of fruits (Moneruzzaman et al., 2008). The color of the fruits depends on their lycopene and B-carotene. Wax apple fruit color range from green to yellow green to pink to red and fully deep red (Moneruzzaman et al., 2011). Anthocyanin pigments are responsible for the red, purple, and blue colors of many fruits, vegetables, cereal grains, and flowers and as a result, research on anthocyanin pigments has intensified recently because of their possible health benefits as dietary antioxidants (Ronald, 2001). Moneruzzaman et al. (2010) reported that application of citokinin increased the color and postharvest quality of Bougainvillea flowers. Fruit pigmentation is one of the significant aspects of fruit quality. The accumulation of anthocyanins, the principal pigments, is developmentally regulated. Chlorophyll, the predominant pigment in immature fruit, disappears at 3 weeks after anthesis (WAA) resulting a white surface, then anthocyanin accumulates until full maturity (Cheng and Breen, 1991). Phenylalanine ammonia-lyase (PAL) is one of the key enzymes in controlling anthocyanin biosynthesis from phenylalanine (Tucker, 1993). Teresa et al. (1998) reported that exogenous treatments of GA_3 improve colour of strawberry fruits, and affect PAL activities. Nevertheless, it must be emphasized that the positive effects of PGRs on the quality of wax apple are dependent on types, dose, and environmental conditions (Khandaker et al., 2012). Yuhua et al. (2001) reported that PAL activity high in young fruits, decreased to a white maturity stage and then increased until the fully ripe stage in strawberry fruits. McGlasson et al. (1978) suggested that these substances have an important incidence on the biosynthesis of the anthocyanins. Scarcity of information about the role of the gibberellic acid on fruit ripening of wax apple is evident.

In this study, we report investigations of PAL activity responsible for wax apple colour during development and ripening and the effects of exogenous treatments with GA_3 on soluble protein, chlorophyll fluorescence and quantum yield of wax apple and also the possible relation between the PAL activity and anthocyanin biosynthesis.

Results and Discussion

Chlorophyll a content in wax apple leaves

Leaf chlorophyll content provides valuable information about physiological status of plants. It has been reported that GA_3 increased the leaf area and chlorophyll content in apple leaves (Lim et al., 2003). As shown in Fig. 1, the chlorophyll content in leaves from all the treated branches was highest (3.73 mg L^{-1}) in the 50 mg L^{-1} GA_3 treated branch, followed by 20 and 100 mg L^{-1} GA_3, with a value of 3.66 and 3.11 mg L^{-1} respectively, whereas control treatment showed the lowest (2.96 mg L^{-1}) chlorophyll content. The data between the treatments and control was found to be statistically significant different. Similar results were found in our previous study, that the application of gibberellins increased the chlorophyll and protein content in the wax apple fruits (Moneruzzaman et al. 2012).

Chlorophyll fluorescence and soluble protein content

Measurements of chlorophyll fluorescence are linearly correlated with the functionality of PSII, where F_0 and F_m are the chlorophyll fluorescence yields corresponding to open and closed PSII reaction centres, respectively (Ilis et al., 2007). Different GA_3 treatments produced the significant effects on chlorophyll fluorescence in the leaves of wax apple (Table 1).The highest fluorescence (F_m) was observed in 50 mg L^{-1} GA_3 branch followed by 20 and 100 mg L^{-1} GA_3 with a value of 3727 and 3330, whereas, leaves of control branch produced the lowest (2647) Fm value. Lower fluorescence (F_0) was highest in 20 mg L^{-1} GA_3 treatment followed control and 50 mg L^{-1} GA_3 treated leaves , whereas, 100 mg L^{-1} GA_3 was the lowest. Variable fluorescence (F_v) was highest in 50 mg L^{-1} GA_3 treated branches followed by 20 and 100 mg L^{-1} GA_3, whereas, control performed least fluorescence (Table 1). GA_3 treatments also produced the significant effects on the quantum yield or photosynthetic yield. From the Table 1, it could be seen that optimum quantum yield [(Photosynthetic yield (F_v/F_m)] was highest (0.78) in 50 mg L^{-1} GA_3 treatment followed by 20 and 100 mg L^{-1} GA_3 treatment with a value of 0.75 and 0.74 while, control produced the least 0.66 photosynthetic yield. Georgia et al. (2010) also suggested that application of GA_3 increased the maximum quantum yield of primary photochemistry (F_v/F_m) and the ratio of F_v/F_m in *Capsicum annum* L. Protein content were measured from the selective leaves of treated branches. From the results, it can be seen that gibberellin treatment had a significant effect on leaves soluble protein content. As can be seen in Fig. 2, 50 mg/ L GA_3 treated leaves produced on average the highest (24.81mg g^{-1} FW) soluble protein content followed by 100 and 20 mg L^{-1} GA_3 with a value of fruits 20.62 and 16.21 mg g^{-1} FW respectively, whereas, control produced the lowest (11.51 mg g^{-1} FW) amount of soluble protein content. Lu et al. (2010) reported similar results in rapeseed and stated that in stress condition gibberellin could increase soluble protein content in leaves.

PAL activity in wax apple fruits

Phenylalanine ammonia-lyase (PAL) is one of the key enzymes in controlling anthocyanin biosynthesis from phenylalanine (Tucker, 1993). McGlasson et al. (1978) suggested that gibberellin (GA_3) have an important incidence on the biosynthesis of the anthocyanins. Results showed that gibberellin treatment had a significant effect on PAL activity in the treated fruits (Fig. 3). Gibberellin (50 mg L^{-1}) treatment increase the PAL activity (89.54 nmol-Cinnamic acid g^{-1} FW) followed by 100 mg L^{-1} and 20 mg L^{-1} GA_3 treatment only, with 59.35 and 56.31(nmol-Cinnamic acid g^{-1} FW) PAL activity, respectively. The control treatments produced the lowest PAL activity 44.84 (nmol-Cinnamic acid g^{-1} FW). These results were found to be in consonance with that of Terasa, et al. (1998) who reported that GA_3 increased the PAL activity in strawberry plant. The application of various concentration of GA_3 had a significant effect on the PAL activity versus time (Fig.4). As can be seen in Fig. 4 gibberellic acid treated fruit produced significantly the highest amount of Cinnamic acid yield versus time.

At the 30 min of observations, the highest cinnamic acid yield was 223 nmol g^{-1} FW observed in the 50 mg L^{-1} GA_3 treated fruits followed by 20 mg L^{-1} GA_3 and control treatment with cinnamic acid yield of 171 and 156 nmol g^{-1} FW respectively, whereas, the lowest cinnamic acid yield of 114 nmol g^{-1} FW was observed in the 100 mg L^{-1} GA_3 treatments (Fig.4). Higher concentration of gibberellin showed the negative effects on PAL activity, probably as a result of decrease in the respiratory activity and a delay in anthocyanin synthesis and chlorophyll degradation. Our results suggest that PAL activity (cinnamic acid yield) increase with the incubation time. Similar findings reported by Yueming and Daryl (2003), they reported that PAL activity increase versus time in strawberry fruits. From the results it was observed that different stages of fruit development had significant effects on PAL activity in wax jambu fruits (Fig.5). As can be seen that, at the early fruit developmental stage PAL activity a little bit high, then decrease up to mature green stage and subsequently increase up to fully ripen red stage. These findings are in

Table 1. The effect of different treatments of GA_3 on chlorophyll fluorescence of wax apple leaves.

Treatment	F_0	F_m	F_v	F_v/F_m
Control	892 ± 55a	2647 ± 208d	1755 ± 185c	0.66 ± 0.035c
GA3 20mg/L	930 ± 54a	3727 ± 522b	2797 ± 235b	0.75 ± 0.026b
GA3 50mg/L	881 ± 63a	4084 ± 169a	3203 ± 231a	0.78 ± 0.025a
GA3 100 mg/L	859 ± 80a	3330 ± 400c	2471 ± 125b	0.74±0.035b
	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 levels, ** Significant at 0.01 levels

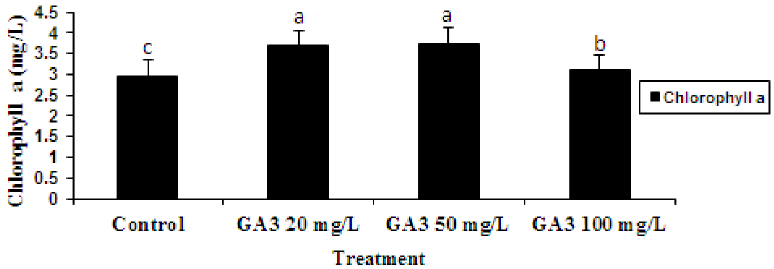

Fig 1. The effect of different treatments of GA_3 on chlorophyll a (mg L^{-1}) content of wax apple fruits.

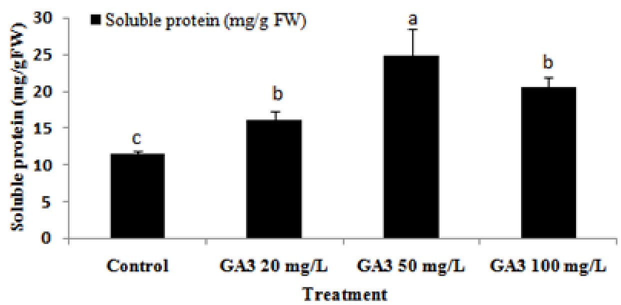

Fig 2. The effect of different treatments of GA_3 on soluble protein (mg g^{-1} FW) of wax apple leaves.

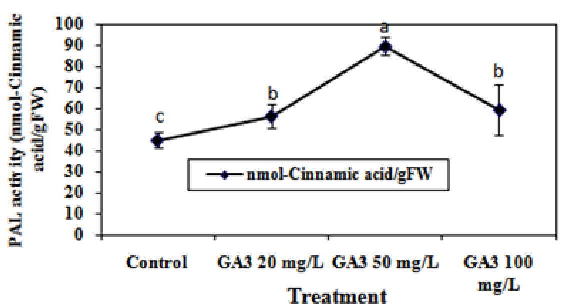

Fig 3. PAL enzyme activity (nmol-cinnamic acid g^{-1} FW) as affected by different treatments of GA_3 in wax apple fruit.

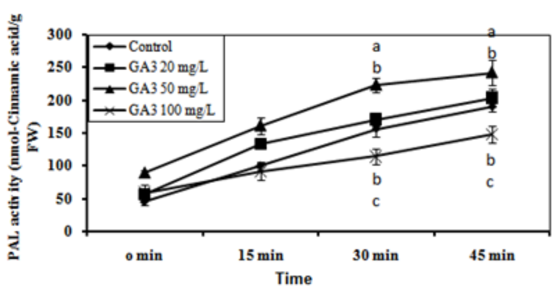

Fig 4. PAL enzyme activity (nmol-cinnamic acid g-1 FW) versus time in GA_3 treated wax apple fruits.

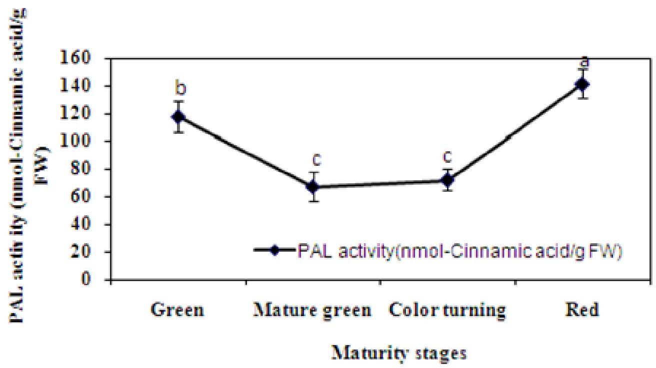

Fig 5. PAL activity (nmol-cinnamic acid g^{-1} FW) at different fruit developmental stages of wax apple fruits.

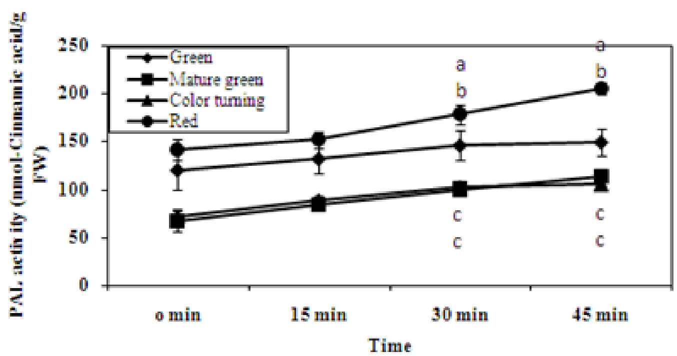

Fig 6. PAL activity (nmol-cinnamic acid g^{-1} FW) versus time at different stages of wax apple fruit development.

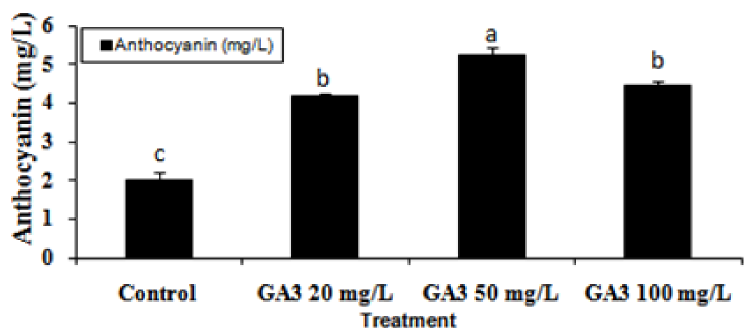

Fig 7. The effect of different treatments of GA_3 on anthocyanin (mg L^{-1}) content in wax apple fruits.

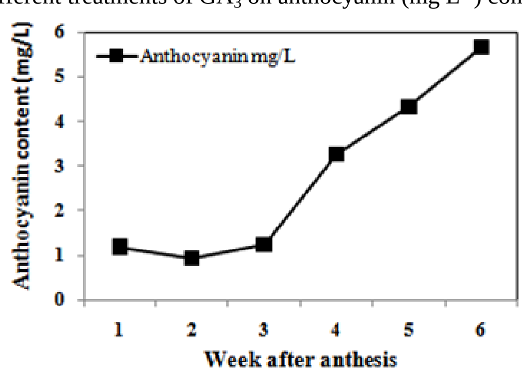

Fig 8. The effect of different stages of fruit development on anthocyanin content in wax apple fruits.

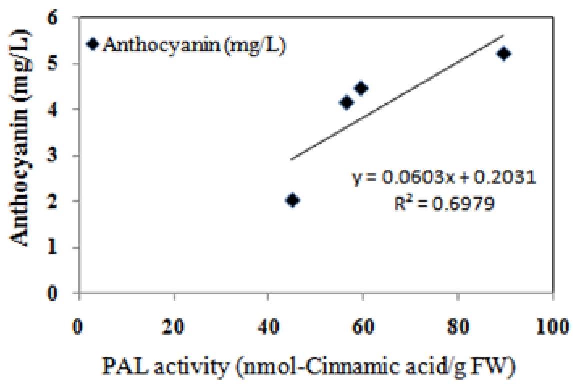

Fig 9. Correlation between PAL activity and anthocyanin content in GA_3 treated wax apple.

agreement with those obtained by Sujitra, et al. (2005). They reported that PAL activity decreased at early stage and increased at the final stage of fruit maturity in mangosteen. PAL can be induced by a wide range of factors including wounding, temperature, lights and chemicals and can be induced at any fruit developmental stages (Tucker, 1993). From the results shown in Fig. 6, it can be seen that PAL activity was highest in ripening stage, followed by green pit stage and colour turning stage to the whole obserbations periods. At the 45 min of observations, the highest cinnamic acid yield was 223 nmol g^{-1} FW, observed in the red ripen fruits followed by green and color turning stage with cinnamic acid yield of 171 and 156 nmol g^{-1} FW respectively, whereas the lowest cinnamic acid yield of 114 nmol g^{-1} FW was observed in the mature green fruit (Fig.6). Cinnamic acid yield was found to be statistically significant among the different stages of fruit. Shigeki et al. (1981) found similar results in *Rhodotorula glutinis.* They reported that PAL activity (cinnamic acid yield) increased with the incubation time.

Anthocyanin content

Anthocyanin pigments are responsible for the red, purple, and blue colors of many fruits, vegetables, cereal grains, and flowers and as a result, research on anthocyanin pigments has intensified recently because of their possible health benefits as dietary antioxidants (Ronald, 2001). The application of various concentration of GA_3 had a significant effect on the anthocyanin content of wax apple fruits (Fig.7).The anthocyanin content of fruits showed a strong correlation with the GA_3 concentrations applied. Results showed that, anthocyanin content in fruits treated with up to 50 mg L^{-1} treatment but thereafter decreased. The highest amount of anthocyanin was observed in 50 mg L^{-1} treated fruits followed by 100 and 20 mg L^{-1} treatment, whereas untreated control fruits showed the lowest anthocyanin content. These results concur with the findings of Roussos et al. (2009) who observed that anthocyanin content in strawberry fruit increased significantly when the plants were treated with GA_3 hormone. Fruit pigmentation is one of the significant aspects of fruit quality. In most of the fruits, the accumulation of anthocyanin is developmentally regulated (Rousses et al., 2009). From the results, it can be seen that different stages of fruit development had a significant effects of anthocyanin accumulation in the fruits (Fig. 8). In early fruit developmental stage anthocyanin accumulation is very low until 3 weeks after anthesis, then accumulation started to increase until full ripen stage. These results were found to be in agreement with that of Cheng et al. (1991) who observed that in early stages of fruit development chlorophyll and phenolic content is high but anthocyanin level is very low. They also stated that anthocyanin accumulation stated to increase at full maturity stages until fruit ripen condition. Tucker (1993) observed PAL activity only in the red parts of the apple skin and concluded that PAL activity was closely related to the formation of anthocyanin.

Correlation between PAL activity and anthocyanin content

Fig. 9, shows the relationship between PAL activity and the anthocyanin content of the *S. samarangense* fruits studied. A high correlation between the PAL activity and anthocyanin content was observed (R^2=0.697). Our results are in agreement with the results of Sujitra et al. (2005). They suggested that PAL controlled the rate of anthocyanin synthesis in mangostene.

Materials and Methods

Experimental site and plant materials

The experiments were performed at a commercial orchards located at Banting, Selangor and at experimental farm in UniSZA tembila campus, Terengganu, Malaysia. The soils of orchard and pot were peat, with a mean pH of approximately 4.6. The area under study has a hot and humid tropical climate. The experiments were conducted between 2010 and 2014. The first season (December 2010 to May 2011) season experiment was conducted at the farm in Banting and the second season (April 2013 to December 2014) experiment in pot conducted at the farm of Tembila campus UniSZA. Twelve-year-old wax apple plants (at Banting) and one year wax apple plants propagated through air layering (Tembila campus, UniSZA) were selected for the study.

Treatment application

The selected uniform branches of plants (around 4 cm diameter and one year old) were sprayed with 20, 50 and 100 mg/l GA_3 and water (control) based on a completely randomized design with three replications once a week at the beginning of flower opening until fruit maturation. A total of eight spraying times were carried out; two times before anthesis and six times after anthesis and 450 ml GA_3 solution was used per treatment. All the selected fruits were harvested and kept in a refrigerator at 4°C prior to biochemical analysis.

Measurement of physiological and biochemical parameters

The chlorophyll content of leaves at the fruit maturity stage was measured by method describe by Hendry and Price (1993). Chlorophyll fluorescence was measured by Plant Efficiency Analyzer (Hansatech Instrument Ltd., England). Protein was assayed, with bovine serum albumin as the standard, by the dye- binding methods of Bradford (1976). PAL activities in the buffer supernatant were determined by the production of trans-cinnamic acids, from L-phenylalanine, during 0, 15, 30 and 45 minutes, as measured by the absorbance change at 295 nm (Zucker, 1965). The molar extinction coefficient of authentic trans-cinnamic acid in assay buffer plus TFA was determined to be 19207. Total anthocyanin contents of the hydrophilic extracts were measured by the pH-differential method described by Rodriguez-Saona et al. (1999).

Statistical analysis

The data obtained were analyzed using MSTAT 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 ($p \leq 0.05$).

Conclusion

From the above results, it can be concluded that to increase the chlorophyll content, photosynthetic yield and soluble protein in wax apple, 50 mg L^{-1} GA_3 was effective compared to other treatment. It can be summarized that GA_3 (50 mg L^{-1}) treatment increase the PAL activity and anthocyanin content in the wax apple fruit. It also be concluded that PAL activity and anthocyanin content high in red ripen stage. Thus, it can be concluded that 50 mg L^{-1} GA_3 treatment are promising for enhancing the chlorophyll fluorescence, protein content and increasing the PAL activity and anthocyanin content of wax apple fruit under field conditions.

Acknowledgements

This research was supported by grant from Universiti Sultan Zainal Abidin (UniSZA), 22200 Besut, Terengganu, Malaysia (Project No. UniSZA/14/GU/024)

References

Basak A, Rozpara E, Grzyb Z (1998) Use of bioregulators to reduce sweet cherry tree growth and to improve fruit quality. Acta Hort. 468: 719–723.

Bradford M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72: 248-254

Cheng GW, Breen PJ (1991) Activity of phenylalanine ammonia-lyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. J Amer Soc Hort Sci. 116: 865–869.

Georgia G, Ilias I, Anastasia G, and Parthena (2010) Comparative study on the effects of various plant growth regulators on growth, quality and physiology of *Capsicum annuum* L. Pak J Bot. 42: 805-814.

Guardiola JL (1992) Fruit set and growth. In: Donadio LC (ed) Second International Seminar on

Citrus," Univ. of Bebedouro, 1–30, Department of Food Science and Technology. Oregon State University, Corvallis, USA.

Hendry GAF, Price AH (1993) Stress indicators: chlorophylls and carotenoids. In: Hendry, G.A.F., Grime, J.P. (Eds.), Methods in Comparative Plant Ecology. Chapman & Hall, London, 148–152.

Ilias I, Ouzounidou G, Giannakoula A, Papadopoulou P (2007) Effects of GA_3 and Prohexadione-calcium on growth, chlorophyll fluorescence and quality of okra plant. Biol Plant. 51: 575-578.

Khandaker MM, Boyce AN, Normaniza O, Hossain ABMS (2012) Physiochemical and phytochemical properties of wax apple (Syzygium samarangense [Blume] Merrill & L.M. Perry) as affected by growth regulator application under field conditions. Sci World J. volume 2012:1-13.

Khandaker MM, Hossain ABMS, Osman N, Boyce AN (2011) Application of girdling for improved fruit retention, yield and fruit quality in Syzygium samaragense under field conditions. Int J Agril Biol. 13:18-24.

Lim SC, Kim SK, Kim YH, Youn CK, Yoon T (2004) Vine growth and fruit quality of 'kyoho' grapes as affected by Mepiquat chloride and GA3. Acta Hort. (ISHS) 653: 145-149.

Lu Z, Zhang GY, Zou XK, Cheng CS, Zheng YPY (2010) Improving drought tolerance of germinating seeds by exogenous application of gibberellic acid (GA3) in rapeseed (*Brassica napes'* L.). Seed Sci Technol. 38: 432-440.

McGlasson WB, Wade DL, Idato I (1978) Phytohormones and fruit ripening In: Phytohormones and Related Compounds. ÈA Comprehensive Treatise, eds Letham D S, Goodwin P B & Higgins T J V. Elsevier, Amsterdam, The Netherlands, 447-494.

Moneruzzaman KM, Hossain ABMS, Sani W, Saifuddin M (2008) Effect of Stages of Maturity and Ripening Conditions on the Physical Characteristics of Tomato. Amer J Biochem Biotechnol. 4: 329-335.

Moneruzzaman KM, Hossain ABMS, Saifuddin M, Imdadul H, Normaniza O, Amru NB (2010) Effects of sucrose and kinetin on the quality and vase life of

Bougainvillea glabra Var. Elizabeth angus bracts at different temperature. Aus J Crop Sci. 4: 474-479.

Moneruzzaman KM, Al-Saif AM, Alebedi AI, Hossain ABMS, Normaniza O, Boyce AN (2011). An evaluation of the nutritional quality of three cultivars of Syzygium samaragense under Malaysian conditions. Afr J Agril Res. 6:545-552.

Rodriguez-Saona LE, MM Giusti, Wrolstad RE (1999) Color and pigment stability of red Radish and red-fleshed potato anthocyanins in juice model systems. J Food Sci. 64:451–456.

Ronald EW (2001) The possible health benefits of anthocyanin pigments and polyphenolics.

Department of Food Science and Technology, Oregon State University, Corvallis.

Roussos PA, Denaxa NK, Damvakaris T (2009) Strawberry fruit quality attributes after application of plant growth stimulating compounds. Sci Hort. 119:138–146.

Shigeki Y et al (1981) Production of L-phenylalanine from trans-cinnamic acid with rhodotorula glutinis containing L-phenylalanine ammonia-lyase activity, Applied Environ Microbial. 54: 773-778.

Sujitra R, Jamnong U, Kobkiat S (2005) Effects of bagging and storage temperature on anthocyanin content and phenylalanine ammonialyase (PAL) activity in mangosteen (Garcinia mangostana L.) fruit pericarp during maturation, Songklanakarin J Sci Technol. 27: 711-717.

Teresa M, Esperanza M, Maria A, Cabrejas M, Francisco JLA (1998) Effects of gibberellic acid (GA_3) on strawberry PAL (phenylalanine ammonia-lyase) and TAL (tyrosine ammonia-lyase) enzyme activities. J Sci Food Agric. 77: 230-234.

Tucker GA (1993) Introduction. In: Biochemistry of Fruit Ripening. Seymour G B, Taylor J E & Tucker G A. Chapman & Hall, London, UK, 1-51.

Yueming J, Daryl CJ (2003) ABA effects on ethylene production, PAL activity, anthocyanin and phenolic contents of strawberry fruit. Plant Growth Reg. 39: 171–174.

Yuhua L, Ryozo S, Hirabumi M, Aneyuki SK (2001) Regulation of anthocyanin biosynthesis during fruit development in strawberry. J Japan Soc Hort Sci. 70: 28-32.

Zen-hong SZ, Meon R, Tirtawinata, Thanarut C (2006) Wax apple production in selected tropical Asian countries. Acta Hort. 773: 161-164.