HAYATI Journal of Biosciences, March 2009, p 9-14 Vol. 16, No. 1
ISSN: 1978-3019

Physiological Responses and Fruit Retention of Carambola Fruit (*Averrhoa carambola* L.) Induced by 2,4-D and GA3

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Received January 8, 2009/Accepted March 11, 2009

One of the problems in cultivation of carambola fruit is the high of flower and fruit drop during fruit development. To understand these problems and to improve fruit retention, the content of indole-3-acetic acid (IAA) and total sugar in carambola fruit and leaves were analysed in response to application of gibberellic acid (GA3) and 2,4-dichlorophenoxyacetic acid (2,4-D). The experiments used 1,5 year old of carambola plants (*Averrhoa carambola* L. var Dewi) grown in polybag of 40 x 50 cm. GA3 with the concentration of 0, 20, 40, and 60 ppm and 2,4-D of 0, 5, 10, and 15 ppm were applied to the flower and the supporting leaves of carambola plant. The parameters analysed were number of flower drop, fruit formation, fruit retention, number of harvestable fruit per cluster, fruit weight per cluster, the content of sugar in the leaves and IAA in the fruit. The result showed that IAA content of the fruit increased in response to single as well as combination of GA3 and 2,4-D application. Sugar content of the leaves also increased in response to GA3 and 2,4-D application; however, the pattern was different with that of IAA. The best treatment to improve fruit retention was a single application of 10 ppm 2,4-D or 60 ppm GA3, and combined application of 5 ppm 2,4-D and 60 ppm GA3.

Key words: fruit drop, fruit retention, carambola fruit, auxin, gibberellin

INTRODUCTION

Carambola fruit, known as star fruit (*Averrhoa carambola* L.), is an Asian original fruit that is usually consumed freshly as table fruit or used in salads, fruit salads, drinks, and as a garnish (Galan-Sauco 1993; Teixeira *et al*. 2007). This fruit, including its residue extract, is also good source of natural antioxidants (Shui & Leong 2006). Some investigation suggested that it contained many antioxidants such as proanthocyanidins, (-)-epicatechin and vitamin C (Shui & Leong 2004).

One of the problems in star fruit cultivation is the high of flower and fruit drop during fruit development that can reach more than 80% of total flower formation. Consequently, less than 20% of the inflorescence retains and becomes fruit (Samson 1992; Galan-Sauco 1993). Even though this phenomenon is common in many tropical and subtropical fruit such as mangoes (Quintana *et al*. 1984), longan (Choo & Ketsa 1992), and litchi (Stern *et al*. 1995), a systematic effort to reduce flower and fruit drop is still needed to improve star fruit production.

Retention or abscission of flower and fruit are influenced by combination of endogenous as well as environmental factors. Plant hormones such as auxin, gibberellin and ethylene are among the endogenous factors controlling abscission organ, including flowers, and fruits (Srivastava 2002; Taiz & Zeiger 2002). Aneja and Gianfagna (1999) suggested that high ethylene concentration and low auxin and gibberellin concentration in plant become a major cause of fruit drop. Application of gibberellin may reduce flower and fruit drop due to the suppression of ABA biosynthesis (Steffens 1988). In addition, concentration of IAA and GA3 in the pedicles and fruitlet of the falling mango is lower than that in the retention fruit; furthermore, the drop fruit had a high content of ABA (Bain *et al*. 1997). Therefore, an application of exogenous auxin and gibberellins might improve fruit retention. Synthetic auxin can be used to induce fruit retention such as 2,4-Dichlorophenoxyacetic acid (2,4-D). This compound has advantages as auxin due to high activity in low concentration and it is stable from degradation of IAA oxidase enzyme (Salisbury & Ross 1995).

This research were aimed to analyse the content of auxin and total sugar in carambola fruit and leaves in response to application of 2,4-D and GA3, and its implication on fruit development and retention.

MATERIALS AND METHODS

In this experiment, 1.5 year old of carambola plants (*Averrhoa carambola* L. var Dewi) were grown in polybag of 40 x 50 cm. These plants were resulted from grafting system with the scion source of 5-6 years old plant, the collection of Unit of Nursery "AGROTEKO" IPB-Farm. Plants that were used for these experiments already had three flowering periods, hence we expected they achieved establishment of flowering system. Fertilizers used were NPK 15.15.15 and KCl. The hormones used in this experiment were GA3 ($C_{19}H_{22}O_6$) G500 Phyto Technology Laboratories and 2,4-D Schuchardt OHG 85662 Hohenbrunn Germany, with the application of Tween 20 as adhesive agent.

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Preparation and Plant Cultivation. Plants were grown in the 40 x 50 cm polybag. For the experiments, we chose 48 plants that were similar in height, number of branches and flowering time. Plants were collected and arranged randomly in the IPB Farm "AGROTEKO" Darmaga-Bogor. The media used in this experiment was a combination of soil and manure (2:1) with the total of about 12 kg. At the beginning of the experiment, 800 g of manure was added for every polybag, and during the experiment, 150 g of NPK fertilizer was given every month before flowering. After flowering, the plants were fertilized by 150 g of KCl per polybag. To protect the plants from pests and diseases, plants were treated with Culacron (1 ml/5 l of water) every two weeks before fruit formation. After fruit formation, the fruits were wrapped using porous plastic bags to protect them from fruit fly. To obtain uniform flowering before hormone application, the flowers were pruned up to 3 times.

Hormone Application. In this experiment, two hormones (GA3 and 2,4-D) were applied with four different concentrations. The concentrations of GA3 were: 0, 20, 40, and 60 ppm, while 0, 5, 10, and 15 ppm for 2,4-D. The hormone was applied using hand sprayer with two periods of application between 06:00-07:30 a.m.: (i) flower bloom (minimal two blooming flowers) and (ii) after fruits were formed indicated by abscission of the petal of the flower. The applications were given to 10 inflorescences including supporting leaves that were chosen and tagged with the total volume of 25 ml for every inflorescence. To support this application, the solution was added with Tween 20 with the dosage of 2 ml/l.

Observation and Parameters Measurement. During the experiment, the seven parameters were analysed i.e. (i) the number of flower drop, (ii) the fruit formation, (iii) the fruit retention, (iv) the number of harvestable fruit per cluster, (v) the fruit weight per cluster, (vi) sugar total in the leaves, and (vii) the content of IAA in the fruit. The number of fruit formation and drop were recorded every 4 days until harvested. Those were calculated by comparing the number of drop flower and retained fruit with the total flowers and fruits formation.

Analysis of IAA Content of the Fruit. The samples were collected from fruits with uniform size, one week after hormones application. The fruits were cut, and 1 g of samples were frozen in liquid nitrogen and stored in -30 ^{o}C until analysed. The IAA content was analysed using method of Unyayar *et al*. (1996) for extraction and Pattern and Glick (2002) for quantification. After upper phase separation and methanol evaporation, the samples were extracted using 15 ml ethyl acetate for three times. Then, it evaporated and incubated for 1 hour in room temperature. One milliliter of extracted sample was then mixed with 4 ml of Salkowsy solution (Pattern & Glick 2002), prior to measure in spectrophotometer in 510 nm.

Analysis of Leaves Sugar Content. Leaves sugar content was analyzed to evaluate physiological sink activities of leaves during fruit development in response to exogenous hormone application. Total sugar was analysed using Anthrone method modified by Apriyantono *et al*. (1989). The samples were prepared by drying them in oven (80 ^{o}C) for 48 hours. The 200 mg of ground samples were mixed with 15 ml aquadest and two drops of 80% ethanol. The mixture was added with 25 ml of 80% ethanol (60 ^{o}C) and was shaked for 5 minutes. Then the solutions were centrifuged for 15 minutes at 4,000 rpm. The supernatant was evaporated at 80 ^{o}C up to 50 ml. One milliliter of sample, 1 ml of water and 5 ml of Anthrone solution were mixed and kept at water bath (100 ^{o}C) for 12 minutes. Subsequently the solution was cooled and analysed using spectrophotometer at λ = 630 nm.

Data Analysis. Data were analysed using SPSS vers. 15.0 to determine ANOVA followed by Duncan Multiple Range Test (DMRT) at the level a of 5%.

RESULTS

The Pattern of Carambola Fruit Drop. Flower appearance and blooming in the inflorescence occurred in different time (Figure 1). In general, fruit drop occurred from day 2 up to 24 after fruit formation, with the maximum on day 4. Then, the fruit drop decreased up to day 24, after which the drop stopped toward to fruit ripening stage.

IAA Content of Carambola Fruit. The treatment using different concentrations of GA3 and 2,4-D significantly increased IAA content of the carambola fruit. The single effect of GA3 treatment from 20 up to 60 ppm showed positive correlation in improving fruit IAA content (r^2 = 0.99). Application of 60 ppm GA3 increased IAA content up to 212 ppm, which was 58% higher as compared to that of control plant (Figure 2a).

Fruit IAA content increased in response to single application of 2,4-D. The increase of 2,4-D concentration from 0 up to 15 ppm improved fruit IAA content (r^2 = 0.95). At the concentration of 15 ppm of 2,4-D, IAA content increased up to 227 ppm, which was 84% higher as compared to the control plants (Figure 2b).

The combination of GA3 and 2,4-D treatments significantly affected IAA content of the carambola fruit as well. There was a synergic effect of GA3 and 2,4-D on IAA content. The maximum IAA content was resulted from the combination of 15 ppm of 2,4-D and 60 ppm of GA3 which improved IAA content of the fruit up to 108% as compared to the control plants (Figure 3).

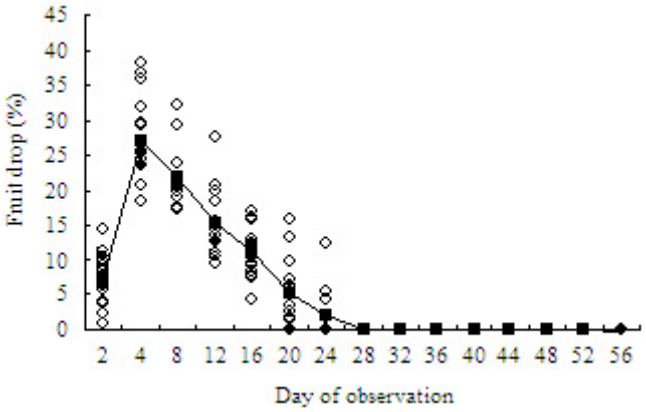

Figure 1. The pattern of carambola fruit drop during the beginning of fruit development. Open circles are cumulative data; solid rectangles connected by line are average data.

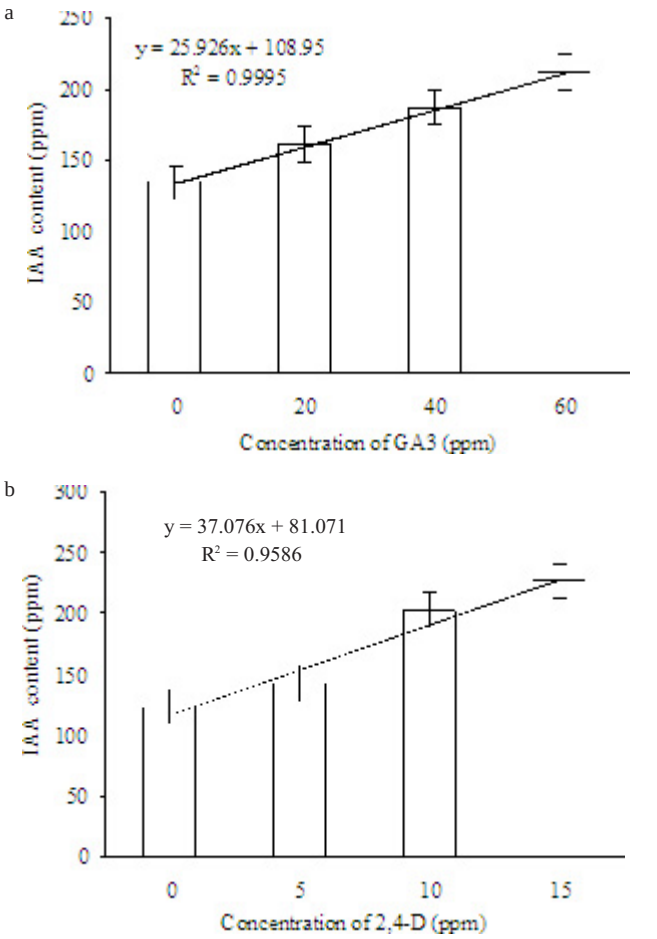

Figure 2. IAA content in carambola fruit in response to application of GA3 (a) and 2,4-D (b) with different concentration. Bar lines indicate standard error.

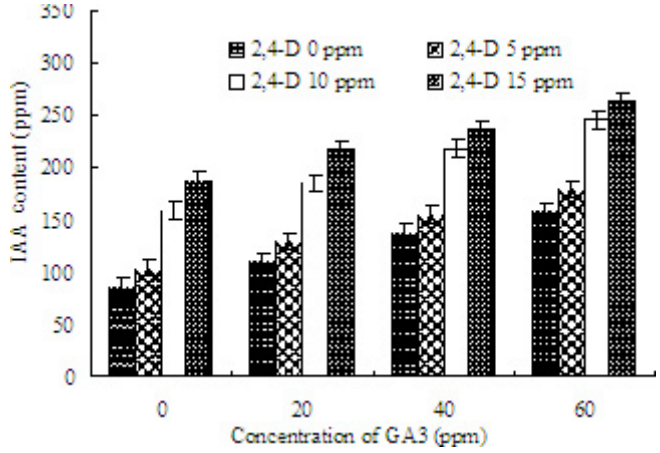

Figure 3. IAA content in carambola fruit in response to interactive application of GA3 and 2,4-D with different concentration. Bar line indicate standard error.

Total Sugar Content of Carambola Leaves. A single application of GA3 and 2,4-D, as well as their combination had significant effect on total sugar content of carambola leaves (Figure 4). The figure showed that application of GA3 especially with concentration of 40 and 60 ppm improved total sugar. The maximum total sugar content was 12.10 mg/g leaf dry weight due to the application of 60 ppm GA3, while 10.96 mg/g leaf dry weight in the leaves without GA3 application (Figure 4).

Application of 2,4-D had different effect than GA3 on total sugar content. Higher concentration of 2,4-D resulted in reduction of total sugar content of carambola leaves. Although at the concentration of 5 ppm, 2,4-D tended to increase sugar content as compared to 0 ppm (Figure 4). The maximum sugar content was achieved by application of 5 ppm, while the minimum sugar content was obtained by application of 15 ppm of 2,4-D.

The interaction effect of GA3 and 2,4-D application showed that there was a different effect of those hormones on sugar content of carambola leaves. The highest sugar content was resulted in the application of 60 ppm GA3 without 2,4-D as well as application of 2,4-D 10 ppm without GA3 (Figure 5).

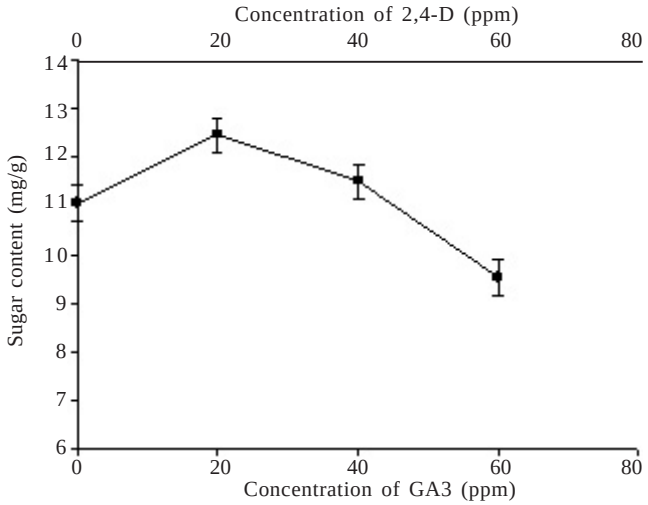

Figure 4. Sugar content in carambola leaves in response to single effect of GA3 (open circles) and 2,4-D (closed circles) with different concentration. Bar line indicate error standard.

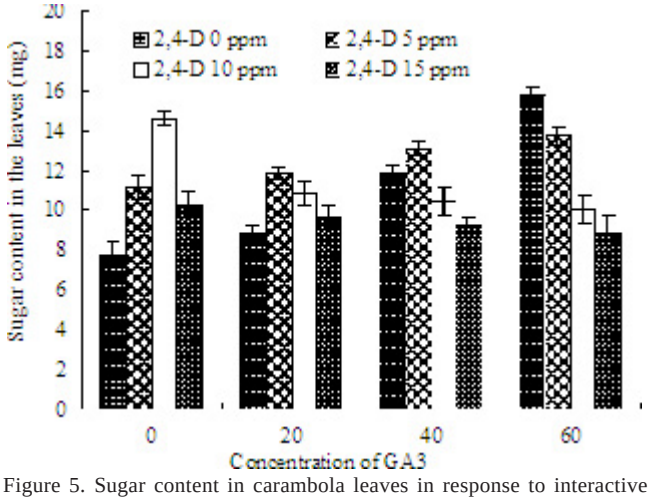

Figure 5. Sugar content in carambola leaves in response to interactive application of GA3 and 2,4-D with different concentration. Bar line indicate standard error.

Effect of GA3 and 2,4-D on Carambola Fruit Drop and Retention. There was an interaction effect of GA3 and 2,4-D application on percentage of fruit drop in carambola fruit. The plants that were not treated by GA3 and 2,4-D (control plants) had the maximum fruit drop (81.9%). On the other hand, the treatment with single or combination of both hormones significantly decreased flower drop (Figure 6). In the absent of 2,4-D, the increase of GA3 concentration was able to reduce fruit drop of carambola fruit dramatically. The reduction of fruit drop was occured by the increase of GA3 concentration in combination with 5 ppm of 2,4-D as well. However, under the higher concentration of 2,4-D (10 and 15 ppm), the increase of GA3 concentration caused an increase of fruit drop (Figure 6).

Our result showed three kind of treatments that were able to reduce fruit drop effectively i.e.: single application of GA3 60 ppm, single application of 2,4-D 10 ppm, and combination of 2,4-D 5 ppm with GA3 60 ppm. These three treatments had the fruit drop of 44.6, 47.4, and 48.0%, respectively, and were not significantly different (Figure 6).

In the same way, interaction of GA3 and 2,4-D also significantly ($P < 0.000$) influenced fruit retention of carambola plant indicated by the number and weight of fruit per cluster (Figure 7). Application of GA3 from 20 ppm until 60 ppm without any addition of 2,4-D hormone dramatically increased fruit weight of carambola per cluster. However, in the combination with 2,4-D especially with the concentration of 10 and 15 ppm, an increase of GA3 concentration tended to reduce on fruit weight per cluster (Figure 7).

The best treatment that was able to improve fruit weight per cluster was a single application of 10 ppm 2,4-D, combined application of 60 ppm GA3 and 5 ppm 2,4-D, or single application of 60 ppm GA3. The fruit weight per cluster produced by these treatments were 710, 691, and 670 g respectively, which were not significantly different ($P < 0.000$) (Figure 7).

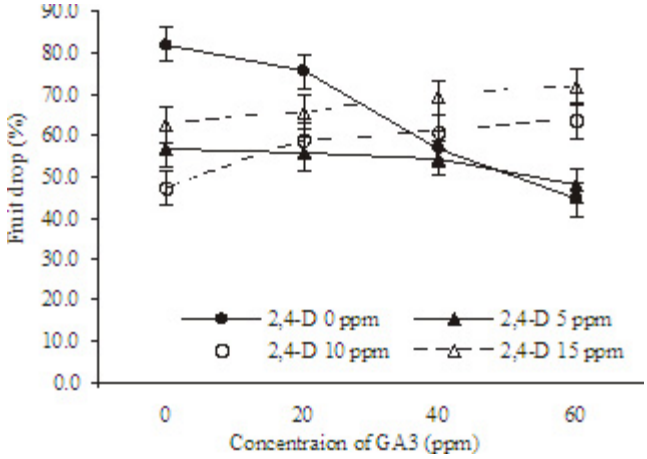

Figure 6. Percentage of fruit drop in response to interactive application of GA3 and 2,4-D with different concentration. Bar line indicate standard error.

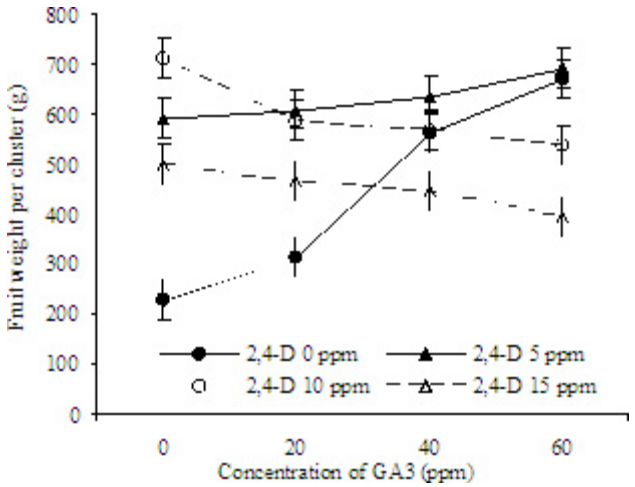

Figure 7. Carambola fruit weight per cluster in response to interactive application of GA3 and 2,4-D with different concentration. Bar line indicate standard error.

DISCUSSION

Fruit Drop and Hormone Activity in Carambola Fruit. A high fruit drop or abscission is one of major constraints in cultivation of carambola fruit. This phenomenon is also occured in many other tropical and subtropical fruits. In lychee fruit for example, the fruit drop might be reach 90-95% Stern *et al*. (1995). Therefore, some attempt to evaluate physiological factors to suppress the flower and fruit drop is very important to improve fruit production especially carambola fruit in Indonesia.

Based on carambola fruit drop pattern (Figure 1), we found that it was a typical drop pattern of common fruit such as mangoes and lychee. The highest drop level of mangoes fruit occurred at the first week of fruit formation, and then the drop level decreased until 7 weeks. On mangoes fruit, the pattern was almost the same with that showed in Figure 1, and the drop sometimes reached up to 90% of total flower (Quintana *et al*. 1984).

Flower and fruit abscission are usually due to high level of ethylene in the flower and fruit (Malik *et al*. 2003; Beno-Moualem *et al*. 2004), and the lower concentration of auxin and gibberellin (Aneja & Gianfagna 1999; Malik & Singh 2006). The auxin is an important hormone to support growth and development of the fruit (Srivastava 2002). The auxin source, at the beginning of fruit growth is from the endosperm, and at the following growth, the source is from seed embryo (Aneja & Gianfagna 1999; Srivastava 2002).

The high level of fruit drop at the first week of fruit formation is possibly due to the formation of growth promoting hormone especially auxin that was in low concentration. Consequently, this hormone level was not enough to keep abscission zone not sensitive to ethylene. The sensitivity of abscission zone to ethylene will induce gene that is able to produce hydrolytic enzymes that cause degradation of cell wall in the abscission zone, and finally the fruit drop (Taiz & Zeiger 2002).

The Increase of Auxin and Sugar Content in Response to Hormone Applications, and its Implication on Carambola Fruit Retention. The data of IAA content in the fruit show that this endogenous auxin increased significantly in response to GA3 or 2,4-D either in combination or solitarily with the highest IAA content exhibited by the plants with application of 60 ppm GA3 in combination with 15 ppm 2,4-D (Figures 2 & 3). This data showed that exogenous application of auxin and gibberellins had positive response on endogenous hormone indicated by increased IAA content. This response is important because endogenous hormone level especially IAA is essential to support fruit retention (Bangerth 2000; Taiz & Zeiger 2002). This data also show us that the level of endogenous IAA content can be controlled by application of exogenous GA3 and 2,4-D with a certain concentration (Figures 2 & 3).

Even though there is a linear correlation between exogenous GA3 and 2,4-D application with IAA content (Figures 2 & 3), this hormone application was only partially able to improve carambola fruit retention. The result was due to the higher level of IAA content was not parallel to fruit retention (Figures 3, 6 & 7). With the application of 2,4-D at the concentration of 10 and 15 ppm, additional GA3 even reduced fruit retention (Figures 6 & 7). The result was probably due to negative impact of IAA content if the concentration exceeds the optimum level. The high level of IAA can induced ethylene accumulation by activation of mRNA to produce ACC enzymes (Mc Keon *et al*. 1995) which activate abscission process.

However, sugar content had variation patterns in response to application of GA3 and 2,4-D, where the increase of sugar content due to GA3 concentration occured in combination with 0 and 5 ppm 2,4-D only. On the other hand, by using 10 and 15 ppm of 2,4-D, additional of GA3 reduced sugar content (Figure 5). It means that the increase of sugar content had similar pattern with fruit retention in ransoms to GA3 and 2,4-D application (Figure 5 & 7), suggested that fruit retention is not only influenced by endogenous IAA but also depend on sugar content in the supporting leaves. The leaves sugar content is one of important indication of photosynthate supply requirement for fruit development. Gibberellin stimulates the synthesis of hydrolytic enzymes such as α-amylase to hydrolyse amylum into glucose and fructose hence improve total sugar in the actively grown tissue (Sandovald *et al*. 1995). This result showed that gibberellin can at least partly work with auxin to support fruit retention might induce α-amylase enzyme to hydrolyse starch to become sugar required for fruit growth and development (Subiyanto 1991). This result was also in accordance to Stopar *et al*. (2001) that the shed apple fruit had higher starch content and lower sugar content as compared to that of retention fruit.

This experiment showed that fruit drop and abscission during fruit development is not only influenced by endogenous auxin but also depend on total sugar accumulation in the supporting leaves. The total sugar in the leaves is an indication of leaves status as a strong sink to support growth and development of fruit. An inadequate of photosynthate supply may result in fruit drop and abscission during fruit development (Bangerth 2000).

ACKNOWLEDGEMENT

The research was funded by Indonesian Ministry of Religious Affairs (2006-2007) in collaboration with Bogor Agricultural University to improve Human Resources and Teaching Quality in Indonesian Islamic High School.

REFERENCES

Aneja M, Gianfagna T. 1999. The role of abscission and ethylene in the abscission and drop of cocoa flower. *Plant Growth Regul* 27:149-155.

Apriyantono A, Fardiaz D, Puspitasari NL, Sedarnawati Y, Budiyanto S. 1989. *Analisis Pangan*. Bogor: IPB Pr.

Bain KS, Bajwa GS, Singh Z. 1997. Abscission of mango fruitlet I in relation to endogen concentration of IAA, GA3 and ABA in pedicels and fruitlets. *Fruit Paris* 52:159-165.

Bangerth F. 2000. Abscission and thinning of young fruit and their regulation by plant hormones and bioregulators. *Plant Growth Regul* 31:43-59.

Beno-Moualem D *et al*. 2004. The effects of ethylene, methyl jasmonate and 1-MCP on abscission of cherry tomatoes from the bunch and expression of endo-1,4-β-glucanases. *Plant Sci* 167:499-507.

Choo WK, Ketsa S. 1992. Euphoria L. In: Verhej EWM, Coronel RE (eds). *Plant Resources of South-East Asia II. Edible Fruits and Nuts*. Bogor: Prosea. p 146-151.

Galan-Sauco V. 1993. Carambola cultivation. FAO Plant Production and Protection Paper No. 108. Rome, p 74.

Malik AU, Agrez V, Singh Z. 2003. Fruitlet abscission of mango in relation to ethylene. *J Hortic Sci Biotechnol* 78:458-462.

Malik AU, Singh Z. 2006. Improved fruit retention, yield and fruit quality in mango with exogenous application of polyamines. *Sci Hortic* 110:167-174.

Mc Keon TA, Macalet JCF, Shang-Fa Y. 1995. Biosynthesis and metabolism of ethylene. In: Davies PJ (ed). *Plant Hormone*. Netherlands: Kluwer Acad Publ. p 119-135.

Pattern CL, Glick BR. 2002. Role of *Pseudomonas putida* indole acetic acid in development of the plant root system. *Appl Environ Microbiol* 68:3795-3801.

Quintana EG, Nanthacai P, Hiranpradit P, Mendoza DB, Ketsa S. 1984. Changes and mango during growth and maturation. In: Mendoza DB, Wills RBH (eds). *Mango: Fruit Development, Postharvest Physiology and Marketing in ASEAN*. Philippines: ASEAN Food Handling Bureau. p 21-38.

Salisbury FB, Ross CW. 1995. *Plant Physiology* 4th Ed. California: Academic Pr.

Samson JA. 1992. *Averrhoa* L. In: Verhej EWM, Coronel RE (eds). *Plant Resources of South-East Asia II: Edible Fruits and Nuts*. Bogor: Prosea Foundation. p 96-98.

Sandovald J, Kerbellec, Cote F, Doumas P. 1995. Distribution of endogenous gibberellins in a giant off-types banana (Musa A.A.A,CV "Grand nain"). Plant from in vitro propagation. *Plant Growth Regul* 17:219-224.

Shui G, Leong LP. 2004. Analysis of polyphenolic antioxidants in star fruit using liquid chromatography and mass spectrometry. *J Chromat A* 1022:67-75.

Shui G, Leong LP. 2006. Residue from star fruit as valuable source for functional food ingredients and antioxidant nutraceuticals. *Food Chem* 97:277-284.

Srivastava LM. 2002. *Plant Growth and Development, Hormones and Environment*. California: Acad Pr.

Steffens GL. 1988. Gibberellin biosynthesis inhibitors comparing growth retarding effectiveness on apple. *Plant Growtth Regul* 7:27-36.

Stern RA, Kigel J, Tomer E, Gazit S. 1995. 'Mauritius' lychee fruit development and reduced abscission after treatment with the auxin 2,5,6-TP. *Hort Sci* 120:65-70.

Stopar M, Resmik M, Pongrac VZ. 2001. Non structural carbohydrate status and CO_2 exchange rate of apple fruitlets at the time of abscission influenced by shade, NAA or IBA. *Hort Sci* 87:65-76.

Subiyanto HS. 1991. *Fisiologi Tanaman Budidaya*. Jakarta: Universitas Indonesia.

Taiz L, Zeiger E. 2002. *Plant Physiology*. 3rd ed. Sunderland, Massachusetts: Sinauer associates Inc. Publisher.

Teixeira GHA, Durigan JF, Alves RE, O'Hare TJ. 2007. Use of modified atmosphere to extend shelf life of fresh-cut carambola (*Averrhoa carambola* L. cv. Fwang Tung). *Postharvest Biol Tech* 44:80-85.

Unyayar S, Topcouglu SF, Unyayar A. 1996. A modified method for extraction and identification of Indole-3-Acetic Acid (IAA), Gibberelic Acid (GA), Absisic Acid (ABA), and Zeatin produced by Phanerochaeta crysosporium ME446. *Bulg J Plant Physiol* 22:105-110.