INDOLE-3-ACETIC ACID, 1-AMINO CYCLOPROPANE-1-CARBOXYLIC ACID, AND CARBOHYDRATE IN RELATION TO FRUIT DROP ON MANGO TREE

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ABSTRACT

Mango (*Mangifera indica* L.) is a highly popular tropical fruit. One of the factors causing the low production of mango is fruit drop. Many factors cause fruit drop, one of them being a phytohormonal imbalance. The objective of this research was to compare the content of indole-3-acetic acid (IAA), 1-amino cyclopropane-1-carboxylic acid (ACC), and carbohydrate between persistent and pre-abscission fruits of two cultivars Gadung 21 and Lalijiwo. Pre-abscission fruit had lower IAA and total sugar, but higher ACC and starch than persistent fruit for both cultivars.

Key words: mango, fruit drop, IAA, ACC, total sugar, starch

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the major fruit crops in tropical and subtropical regions. This fruit is preferred because of its good flavour and taste. An increase in human populations, income and awareness of the importance of fruit-derived nutrients has led to an increase in demand for mango. Since it is a climacteric fruit, pre-harvest practices can affect post-harvest quality (Fabi et al., 2010). Indonesia is the fifth exporter after India, Pakistan, China and Mexico. Mango exports from Indonesia increased from 564 tons in 1999 to 1,908 tons in 2008, account-

ing for US$ 1,645,948 (US Department of Agriculture 2010). Therefore, there is an urgent need to increase mango production in some way, one of which is by reducing fruit drop.

Fruit drop is a natural phenomenon in which the number of fruits on a tree is reduced by a lack of photosynthates caused by auto regulation (Marcelis et al., 2004). However, fruit drop is particularly high for mango, causing a reduced yield (Chattha et al., 1999). Fruit drop, particularly during the first two weeks after fruit set, is severe with more than 80% of the initial fruitlets being lost before maturity. Some cultivars such as 'Tommy Atkins', 'Haden' and 'Gadung 21' usually bear one fruit per panicle through to maturity. Others, like 'Irwin', 'Nam Dok Mai' and 'Lalijiwo' often retain two or more fruits per panicle to maturity (Yeshitela, 2004).

Reducing fruit drop, especially for cultivars with high economic value, like 'Gadung 21', is important. There are many factors that cause fruit drop: high precipitation and humidity, pests and diseases, low levels of carbohydrates (Iglesias and Tadeo, 2006), nutrient deficiency, and phytohormonal imbalance (Chen et al., 2006).

Ethylene and auxin are the primary hormonal mediators of fruit drop (Racsko et al., 2006). Auxin appears to be an important regulator of ethylene sensitivity and fruit abscission. Natural ethylene and/or 1-amino cyclopropane-1-carboxylic acid (ACC) levels are often higher during periods of fruit drop, including during early drop and ripening. Sugar translocation from the leaves (Sexton, 1995) and an increase in ethylene production (Dal Cin et al., 2007) interfere with fruit drop.

Our objective was to identify one or more factors that might cause the low production of mango leading to fruit drop.

MATERIAL AND METHODS

Research was carried out at a mango plantation belonging to PT Fajar Mekar Indah, in the village of Jarangan, Rejoso District, Pasuruan Regency, East Java and in the Laboratory of the Study Center for Plant Breeding, Bogor Agricultural University Bogor, Indonesia from July until December. For this experiment, 12 mature, 15-year-old mango trees each for 'Gadung 21' (high fruit drop) and 'Lalijiwo' (low fruit drop) cultivars were used. Fruits were sampled 6 and 9 days after anthesis for 10 persistent fruits and 10 pre-abscission fruits from each tree, corresponding to the peaks of fruit drop for 'Gadung 21' and 'Lalijiwo', respectively. The experiment was factorial, consisting of 2 factors: cultivar ['Gadung 21' (high fruit drop), 'Lalijiwo' (low fruit drop)] and fruit condition (persistent, pre-abscission).

Mangoes were collected in July (6 and 9 days after anthesis (DAA)) from the trees, placed immediately in a cooler box, and then freeze-dried. Persistent fruits were a fresh green colour while pre-abscission fruits were not. Samples were stored at

-18 °C. When needed for analysis, they were homogenized in cold 70% methanol at room temperature and were temporarily stored at 7 °C. The extracts were filtered through Whatman No. 5 filter paper and the supernatant was re-homogenized with the same solution. All extracts were combined. There were 4 samples for each treatment or 4 × 8 treatments = 32 samples for each variable. The treatments were: (1) persistent fruit at 6 DAA of cultivar 'Gadung 21', (2) pre-abscission fruit at 6 DAA of 'Gadung 21', (3) persistent fruit at 6 DAA of 'Lalijiwo', (4) pre-abscission fruit at 6 DAA of 'Lalijiwo' (5) persistent fruit at 9 DAA of 'Gadung 21' (6) pre-abscission fruit at 9 DAA of 'Gadung 21' (7) persistent fruit at 9 DAA of 'Lalijiwo', and (8) pre-abscission fruit at 9 DAA of 'Lalijiwo'. There were 4 variables, so the total number of samples was 32 × 4 = 128. The variables measured were: indole-3-acetic acid (IAA), 1-amino cyclopropane-1-carboxylic acid (ACC), total sugar, and starch. IAA analysis was conducted on seed using the method of Sanberg et al. (1987). Ten grams of seed homogenized in 200 ml of methanol containing 20 mM sodium diethyldithiocarbamate and 1.2 kBq of [1-^{14}C]IAA. After extraction at 4 °C for 24 h, the extract was filtered and mixed with 20 ml of 0.5 M sodium phosphate (pH 8.0) and reduced to the aqueous phase *in vacuo* at 40 °C. The aqueous extract was applied to a 150 × 20 mm PVP column and eluted with 200 ml of 0.1 M sodium phosphate (pH 8.0). Fraction 0-125 ml was collected, acidified with 1 M HCl to pH 2.7 and partitioned against diethyl ether (5 × 0.5 volumes). The samples were further purified on a semi-preparative 250 × 4.6 mm, 10 µm Nucleosil CN column eluted with ethyl acetate: hexane: acetic acid (35: 64: 1 v/v/v). The final quantification was performed on 250 × 4.6 mm, 5 µm Nucleosil C_{18} column eluted isocratically with 30% methanol in 0.01 M sodium phosphate and 0.1 M tetrabutylammonium hydrogen sulphate (pH 6.5). The mobile phase was delivered at a flow rate of 1 ml min^{-1} by a Milton Roy mini pump. Samples were introduced off column by means of a Valco injector (100 µl loop). Column eluent was monitored with a Spectra Physic SF 970 spectrofluorimeter (excitation 285 nm, emission 360 nm). Quantitative estimates were based on the fluorescence signal related to a standard curve. Correction for losses was made by liquid scintillation counting of IAA fractions from the final HPLC step. ACC was analysed from pericarps by the Lizada and Yang (1979) method. About 1 g fresh weight of pericarp was homogenized in 8 ml 80% ethanol. The shaft was washed with an additional 2 ml 80% ethanol. The extract and washing were combined, filtered through glass wool, and centrifuged for 10 min at 10,000 × *g*. The pellet was discarded and the supernatant was evaporated to dryness under reduced pressure at 50 °C. The dry residue was dissolved in 2 ml of water and an equal volume of chloroform was added. The tubes were vigorously shaken, and centri-

fuged as above. One µmol of $HgCl_2$ was added to 0.6 ml extract in a 15 × 125 mm test tube and water was used to bring the volume to 0.9 ml. The tube was sealed with a rubber serum cap and kept in ice. Approximately 0.1 ml of a cold mixture of 5.5% NaOCl and saturated NaOH (2:1, v/v) was injected into the test tube through the rubber cap. The mixture was agitated for 20 s on a Vortex. After incubation for 2.5 min in ice, the tube was again agitated and a 1 ml gas sample was removed for ethylene analysis by GC. The conversion efficiency of ACC to ethylene in each sample was determined separately with a replicate sample containing a known amount (2.5 nmol) of ACC as an internal standard. The amount of ACC in the sample is calculated as the amount of ethylene released from the sample divided by the conversion efficiency. Total sugar and starch were analysed in endocarps using the method described by Apriyantono et al. (1994). To analyse the amount of total sugar and starch, about 0.1-0.5 g of the sample was homogenized in hot 80% ethanol. Next, it was centrifuged and the residue was retained. This residue was washed repeatedly with hot 80% ethanol until washing did not give a positive colour reaction with anthrone reagent. The residue was dried well over a water bath. Then, 5.0 ml of water and 6.5 ml of 52% perchloric acid were added to the residue. This mixture was extracted at 0 °C for 20 min and centrifuged. The supernatant was saved. The extraction was repeated using fresh perchloric acid and re-centrifuged. The supernatants were pooled and made up to 100 ml. About 0.1-0.2 ml of supernatant was pipetted out and made up to 1 ml with water. The standards were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard and made up to 1 ml in each tube with water. To each tube, 4 ml of anthrone reagent was added, heated for 8 min in a boiling water bath, and then cooled rapidly. The green to dark green colour intensity was read at 630 nm. The glucose content in the sample was determined using a standard graph. The value was multiplied by a factor 0.9 to give the starch content. All data were analysed using the *t*-test ($p = 0.05$).

RESULTS

The results are presented in Tables 1-4. For both cultivars, pre-abscission fruit had significantly less IAA (Tab. 1) and higher ACC levels (Tab. 2) than persistent fruit throughout the entire period of observation. Pre-abscission fruit has significantly lower total sugar than persistent fruit (Tab. 3), but higher starch content (Tab. 4).

DISCUSSION

The developmental stage of mango fruit on a tree determines its levels of IAA, ACC, sugar and starch. Pre-abscission fruit has a lower IAA. Previous research showed that pre-abscission fruit has lower fresh weight, diameter, and length than persistent fruit

Table 1. Indole-3-acetic acid (IAA) content (10^{-1} µg/g sample fresh weight) on persistent and pre-abscission fruits (n = 32)

Cultivar	6 DAA*	9 DAA
Gadung 21		
Persistent fruit	10.32 a**	8.12 a
Pre-abscission fruit	5.96 b	3.86 b
Lalijiwo		
Persistent fruit	10.08 a	7.98 a
Pre-abscission fruit	4.48 b	4.12 b

*DAA = days after anthesis
**Means followed the same letter within a column and cultivar are not significantly different (*t*-test, 5%)

Table 2. 1-aminocyclopropane-1-carboxylic acid (ACC) content (ng/g sample fresh weight) on persistent and pre-abscission fruits (n = 32)

Cultivar	6 DAA*	9 DAA
Gadung 21		
Persistent fruit	20.95 b**	14.12 b
Pre-abscission fruit	36.15 a	44.24 a
Lalijiwo		
Persistent fruit	13.48 b	11.78 b
Pre-abscission fruit	30.05 a	36.35 a

*, **Explanations: see Table 1

Table 3. Total sugar content (mg/g sample fresh weight) on persistent and pre-abscission fruits (n = 32)

Cultivar	6 DAA*	9 DAA
Gadung 21		
Persistent fruit	4.22 a**	4.98 a
Pre-abscission fruit	2.06 b	2.84 b
Lalijiwo		
Persistent fruit	6.10 a	6.72 a
Pre-abscission fruit	2.22 b	2.54 b

*, **Explanations: see Table 1

Table 4. Starch content (mg/g sample fresh weight) on persistent and pre-abscission fruits (n = 32)

Cultivar	6 DAA*	9 DAA
Gadung 21		
Persistent fruit	2.25 b**	5.55 b
Pre-abscission fruit	5.68 a	7.72 a
Lalijiwo		
Persistent fruit	6.08 b	5.84 b
Pre-abscission fruit	9.65 a	9.82 a

*, **Explanations: see Table 1

(Sakhidin et al., 2004). Prakash and Ram (1984) claimed that auxins appear to play a major role in the growth of fruit. Seeds, especially their endosperm, are the sites of synthesis, where auxin is produced. The IAA content of sepals in shaded trees, 20 days after full bloom, is lower (1.0 µg per g fresh sample) than that in unshaded (1.9 µg per g fresh sample) trees (Takashi et al., 2002).

Fruit drop is closely related to fruit size in several fruit trees. Koukourikou-Petridou (2003) reported that small almond (*Prunus dulcis* [Mill.] D.A. Webb) fruits had lower levels of extractable and diffusible free IAA than intermediate and large size fruits. The percentage of small fruits that abscised was five times higher than that of the other size categories of fruits. Modise et al., (2009) stated that small-sized fruits of navel oranges (*Citrus sinensis* L.) were more susceptible to fruit drop than larger fruits.

This research showed that pre-abscission fruit has higher ACC than persistent fruit. Higher ACC levels leads to high ethylene (Zheng et al., 2005) levels since ACC is the precursor of ethylene (Lechaudel and Joas, 2007). A high content of ethylene and a low content of auxin increase the sensitivity of the abscission zone to ethylene. This increased sensitivity leads to an increase in the secretion of hydrolytic enzymes, resulting in cell separation and fruit drop (Bangerth, 2000).

One of the potent inhibitors of ethylene biosynthesis is aminoethoxyvinylglycine (AVG). AVG inhibits the conversion of adenosyl methionine to ACC. AVG reduced fruit drop, delayed starch conversion and decreased ethylene production of 'Kogetsu' apples (Rath et al., 2006). Similarly, do Do Amarante et al. (2002) noted that spraying AVG reduced fruit drop to 10% in 'Gala' apples. Fruit drop in the control treatment was 85%.

Pre-abscission fruit has lower total sugar, but higher starch than persistent fruit (Tab. 3, 4). Stopar et al. (2001) noted the same trend for apple. Yuan and Greene (2000) claimed that pre-abscission fruit has lower metabolic activity; thus, total sugar content was lower than persistent fruit. The higher starch and lower total sugar in pre-abscission fruit is related with the slow mobilization of starch reserves (Ruiz et al., 2001).

Rai et al. (2008) reported similar results for mangosteen, in which fruit drop was caused by low IAA and total sugar. In pre-abscission fruit, IAA and total sugar were significantly lower than in persistent fruit.

Since pre-abscission mango fruit has a higher content of ACC and starch but a lower content of IAA and total sugar than persistent fruit, the application of a synthetic auxin such as NAA or other plant growth regulator is recommended. Such applications would be able to suppress ethylene synthesis and consequently reduce fruit drop leading to higher yields and economic returns.

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WPŁYW ZAWARTOŚCI KWASU INDOLILO-3-OCTOWEGO I 1-AMINO CYKLOPROPANO-1-KARBOKSYLOWEGO ORAZ WĘGLOWODANÓW NA OPADANIE OWOCÓW MANGO

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S T R E S Z C Z E N I E

Mango jest jednym z najbardziej popularnych owoców tropikalnych. Jednym z czynników wpływających na obniżenie jego produkcji jest opadanie owoców, które spowodowane jest między innymi zaburzeniem równowagi fitohormonalnej. Badaniami objęto dwie odmiany – Gadung 21 i Lalijiwo. Celem ich było określenie zawartości kwasów indolilo-3-octowego (IAA) i 1-amino cyklopropano-1-karboksylowego (ACC) oraz węglowodanów w owocach mocno trzymających się drzewa i przed opadaniem. W przypadku obydwu odmian owoce przed opadaniem miały niższą zawartość IAA i cukru, natomiast wyższą zawartość ACC i skrobi niż owoce we wcześniejszym stadium rozwoju.

Słowa kluczowe: mango, opadanie owoców, IAA, ACC, cukry ogółem, skrobia