International Journal of Molecular Biology and Biochemistry
www.biologyjournals.net
Online ISSN: 2664-651X; Print ISSN: 2664-6501
Received: 05-09-2019; Accepted: 06-10-2019; Published: 13-10-2019
Volume 1; Issue 2; 2019; Page No. 16-20

Total phenol, flavonoid, anthoayanin and nutrient content development of water apple fruit affected by gibberellic acid

ABM Sharif Hossain[1], Adel M Alsaif[2], Rosna M Taha[3]
[1] Biotechnology Program, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia
[2] Plant Production Dept., College of Agriculture and Food Science, King Saud University, KSA, Saudi Arabia
[3] Department of Biology, Faculty of Science, University of Hail, KSA, Saudi Arabia

Abstract
The study was carried out to investigate the pigments (chlorophyll and anthocyanin), total phenol, flavonoid and mineral (K+) content in water apple fruit affected by gibberellic acid. The lowest amount of chlorophyll was observed in the control treatment. The highest chlorophyll content was found at 60 ppm GA3. The most effective concentration to earlier maturity of water apple fruit was 60 ppm of GA3. Moreover, flavonoid content was observed higher in 60 ppm GA3 than control, 30 and 90 ppm GA3 concentration. However, the most effective concentration for flavonoid content was in the 60 ppm GA3. Fruit K+ content was significantly increased by GA3 treatments, especially at 60 ppm. Anthocyanin compounds were found increasing trend while increasing the maturity development.

Keywords: GA3, total phenol, flavonoid, anthoayanin, nutrient content

Introduction
Water apple (Syzygium samarangense) is a common fruit in Malaysia as well as other Asian countries. The fruit is widely cultivated and grown throughout Malaysia mainly as small scale gardener ranging from 1 to 5 ha with its hectare average estimated at 1,500 ha in 2005 (Zen-hong shu *et al*, 2006). Fruit development and repining have been considered as the most important phenomena in agriculture and fruit production. Idea to develop fruit growth was very old and increase of yield or weight using horticultural practices were reported by many researches. Some of the old used techniques were the pruning, hormone application by spray of trees increased fruit growth and development (Savage and Cowart, 1942; Elfving and Forshey, 1976). Phytohormones contribute in a large range of phenomena that occur during the growth, and the development of plants (Taiz and Zeiger, 1998).
Spraying of plant growth hormone or chemicals is a traditional method. Nowadays Environmental Scientists do not suggest for using this technique too because of the pollution of the air in the environment, water and human health and also not cost effective (Miller, 2004; Tashkent, 1998). An innovative technique swabbing method has been developed because of using small quantity to get more output compared to spray and dipping methods. Swabbing method does not create any droplet and spray dirft which caused by spray and dipping method also. Hossain and Mizutani (2008) [8] developed swabbing technique and resulted excessive flowering in peach plants. They also reported that swabbing method enhanced early flowering (blooming) by dwarfing plant growth while ABA (abscissic acid) was applied to the bark in peach plant. The size of the fruit can be affected by certain horticultural cultural practices, such as application of plant growth hormones. Gibberellic acid (GAs) has been shown to increase fruit set and growth in apples, pears (Weaver, 1972,). It has been reported that the spraying of auxins prevented the senescence of fruits presumably by maintaining the cell turgidity at the zone of abscission, which prevents the synthesis of hydrolytic enzymes, such as cellulase, which hydrolyze cell walls (Onguso *et al*. 2004).
Gibberellic acid (GAS) has been shown to increase fruit set and growth in clementine orange Van Rensburg *et al*. (1996). Choi *et al*. (2002) reported that spraying GA3 increased the fruit size and firmness in cherry fruits. In addition to this El-Sese (2005) working on Balady mandarin trees reported that treatment with GA3 increased the yield of fruits. GA3 increased fruit firmness, soluble solids, nutrient content, and fruit weight (Basak, *et al*. 1998).
Very little scientific information is available and known about the growth and development of water apple fruit. A search in the Thomson-Reuters and Scopus database revealed only a few articles reporting on its chemical constituents as cited above. The following objectives were undertaken:

1. To investigate the effectiveness of swabbing method using plant growth regulator.
2. To investigate the pigment (anthocyanin) phenol, flavonoid and biochemical characteristics of the fruits.

Materials and Methods
Study area
This study was carried out in a private orchard located at a commercial farm in Banting, Malaysia, 20 30N, 1120 30E and 1028 N, 1110 20 E at an elevation of about 147 ft from sea level.

Plant Materials
Twelve years old water apple trees (wax jambu) were selected for the study. The trees were spaced at 20.25 m2 (square pattern). Tree to tree distance was 4.5 m and row to row distance was 4.5 m. 12 trees were used in the study. Three trees were used for each treatment. Five branches from each tree were used for each unit.

All the insects and diseases infected branches were removed before the experiment launched. Sixty uniform branches of the same length, diameter and number of leaves were maintained from the twelve trees for the experiment.

Tree Management

Field was maintained properly and irrigation was done when necessary. Pesticides were applied once at growing season. Weeding was done at one month interval. Plant hormone was applied in the sunny day. Fertilizer was applied at the rate of 15-15-15% (N-P-K) yearly (Hossain *et al*., 2004).

Design of experiment

The five selected uniform branches n swabbed with 30, 60 and 90 mg/L GA3 and water (control) in three plants. Five branches were considered as replication per tree, total 60 branches. 15 fruits were selected in each branch to make swabbing instate of spray. Total number of fruit was 15×15=225 per treatments [n= (10×15) for fruit and n=15 for branch]. The design used in the experiment was Completely Rendomized Design (CRD). The swabbing method was applied to the branches once a week starting from bud formation stage to flower opening stage (blooming) and continued until fruit set stage.

Swabbing technique

In this work we have applied a new technique called swabbing (Figure 1). This method consists to swab PBRs with wetting cotton and forceps without any contamination of fruits. This method was applied successfully followed by Hossain *et al*. (2007), where aqueous solutions of growth iregulator were applied by swabbing two-to-three times with cotton wool held with forceps.

Bud stage initial Bud stage final Flower stage

Fig 1: Swabbing, by cotton applied, at bud flower and flower blooming stage of water apple, by GA3

Chlorophyll content (Represented by SPAD unit)

Chlorophyll content in leaves of treatment branches was determined using a Minolta SPAD meter and measured usually after 1.5 month of treatment application. SPAD value of the leaves were expressed the chlorophyll content.

Measurement of biochemical parameters

Fruit grinding (Collection of fruit juice)

Three fruit were selected randomly from each branch. Total of 3×15=45 fruits were ground separately for each treatments. Total of 180 fruits (4×45) were used for 4 treatments. Fruit was cut into pieces and blender machine was used for grinding. The juice was centrifuged and supernatant (Clear juice) was collected and it was placed in airtight glass bottles, stored in an ice filled cooler and transported to the laboratory to keep at cold temperature (4±1 °C) for biochemical analysis.

Total phenols

The total phenolic content of water apple fruits were determined by using the Folin-Ciocalteu assay (Singleton and Rossi, 1965). Folin-Ciocalteau (FC) colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The intensity of light absorption at that wavelength is proportional to the concentration of phenols.1ml of fruit juice, gallic acid calibration standards, folin-Ciocalteau (FC) reagent stored in the dark and discarded if reagent becomes visibly green, Sodium carbonate solution, 100-ml were used volumetric flask. Spectrophotometer was set to 765 nm, with 1-cm, 2-ml plastic or glass cuvettes. 1ml of fruit extract was added to 25 ml of volumetric flax, containing 9 ml of distilled water. A reagent blank also prepared. 1 ml of Folin –Ciacalteu's phenol reagent was also added to the mixture. The solution was diluated with distilled water and mix. Incubation at room temperature at room temperature. The absorbance against reagent blank was determined at 750 nm with an UV-Vis Spectrophotometer Lambda 5. And expressed at as mg gallic acid equivalent. GAE/ 100g fresh weight.

Total flavonoid

Total flavonoid content was measured by the aluminum chloride colorimetric assay (Zhishen *et al*., 1999). An aliquot (1 ml) of extracts (0.5 g dried shredded peel in 50 ml 80% aqueous MeOH) or standards solution of quercetin (3, 6, 14 mg/ml) was added to 10 ml volumetric flask containing 4 ml dd H2O. To the flask 0.3 ml 5% NaNO2 was added. After 5 min, 0.3 ml 10% AlCl3 was added. At the 6th min, 2 ml 1M NaOH solution was added and the total volume was made up to 10 ml with dd H2O. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content was expressed as mg Catechin equivalents (CE) / 100 g fresh mass. Samples were analyzed in triplicates.

Total anthocyanin content

Total anthocyanin contents of the hydrophilic extracts were measured by the pH-differential method described by Rodriguez-Saona *et al*. (2001). The matured water apples were harvested and the crude extract was prepared in the following manner. The water apple was washed thoroughly with distilled water at room

temperature (27 ± 2 °C). The outer layer of water apple (skin, containing color) was removed manually with the help of peeler. The pigment from peels was extracted with water using food processor (Singer, FP-450). The pigment extract was filtered to remove the fibrous particles and then it was centrifuged at 10,000 rpm for 5–10 min to remove the tiny suspended solid particles. The color extract was then stored at 4–5 °C in the refrigerator and used for the experiments. The anthocyanin content was determined from pH-differential method using the following equation (Ronald *et al*., 1982 and Rodriguez-Saona *et al*., 2001). Development and process optimization of water apple concentration extract as potential natural red colorant,

$$\text{Anthocyanin content (mg/L)} = \frac{A \times \text{Mw} \times \text{DF} \times 10^3}{\varepsilon \times L}$$

Where, A = A510 (pH 1.0) – A510 (pH 4.5), Mw is the molecular weight of anthocyanin (433.2 g mol−1), DF is the dilution factor, ε is the extinction coefficient (31,600 L cm−1 mol−1) and L is the path length (1 cm). It was employed by coupling reaction of 2, 4-dinitrophenyl hydrazine dye with vitamin C and followed by spectrophotometric determination.

K+ content

Fruit juice was taken for K+ determination from each treatment. Then 3 to 5 drops of the supernatant liquid of centrifuged juice (4000 rpm for 10 min) were dropped onto the calibrated sensor pad (Cardy Potassium Meter, Model-2400, USA), on a sampling paper placed on the sensor. The reading in ppm was taken from the display pad after it stabilized (30 to 43 sec).

Statistical analysis

The data were plotted and analyzed using MSTAT statistical software. Least significant difference (Fisher's protected LSD) was calculated, following significant F-test (p=0.05).

Results

The influence of treatments on maturity development was observed throughout the experiments (Table 1). All concentrations were able to enhance the color associated component with respect to experimental periods. The photosynthetic pigment, chlorophyll (SPAD) showed a significant difference with respect to the applied hormone treatments. The accumulation of chlorophyll was significantly higher in plants which underwent in GA3 application than control (Fig. 1). The lowest amount of chlorophyll was observed in the control treatment. Hence, it was visualized that 60 ppm GA3 was the optimum rate for water apple leaves to maintain the highest chlorophyll content.

Table 1: Fruits maturely development by color (%) in different GA3 treatments. Values are means ± S.E. (Different alphabets mark significant differences, P < 0.05)

Treatments (ppm)	Maturely development by color (%)
Control	88.3±0.04d
GA 30	94.3±0.03b
GA 60	98±0.09a
GA 90	93±0.01bc

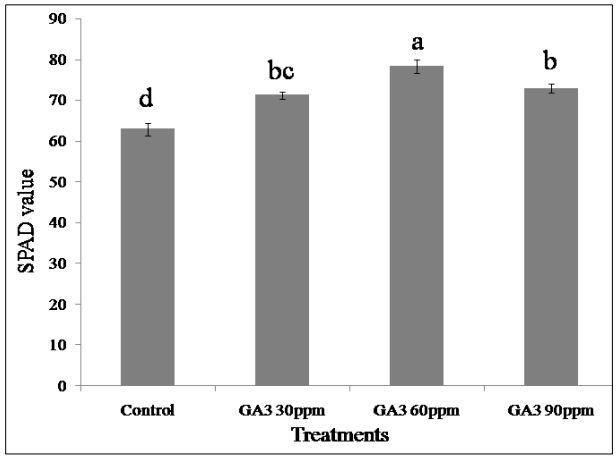

Fig 1: The leaf chlorophyll or SPAD value of leaves in different treated branches

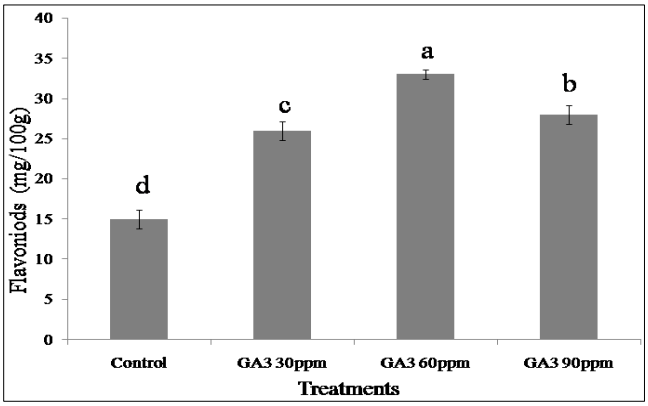

Fig 2: Flavonoid content as affected by different treatments applied to water apple fruit

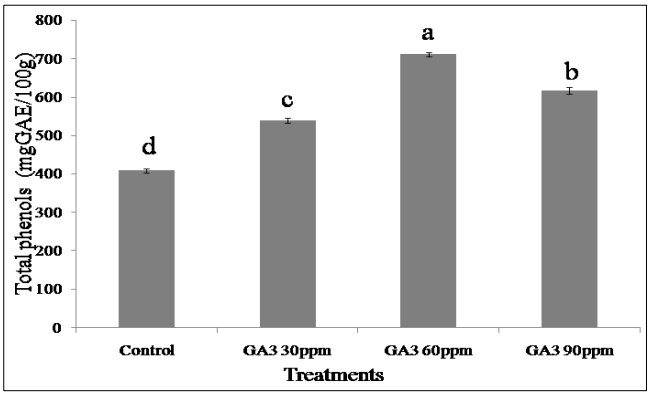

Fig 3: Total phenol content in different treatments

The most effective concentration to earlier maturity of water apple fruit was 60 ppm of GA3. In the case of flavonoid, lower content was observed in control, 30 and 90 ppm GA3 than 60 ppm GA3 concentration (Fig 2). However, the most effective concentration for flavonoid content was in the 60 ppm GA3. In contrast, the maximum anthocyanin content was observed in 60 ppm GA3 and the minimum was observed in water control (Table 3). The results showed that total flavonoid, and total phenolic compounds were significantly increased by GA3 treatment (Fig.

2 and Fig. 3). It was found that total phenolic content also followed the same trend as total flavonoid and anthocyanin content in case of all treatments. It was clear that 60 ppm GA3 had a positive effect on anthocyanin and maturity colour improvement compared to 30 and 90 ppm GA3. Fruit K+ content was significantly increased by GA3 treatments, especially at 60 ppm (Fig. 4). However, no significant differences were found between K+ content at 30 and 90. Thus, there were differences in sensitivity of fruits to GA3 at various fruit development stages.

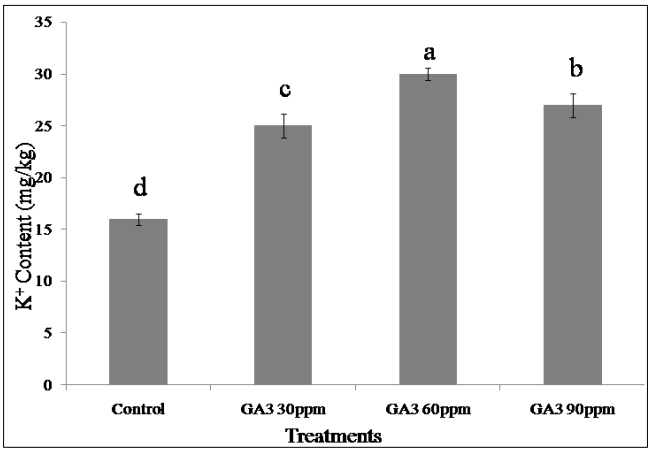

Fig 4: K+ content of water apple fruit as affected by different treatments

Anthocyanin compounds were increasing trend while increasing the maturity development. Positive or highly correlation was made between them (Fig. 5). Fig. 6 shows the fruit maturity color developmental of water apple fruits.

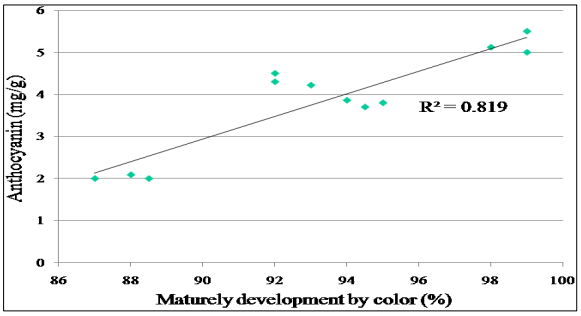

Fig 5: Correlation between fruits maturely development by color (%) and anthocyanin

Fig 6: Photograph shows the fruit maturity color developmental of water apple fruits

Discussion

This research is focused on offering a wide view about physiological parameter as well as chemical analysis and the effects related to plant hormone. A new hormone application method (Swabbing technique) has been introduced under natural sunlight growing condition.

The transition from vegetative to reproductive growth is a critical event in the life cycle of plants. Plant hormones play an integral role in controlling the growth, development, metabolism and morphogenesis of higher plants (Claus, 2008) [5]. Flavonoids are the most important plant pigments for flower and fruit coloration producing yellow or red/blue pigmentation in petals and fruits skin. An important role of flavonoids is to serve as visual signals for animals in attracting pollinators in flowers, and later for animals eating the fruits and thereby helping in seed dispersal. In fruits, flavonoids may contribute in a number of ways to fruit quality, for instance to traits such as color, flavor, and bitterness or texture (Amiot *et al*. 1997) [1]. The composition of flavonoids in different fruit species varies greatly. Anthocyanins are pigments that give most fruits their red, violet and blue color. In addition, environmental factors such as nutrients, temperature and light conditions can have an effect on flavonoid composition and on the final hue of the fruit. In addition, phenolic component, as well as other molecules, such as purines, has the ability to function as co-pigments. Also, the temperature and pH of the vacuolar solution may affect the final color (Brouillard & Dangles 1994) [2]. The change in color in these cultivar mutants might be due to mutations in structural or regulatory genes involved in anthocyanin biosynthesis (Fig. 10).

Potassium (K+) is importance for its role of an activator of many enzymes and to regulate osmotic potential in cell (Bussakorn *et al*., 2003) [3]. Known as a 'quality element', K+ could increase fruit development of apple by enhancing synthesis and translocation of carbohydrates in plants (Han *et al*., 1995) [7], citrus (Chen *et al*., 2000) [4]. Generally, application of plant hormones could increase both fruit setting rate and content of the soluble solids, sugars (Zhang *et al*., 1998; Huang *et al*., 2000; Gao *et al*., 2001) [14, 9, 6].

In this experiment following GA3 application, however, it was found that fruit K+ was significantly increased. Niu *et al*., (2008) [11] reported that at early stage, it is required for sufficiently large molecular substrates like carbohydrates and fruit tissues such as peel and seeds to develop its normal cell division and cell enlargement. As fruit species with high demand for K+, nutrient contents in grape fruits have an important effect on their quality. Niu *et al*., (2008) [11] reported that the application of GA3 to grape fruits enhanced K+ and fruit growth as well as the endogenous hormones (IAA) during fruit growth and development. This result was also attributed to growth acceleration by the GA hormone (Chen *et al*., 2000; Ma and Liu, 1998; Huang *et al*., 2002) [4, 10], which enhanced both the enlargement of grape fruits and sink capacity of grape fluster to absorb water or nutrients, such as K+.

Conclusion

It can be conclude dthat K+ was increased and related with color content. Gibberellin acid (60 ppm GA3) improved the quality of water apple fruit by increasing total phenol, flavonoid and anthocyanin content of water apple fruit.

References

1. Amiot MJ, Fleuriet A, Cheynier V, Nicolas J. Phenolic compounds and oxidative mechanism in fruits and vegetables. In Thomas-Barberan F (ed) Phytochemistry of Fruits and Vegetables. Oxford University Press, New York, 1997, p51-85.
2. Brouillard R, Dangles O. Flavonoids and flower colour. In: Harborne JB (eds) The Flavonoids: Advances in Research since 1986. Chapman and Hall, London, 1994, p565-588.
3. Bussakorn SM, Daniel PS, Michael TT, Mark RT. A review of potassium in grapevines with special emphasis on berry accumulation. Aust. J. Grape Wine Res. 2003; 9:154-168.
4. Chen SY, Peng YJ, Yang ZY. Study of balanced applying fertilizer of nitrogen, phosphorus and potassium on improving fruit quality at the rapidswelling stage of citrus fruit. Guangxi Hortic. 2000; 4:3-4.
5. Claus S. Understanding gibberellic acid signaling- are we there yet. Current Opinion in Plant Biology. 2008; 11:9-15.
6. Gao JF, Sun HB, Zhao ZK, Chen XB. Effects of gibberellin, borax and diahydro potassium phosphate (KH2PO4) on fruit developing and quality of grape. Northern Hortic. 2001; 1:22–23.
7. Han ZH, Liu S, Zhang XA, Wang Q. Micro-girdling—a new method on studying the entering path of fruit nutrition. China Association for Science and Technology. In: Second Session of Youth Academic Annual Meeting Horticultural Collection, Beijing Agricultural University Press, Beijing, 1995, p45-48.
8. Hossain ABMS, Fusao M. Determination of abscisic acid hormone (ABA), mineral content, and distribution pattern of 13C photoassimilates in bark-ringed young peach trees. Mj. Int. J. Sci. Tech. 2008; 2:274-284.
9. Huang XG, Wang Q, Zhao TC. Effect of potassium fertilizersfor improving quality and production of fruit crops. J. Fruit Sci. 2000; 17(4):309–313.
10. Ma HP, Liu ZM. Gibberellins and fruittree development. Chin. Bull. Bot. 1998; 15(1):27–36.
11. Niu Z, Xu X, Wang Y, Li T, Kong J, Han Z. Effects of leaf-applied potassium, gibberellin and source–sink ratio on potassium absorption and distribution in grape fruits. Scientia Horticulturae. 2008; 115:164-167.
12. Sasaki H, Yano T, Yamasaki A. Reduction of high temperature inhibition in tomato fruit set by plant growth regulators. JARQ. 2005; 39:135-138.
13. Sharma RR, Room S. Gibberellic acid influences the production of malformed and button berries, and fruit yield and quality in strawberry (Fragaria × ananassa Duch.). Scientia Horticulturae. 2009; 119:430-433.
14. Zhang SM, Yan H, Liu SH, Yan XS. Effect of compound fertilizer of potash and magnesium on winegrape yield and quality. Viticult. Enol. 1998; 2:7–9.