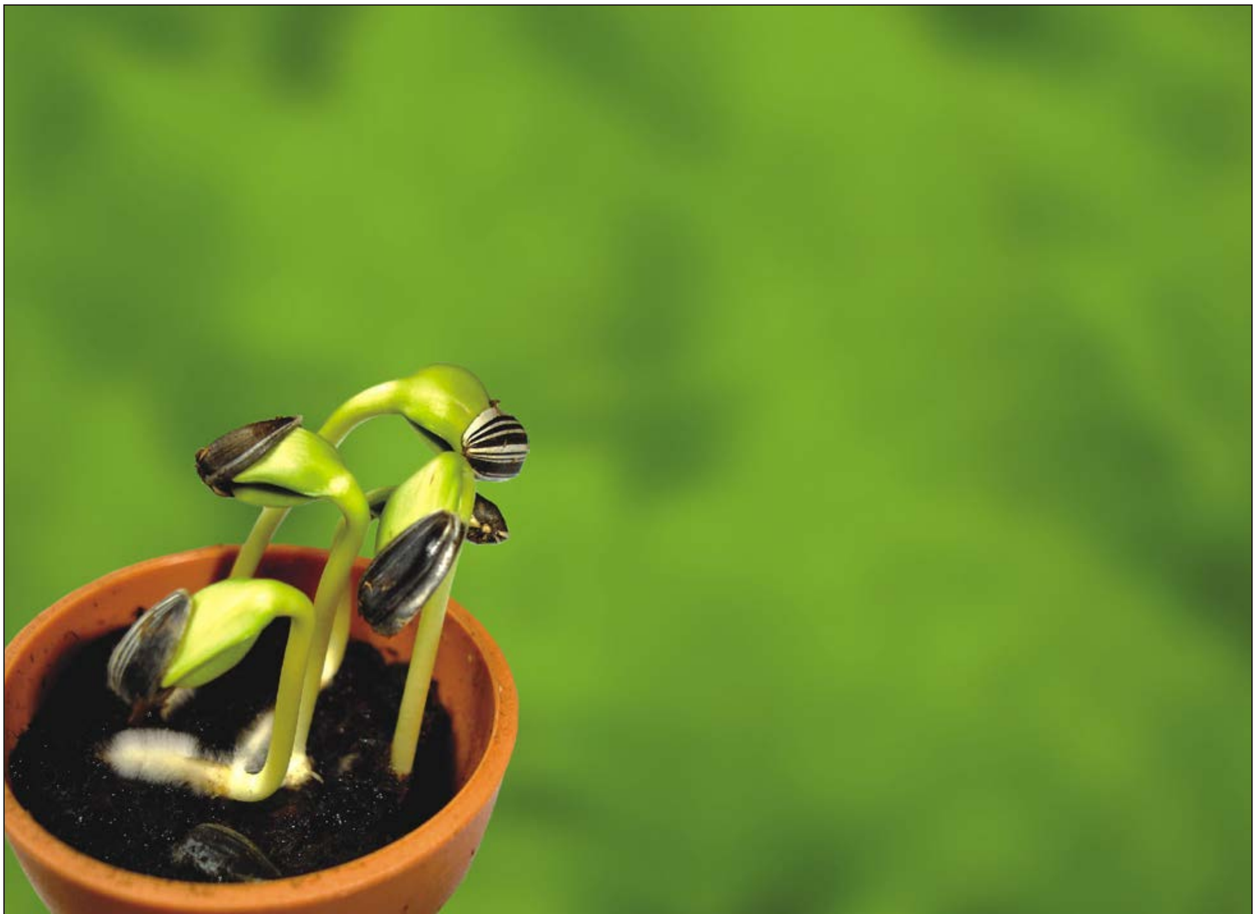

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Research Article

Development of Seedless Star Fruit and its Antioxidant, Biochemical Content and Nutritional Quality by Gibberellic Acid Hormone as Genetically Modified Component

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Abstract

Star fruit is one of the most important fruit that meets the requirements of healthy nutrition. It is a tasty and valuable fruit crop, containing a lot of biologically active substances and significant for dietary qualities. Plant hormone keeps a significant role to enlarge the fruit size and quality development. Fruit growers have a lot of interest in making fruit enlargement and shorten maturity to harvest fruit earlier and to make it marketable soon. The study was carried out to investigate the seedless effect of GA3 150 ppm on star fruit and biochemical and nutritional quality by applying dipping method. Gibberellic acid (GA3) and bark stress play a significant role in fruit enlargement and development. It had been found that the GA3 150 ppm showed bigger sized fruit and made 75% seedless (aborted seed) as compared to the bark stress and control. Fruit/bunch was higher at GA3 150 ppm as well as lower fruit drop percent compared to other treatments. Glucose, inverted sugar, fructose and pH were found higher in the distal end of the fruit than in the middle and proximal end of the fruit. Total Soluble Solid (TSS) content was higher in GA3 150 ppm and bark stress treated fruit than the control. Moreover, antioxidant as vitamin c and polyphenol content were higher in GA3 150 ppm and bark stress treated fruit than the control. The results showed that GA3 150 ppm using dipped technique was the best for seedless fruit production, enlargement of fruit size and improve the better biochemical content.

Key words: Seedless star fruit, nutrient and biochemical content, GA3, bark stress

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Star fruit [Carambola] (*Averrhoa carambola* L.) is a tropical tree fruit with a distinctive star shape in cross-section. The fruit is a native of Asia, but was introduced in Florida more than a century ago and is a very popular fruit in many parts of the world (Campbell, 1987; Campbell *et al.*, 1985; Crane, 1993). It is grown for edible as well as medicinal fruits. Star fruit and its juice is often recommended in many folk medicine in Brazil as a diuretic (to increase urine output), expectorant and to suppress cough (Umesh, 2009). Star fruit is rich in antioxidant phytonutrient polyphenolic flavonoids. Some of the important flavonoids present are quercetin, epicatechin and gallic acid. Total polyphenol contents (Folin assay) in this fruit is 143 mg/100 g. Altogether, these compounds is helpful to protect from deleterious effects of oxygen derived free radicals by warding them off the body (Umesh, 2009). In Indonesia, it is added in some dishes, for substituting tamarind or tomato. Besides, the fruit can be preserved, which reduces its acidity. It can be replaced by mango in making chutney. In Malaysia, it also used for making jam which is rather sweet. It is used for making pickles (Hossain *et al.*, 2008). It was reported that the fruit contains high levels of oxalate. Due to tubular necrosis, acute renal failure caused by oxalate has been recorded in several people and made treatment hypercholesterolemia by the concentrated juice (Bakul *et al.*, 2013). Plant hormones (auxin, gibberellic acid and cytokinins) used in order to promotes the growth of fruits (Hossain *et al.*, 2008; Secer, 1989). Gibberellic acid (GA3) is an important growth regulator that may have many uses to modify the growth and yield contributing of plant (Secer, 1989). Gibberellic acid (GA3) includes a large range of chemicals that are produced naturally within plants. Gibberellin plays a major role in seed germination, affecting enzyme production that mobilizes food production that new cells need for growth. This is done by modulating chromosomal transcription. Gibberellic acid (GA3) causes the remarkable elongation has been reported by several workers (Saifuddin *et al.*, 2009; Kkandaker *et al.*, 2011). Gibberellin promotes the plants growth. Stimulation of cambial growth in gibberellin has been reported in shoots (Hossain *et al.*, 2007). The obvious effect of GA3 had shown the increased in the number of internodes (Thompson and Guttridge, 1959). Some remarkable growth stimulatory effects of gibberellin (GB) on dwarf type plants have been reported (Brian *et al.*, 1954; Phinney, 1956). While the hyperelongation of internodes and increase in fresh weight and dry matter are easily identified, little if any attention has been directed to the effects on the growth and development of carambola. The potential offered bv these substances in control of plant growth may not only be useful in promoting greater.

Maximum number of flowers in a hibiscus flower was obtained by spraying the GA3 at specific concentration (Saifuddin *et al.*, 2009). Hossain *et al.* (2006a) reported that fruit quality might improve by different stresses like phloem stress in bark, temperature, heat and thinning, etc. Hossain *et al.* (2007) reported that swabbing techniques other than spray was better, environmentally safe and cost effective method. Partial ringing as represented by partial ringing is a horticultural practice used to manipulate tree growth, development and fruit growth in a variety of fruit species. Small, compact, dwarfed or size controlled fruit trees provide for easier pruning, thinning, spraying, harvesting, high production of high-grade fruit and lower cost of production (Tukey, 1964; Hossain *et al.*, 2006b, 2007). There were a few literatures found in this regard in different fruit, but no literature found for star fruit. That is why the objectives of this research work were undertaken to study the effect of gibberellic acid on the production of nutritional seedless star fruit and investigate the new information on antioxidant, biochemical content and nutritional quality as health benefits.

MATERIALS AND METHODS

This research work was carried out in the Institute of Biological Sciences orchard in the University of Malaya, Kuala Lumpur, Malaysia and Department of Biology field and laboratory, Hail University, KSA.

Plant materials: The experiment was conducted from April to September, 2011 and 2012. Ten year-old four star fruit trees were selected in the University of Malaya Botanical garden, Kuala Lumpur. Then, 5 branches of each tree were selected.

Treatment setting: The GA3 150 ppm, bark stress and water control (control) were applied as treatments. Five branches were used for each treatment in each tree.

GA3 150 ppm application: Five branches were used for GA3 150 ppm treatment in one tree by which flower bud were dipped at 3-4 times (twice per week until 2 weeks) with GA3 (150 ppm) during the growing season of the flower bud formation.

Bark or phloem stress: Bark stress was employed at the base of the branches by removing bark except leaving I-shape bark (1.5 cm of length remaining 2 mm connecting strips) junction with lower to upper level of shoot.

Each selected branches were then marked for the identification. Before harvested, the fruit diameter, fruit length,

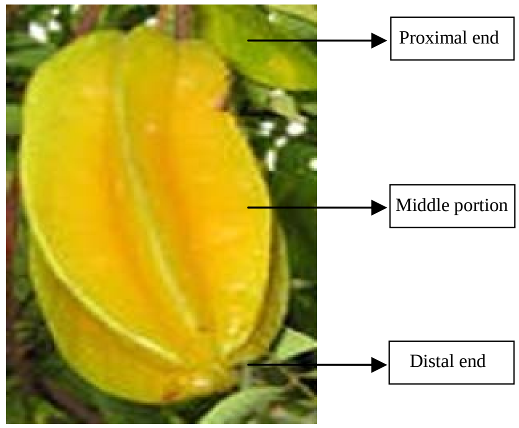

Fig. 1: Different portions of *Averrhoa carambola* fruit

number of fruits drop and set finally were measured during the harvesting. After six weeks, the fruits were harvested.

Measurement the fruit: The fruit length and diameter was measured using the vernear scale.

Juice collection: Fruit juice was collected manually using hand immediately after harvest using hand threaser and also cheesecloth and preserve in the freezer to determine the TSS and pH. Fruit juices were collected according to each portion of the fruits like proximal end, middle portion and distal end (Fig. 1). Then, it was kept in the vials and then stored in the refrigerator.

Total Soluble Solids (TSS) determination: Total Soluble Solid (TSS) content in the *Averrhoa* sp., was determined by using an Atago refractometer (Japan) by placing a drop of fruit juice on its prism. The percentage of TSS was obtained from direct reading of the refractometer. In order to obtain the accurate result, the experiment was repeated and the average reading was recorded.

pH measurement: pH was measured using the pH meter. One drop of sample was homogenized in 1 mL of boiled distilled water and 1 mL of de-ionized water of pH 7.0. The pH of star fruit juice was recorded by using an electronic pH meter. The pH meter was standardizing with the help of buffer solution.

Glucose content determination: Glucose was determined by using glucose refractometer. Three drops of juice sample were put on the disc of the meter and data were displayed and recorded.

Inverted sugar determination: Inverted sugar was determined by using inverted sugar refractometer. Three drops of juice sample were put on the disc of the meter using small syringe dropper and data was displayed and recorded.

Fructose content determination: Fructose content was determined by using fructose refractometer. Three drops of juice sample were put on the disc of the meter and data was displayed and recorded.

Nutrient content determination: Nutrient content potassium (K) and calcium (Ca) were determined by Horiba Scientific potassium meter (Japan). The 2-3 drops of juice sample were put on the disc sensor of the meter using small dropper and data were displayed and recorded.

Vitamin C determination

Starch indicator solution: About 1% of 0.50 g of soluble starch was weighed and added it to 100 mL of boiling water in a 150 mL conical flask. It was stirred to dissolve and cool before using.

Iodine solution: 1-5 g potassium iodide (KI) was dissolved and 0.268 g potassium iodate (KIO_3) in 200 mL of distilled water. Thirty milliliter of 3 M sulfuric acid was added. This solution was poured into a 500 mL graduated cylinder and diluted it to a final volume of 500 mL with distilled water. The solution was mixed. The solution was transferred to a 600 mL beaker.

Vitamin C standard solution: About 0.250 g vitamin C was dissolved in 100 mL distilled water and diluted to 250 mL with distilled water in a volumetric flask.

Standardizing solution: Twenty five milliliter of vitamin C standard solution was added to a 125 mL Erlenmeyer flask and added 10 drops of 1% starch solution. The buret with a small volume of the iodine solution was ringed and then filled it. The initial volume was recorded. The solution was titrated until the endpoint was reached. The final volume of iodine solution was recorded.

For star fruit juice: The homogenate juice was centrifuged at 2,500 rpm for 8 min. Make the extracted solution up to 100 mL with distilled water. The supernatant was used for analysis of ascorbic acid.

Titrating star fruit juice: About 25 mL of juice was added to 125 mL Erlenmeyer flask and then it was titrated until the endpoint reached. Iodine solution was added until a blue-black color was appeared. The titration was repeated at least three measurement that agree within 0.1 mL.

Titration calculations: The milliliter of titrant used for each flask was calculated. The measurements were taken:

$$\text{Average volume} = \frac{\text{Total volume}}{\text{Number of trails}}$$

$$\frac{\text{Iodine solution (mL)}}{\text{Vitamin C (0.250 g)}} = \frac{\text{Iodine solution (mL)}}{\text{Vitamin C (mL)}} = \text{Vitamin C (g) in that sample}$$

Antioxidant (polyphenol) determination: The total polyphenolic content of water apple fruits were determined by using the Folin-Ciocalteu assay. Folin Ciocalteu (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. One milliliter of fruit juice, gallic acid calibration standards, Folin Ciocalteu (FC) reagent stored in the dark and discarded if reagent becomes visibly green, sodium carbonate solution 100 mL was used in volumetric flask. Spectrophotometer was set to 765 nm, with 1 cm, 2 mL plastic or glass cuvettes. One milliliter of fruit extract was added to 25 mL of volumetric flax, containing 9 mL of distilled water. A reagent blank also prepared. One milliliter of Folin-Ciacalteu's phenol reagent was also added to the mixture. The solution was diluted with distilled water and mixed properly the it was incubated at room temperature. The absorbance against reagent blank was determined at 750 nm with an UV-Vis Spectrophotometer Lambda 5 and then expressed at as milligram gallic acid equivalent (GAE/100 g fresh weight).

RESULTS

Physiological data analysis: It has been shown from the Table 1, fruit per bunch and fruit drop percentage were higher in the treated trees than the control tree. The highest fruit per bunch was found in the GA3 treated fruit and drop percentage was the lowest in the GA3 treated fruit and the highest fruit drop percent was found in the water control (Table 1). Fruit length and diameter were higher in the GA3 and bark stress treatment than the water control tree (Table 1). In the Table 2, it has been shown that fruit weight was higher in the GA3 treated fruit than in control. However, seed number, length and per seed weight were higher in the control than the GA3 treated fruit. Moreover, 75% seedless (aborted seed) star fruit was found by the treatment of GA3 compared to the control (Table 2). Results of fruit growth, maturity and color at different treatments are given in Fig. 2.

Biochemical analysis: pH and fructose content were determined from the proximal, middle and distal end of the bilimbi fruit shown in Table 3. The pH content was found higher in treated fruit than the water control fruit in the case of proximal, middle and distal end. It was the highest in the

Table 1: Effects of different treatments on fruit growth and drop percent

Treatments	Fruit/bunch (starting)	Fruit/bunch (during harvest)	Fruit drop (%)	Fruit length (cm)	Fruit diameter (cm)
Control	5	3.0±0.7	40±1.0	8.12±0.05	4.02±0.01
Bark stress	5	3.3±0.6	34±0.6	8.61±0.06	4.32±0.02
GA3	5	3.4±0.7	32±0.6	8.58±0.03	4.972±0.01

Values are given as Mean±SE (n = 5)

Table 2: Fruit weight and seed number measurement

Treatments	Fruit weight (g)	Per seed weight (mg)	Seed number	Seed length (cm)	Seedless (%)
Control	49.1±0.4	2.3±0.2 0	4.0±0.5	0.60	0
Bark stress	51.0±0.4	2.0±0.10	4.0±0.1	0.58	0
GA3	52.2±0.3	1.7±0.01	1.0	0.30	75±0.1

Values are given as Mean±SE (n = 5)

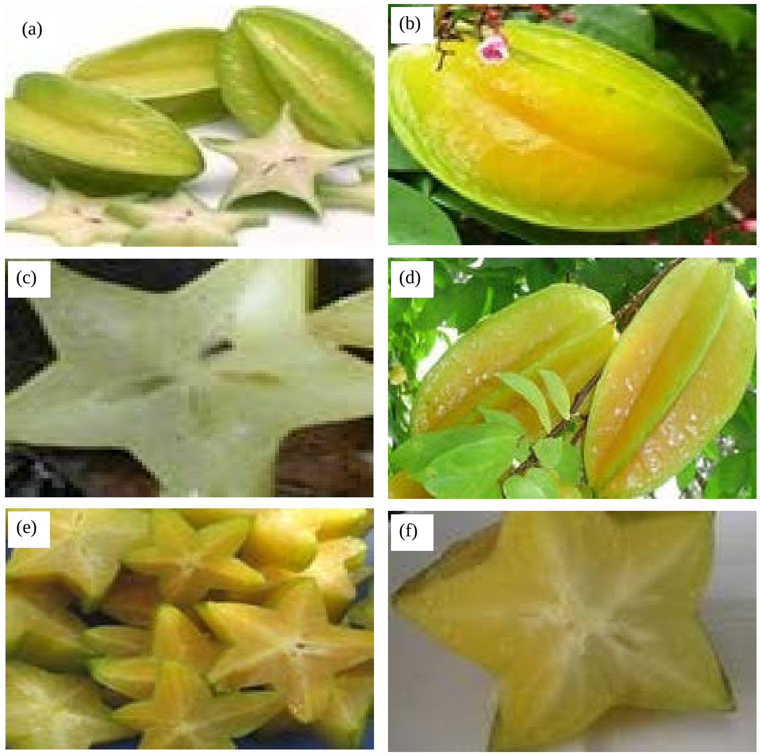

Fig. 2(a-f): Fruit growth, maturity and color at different treatments, (a) Water control, (b) Bark stress, (c) Phloem stress, (d) GA3 150 ppm and (e-f) Seedless fruit by GA3 150 ppm

Table 3: Biochemical content of *Averrhoa carambola* at different treatments

	Proximal end		Middle portion		Distal end	
Treatments	pH	Fructose	pH	Fructose	pH	Fructose
Control	4.7±0.3	8.0±0.4	4.9±0.2	8.3±0.3	4.9±0.1	8.5±0.3
Bark stress	4.8±0.3	9.2±0.3	5.0±0.05	9.5±0.3	5.1±0.1	9.9±0.2
GA3	4.9±0.2	10.0±0.4	5.2±0.2	10.3±0.4	5.5±0.2	10.7±0.2

Values are given as Mean±SE (n = 5)

Table 4: Biochemical content like glucose, inverted sugar and fructose determination

Treatments	Glucose content (%)	Inverted sugar content (%)	TSS (%)
Control	8.0±0.3	5.3±0.2	6.0±0.5
Bark stress	9.0±0.2	6.0±0.1	7.0±0.5
GA3	9.9±0.2	7.8±0.3	7.6±0.5

Values are given as Mean±SE (n = 5), TSS: Total soluble solid

distal end (Table 3). Accordingly, fructose content was found higher in the treated (bark stress and GA3) fruit than the water control fruit in the case of proximal, middle and distal end. It was the highest in the distal end (Table 3). Glucose and inverted sugar content were found higher in the GA3 treated fruit than the bark stress and control (Table 4). It has been shown, Total Soluble Solid (TSS) was higher in the GA3 150 ppm and bark or phloem stress than in water control. The TSS was the highest in GA3 150 ppm.

In Table 5, it has been shown that sodium, potassium and calcium content were higher in the GA3 treated fruit than in control. Antioxidant as vitamin C and polyphenol content were higher in GA_3 150 ppm and bark stress treated fruit than the control (Fig. 3 and 4). Vitamin C was the highest in in the

Table 5: Nutrient content like potassium and calcium content determination

Treatments	Sodium (ppm)	Potassium content (ppm)	Calcium content (ppm)
Control	2±0.01	135.2±0.4	6.1±0.2
Bark stress	3±0.10	140.5±0.3	7.2±0.1
GA3	3±0.20	146.5±0.3	7.5±0.1

Values are given as Mean±SE (n = 5)

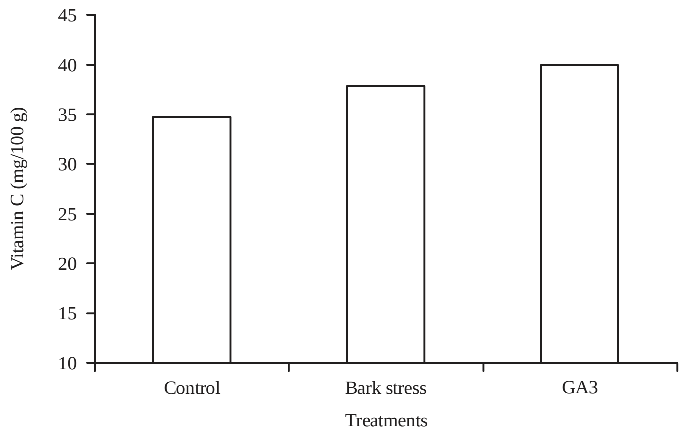

Fig. 3: Vitamin C determination at different treatments. Values are given as Mean±SE (n = 5)

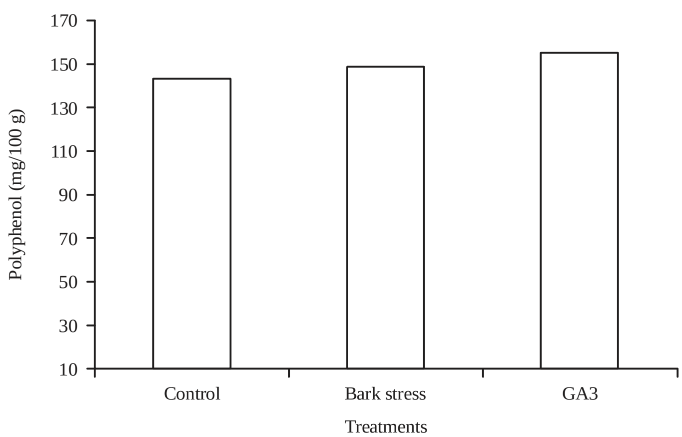

Fig. 4: Polyphenol as antioxidant determination at different treatments. Values are given as Mean±SE (n = 5)

GA3 150 ppm (Fig. 3). In addition to that polyphenol was found 155, 148.5 and 143.5 mg/100 g in GA3 150 ppm, bark stress and water control fruit respectively (Fig. 4).

DISCUSSION

It was possible to make 75% seedless or aborted seeded fruit by genetically modified technology applying gibberellic acid hormone. It might be due to the gene characters had been modified by GA_3. Fruit length and diameter were found higher in the GA3 150 ppm and bark stress. This was due to the effects of bark or phloem stress and GA3. Xylem tissue is responsible for the transportation of water and soluble mineral nutrients from the roots throughout the plant and phloem tissue is responsible to transport carbohydrate (glucose, sucrose) from leaf to shoot and the final to the root (Hossain *et al*., 2006a, 2007). By doing the bark stress or phloem stress treatment to the fruits, the carbohydrate that was not transported from upper part to lower part of the shoot where the treatment was set (Moneruzzaman *et al*., 2010a). Consequently the fruit size become higher compared with the control. For the biological treatment GA3 (one type of plant hormone) was used. It was reported that GA3 influenced the fruit growth and development (Kkandaker *et al*., 2011; Moneruzzaman *et al*., 2010b; Saifuddin *et al*., 2009). Fruit growth is characterized by cell division followed by cell differentiations which were influenced by cytokinins, gibberellins and auxins (Moneruzzaman *et al*., 2010b). Gibberellins can promote cell division and elongation respectively (Wismer, 1994). The increase of fruit weight was attributed to the effect of GA3, acting synergistically increasing fruit diameter and size. Localized application of GA3 to bilimbi is known to increase the sink strength enabling them to attract photoassimilates from the foliage and to develop fully formed fruits (Luckwill, 1981). In this experiment, 50 ppm concentration of GA3 was used. The 150 ppm was chosen because of the previous research, the result showed that 150 ppm concentration had the better effect in terms of other fruits growth and developments (Hossain, 2015). The spraying of GA3 on the *Averrhoa carambola* fruits probably satisfied the initial requirement for fruit. While, for the GA3 treatment, it just promoted the fruit elongation in terms of cell division and cell differentiation (Wismer, 1994). The plants, which are rich in gibberellin, have long internodes. Gibberellins are less sensitive to light compared to auxins and they show less depressive effect in high-dose applications. The gibberellins encourage germination by breaking the dormancy of the seeds within the botanical organs is proportional to the amount of increase in gibberellin. Gibberellins are known to increase the parthenocarpic fruit production like auxins and even they are sometimes more efficient (Secer, 1989; Westwood, 1993; Erio, 1998; Bora and Sarma, 2006). Fruit shape is affected by chemical thinners differently, e.g. gibberellins and some cytokinins increase fruit length and kinetin and auxin do not affect fruit shape (Westwood, 1993).

Phloem stress also made response to fruit size. Jose (1997) found that girdling treatments cause lower vegetative growth in relation to control in mango trees. Arakawa *et al*. (1997) reported that trunk growth of apple trees was significantly increased above the girdling point and reduced below it. Onguso *et al*. (2005) reported that the increase of trunk circumference above the girdle might be caused by swelling of the trunk due to the accumulation of carbohydrates. They also stated that girdling blocks the translocation of sucrose from leaf to root through the phloem bundles. The block decreases the starch content in the root and accumulates sucrose in the leaf (Rose and Smith, 2001).

Total Soluble Solid (TSS) and pH were found higher in middle and distal end than proximal end of the fruit. It is done because every portion in the fruits would have different carbohydrate contents, sugar content as well as different taste. It is due to the different exposure to sunlight. Normally, the middle portion and the distal end exposed more to sunlight compared to the proximal end. Thus, photosynthesis rate occurred rapidly at these portions which led to the higher levels of carbohydrate in the fruits. Brix is a measure of TSS in a given weight of fruit juice. It is often expressed as the percentage of sucrose. However, the sucrose here is actually the sucrose, fructose, vitamins, amino acids, proteins, hormones and other solids. The higher the brix, the better its quality (Hossain *et al*., 2004, 2005, 2009; Onguso *et al*., 2004). Umesh (2009) reported that star fruit contained good quantities of vitamin C. Vitamin C is a powerful natural antioxidant. Hundred gram of fresh fruit provided 34.7 mg or 57% of daily required levels of vitamin C. He also reported that star fruit was rich in antioxidant phyto-nutrient polyphenolic flavonoids. Total polyphenol contented in this fruit was of 143 mg/100 g. This results supports our results.

CONCLUSION

From the result it can be concluded that GA3 150 ppm is the best treatment compared to others showing the 75% seedless as aborted seed, biggest size (length and diameter) and highest TSS and biochemical content of star fruit. In addition, the nutrient like Na, Ca and K was higher in GA3 150 ppm than bark stress and control fruit.

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