Journal of Applied Horticulture, 18(3): 213-216, 2016

Relationship between IAA, sugar content and fruit-set in snake fruit (*Zalacca salacca*)

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Abstract

The main problem of snake fruit production in Indonesia is high fluctuation of fruit production between different harvesting seasons, due to fruit-set failure in some parts of the year. The objective of this research was to find out the relationship between IAA and sugar content of leaves and flowers in relations to the failure of fruit-set in three flowering seasons of snake fruit (April, July, and October). The study was conducted at the production center of snake fruit in Bali (Karangasem Regency) by using Completely Randomized Design. The results showed that fruit-set in April, July and October was 54.16, 47.00 and 70.10%, respectively. Low fruit-set was found associated with low IAA content both in leaves and flowers. The lowest percentage of fruit-set found in July (47.00%) was related to the lowest IAA content in the leaves (10.06 ng g^{-1}) and flowers (20.60 ng g^{-1}). However, the highest percentage of fruit-set in October (70.10%) was correlated to the highest IAA content in the leaves (29.67 ng g^{-1}) and flowers (52.56 ng g^{-1}). Low fruit-set was also closely related to the low content of sugar in the leaves. The lowest percentage of fruit-set in July was caused by the lowest total sugar (24.54%) and reducing sugar (6.56%) content in the leaves, whereas the highest percentage of fruit-set on October related to high total sugar (30.58%) and reducing sugar (12.22%) content in the leaves. It can be concluded that failure of fruit-set in snake fruit was associated with low IAA and sugar content in leaves and flowers.

Key words: Snake fruit, IAA, fruit-set, sugar, flowering season

Introduction

Snake fruit (*Zalacca salacca*) is one of an important fruits in Indonesia and comercially grown in several islands in Indonesia such as Java, Sumatra, Sulawesi, Kalimantan, Papua, Maluku, and Bali. The major problem during snake fruit production is the high fluctuation in the amount of fruit produced between fruiting seasons and non-fruiting season, so it affects the continuity and availability of the fruit in the market. This may be caused by the failure of flowers to develop into fruit during non-fruiting season.

Seasonal fruit production causes discontinuity of fruit supply. During the fruiting season, which is around 2-3 months, the availability of fruits is abundant with very low price. However, during the non-fruiting season, with similar demand, low fruit production causes inadequate supply to fulfill the demand. A short fruit supply during fruiting season is due to short shelf life time of the fruits *i.e.* 7-10 days at room temperature. This condition is major reason for weak bargaining power of farmers in the marketing system, therefore they have to sell their product immediately at low prices. The effort to reduce the imbalance of supply and demand throughout the year is necessary, so that the income of the farmers could rise.

Rai *et al*. (2010) found that snake fruit in Indonesia flowers four times in a year, *i.e.* in January, April, July, and October. However, the flowers produce fruit only in April and/or October flowering seasons. Flowering and fruiting of fruit trees are affected by the environmental condition and endogenous crop factors, such as carbohydrate content (Luis *et al*., 1995; Miller *et al.,* 2015), growth hormone, internal water condition, and nutritional status (Bernier *et al*., 1998). Ogaya and Penuelas (2007) proposed that the environmental factors that influenced fruit-set were air temperature, humidity, rainfall and light intensity.

Generally, success of flowers to develop into fruits is determined by the floral induction (Thirugnanavel *et al*., 2007; Hanke, 2009, Burondkar *et al*., 2013). Different from other plant producing fruits, fruit productions of snake fruit is not affected by the success of flower induction. Naturally this plant is flowering four times a year. Mogea (1990) mentioned that snake fruit belong to the palmae family that can flower all year, as in other palm trees. With such of a flowering habit, the effort is required for fruiting of snake fruit or making the flowers at each flowering season develop into the fruit.

Physiologically, the abscission of flowers on fruit trees is determined by the adequacy of photosynthate supply (Luis *et al*., 1995), and hormonal regulation particularly the adequacy and balancing of endogenous hormones such as IAA (Koshita *et al*., 1999). Bangerth (2000) hypothesized, high concentration of IAA in flowers would increase the ability of flowers to attract assimilates. IAA stimulates photosynthetic activity, thus increasing the supply of assimilates. Inadequate supply of assimilate as a result of limited production of assimilates, as well as low allocation of assimilates to flower can cause flowers to fall off.

Baker *et al*. (1997) also reported that compatible flower pollination on cocoa was closely related to high concentration of endogenous IAA, and flower retention. However, unpollinated flowers or pollinated flowers without compatible pollinations

die due to the low concentration of endogenous IAA. Based on the differences of endogenous IAA concentrations, which was found by Baker *et al*. (1997) in cocoa, we presumed that failure of fruit-set in snake fruits may also caused by low endogenous IAA content. Research by Aneja *et al*. (1999) confirmed that treatment of cocoa with auxin, naphtalene acetic acid (NAA) prevented abscission of flowers. The objective of the current research was to investigate the relationships between the IAA and sugar content of leaves and flowers in relation with failure of fruit-set of snake fruit in the three flowering seasons (April, July and October).

Materials and methods

Field experiment was carried out at the production center of snake fruit in Bali that is in Bebandem District, Karangasem Regency, from February to December, 2014. Fifteen years old snake fruit (*Zalacca salacca* var. Gula Pasir) cultivars with similar growth vigor were selected. Snake fruit plants used in this experiment were maintained in accordance with that of farmers. Plants were fertilized by organic fertilizers (cow manure) at a dose of 5 t ha^{-1} (without inorganic fertilizers), and the irrigation was mainly from rainfall. Routine maintence by farmers included weeding around the plants and pruning of the old-dried midrib leaves. Leaf sheaths were embedded around the plants as an organic fertilizer and retained the soil moisture.

The experiment was designed as randomized complete design (RCD), with one factor as the dependent variable with 30 replicates. Factor of the dependent variable was the flowering seasons, consisting of three (3) levels *i.e.,*: April, July, and October. Variables observed were the percentage of fruit-set, the number of fruit bunches per plant, IAA content of leaves and flowers, sugar content of leaf which consisted of total sugar, reducing sugar (R-sugar), and sucrose, and relative water content (RWC) of leaves. Samples of mature leaves and blooming flowers for analyzing IAA were collected three times (early, mid, and late month in each flowering season) at 10-day intervals, while sugar content and RWC were analyzed only from mature leaves at similar collection times.

Percentage of fruit-set: Percentage of fruit-set in each flowering season was calculated by dividing the number of flowers develop into fruits with the number of flowers, then multiplied by 100, while the number of fruit bunches per plant was obtained by calculating all bunches of flowers that developed into bunches of fruits.

IAA extraction, purification and quantification: With slight modifications, the extractions, purification and quantification of endogenous IAA were performed by employing HPLC as described by Sandberg and Ernstsen (1987), conducted at laboratory of Indonesian Agency for Agricultural Postharvest Research and Development, Bogor. Samples (mature leaves and blooming flower) which were collected from plants were immediately placed in an ice box containing dry ice, then freeze-dried. Freeze dried samples were stored in a freezer at -80 °C until further analyses. IAA extraction was conducted by placing approximately 2 g of dried sample that has been homogenized (with a pestle and mortar), into a separating funnel, then extracted with 3 x 10 mL of methanol. The residue was dissolved in 30% acetic acid and 23 mL of acetonitrile. This mixture was centrifuged at 4000 rpm for 30 minutes. The supernatant was filtered on a milipore filter paper, then analyzed using HPLC. Analysis was done using a mobile phase of acetonitrile and acetic acid (60:40), the stationary phase (column) C-18, flow rate of mobile phase of 1 mL min^{-1}, the injection pressure at 900 psi and detected with a UV-VIS detector models 440 at a wavelength of 254 nm. Quantification was done by using peak area under sample corresponding with standard compound.

Total sugar, R-sugar and sucrose analysis: Total sugar, R-sugar and sucrose were determined by the method proposed by Apriyantono *et al*. (1994). The total sugar was analyzed by employing Anthrone method, R-sugar by Nelson-Somogyi method, while content of sucrose was calculated by subtracting the value of total sugar content with value of R-sugar, then multiplied by 0.95. In order to analyze total sugar and R-sugar, 0.2 g sample of leaves that has been finely ground was homogenized in 5 mL of water and 20 mL of 80% ethanol, and then shaken. After centrifugation at 6000 rpm for 20 min, the supernatant was evaporated and continued by dissolving in water up to 100 mL in volume. One mL of that 100 mL solution was taken to analyze the total sugar and another 1 mL to analyze the R-sugar. The total sugar was analysed by adding 1 mL of the sample with 1 mL H_2O and 5 mL 0.1% Anthrone. This mixture was heated at 100 °C for 12 minutes, then cooled down. The total sugar content was measured by UV-VIS spectrophotometer at a wavelength of 630 nm. The R-sugar was analysed by adding 1 mL reagent Nelson in 1 mL prepared sample, and heated at 100 °C for 12 minutes. After the sample was cool, 1 mL of arsenic molybdate and 7 mL H_2O were added, then shaken to homogenized. The R-sugar content was measured in UV-VIS spectrophotometer at a wavelength of 540 nm. Nelson Reagent consisted of solution a and b with ratio of 25:1. Solution a contained of 12.5 g Na_2SO_4 + 12.5 g Na-K tartrate + 10 g $NaHCO_3$ + 100 g Na_2SO_4, which was dissolved in 400 mL of water, while solution b contained 7.5 g $CuSO_4.5H_2O$ + 1 drop of concentrated H_2SO_4, which was dissolved in 50 mL H_2O.

Relative water content (RWC) was measured in matured leaves, carried out in the Laboratory of Agronomy and Horticulture, Faculty of Agriculture, Udayana University. The sample of leaves were immediately wrapped in aluminum foil, stored in the ice box, then transported to the laboratory for the analysis. Thirty pieces of leaf samples (10 pieces from the tip of the leaf, 10 pieces form the middle and 10 pieces from the bottom) were taken from leaf sheaths using a round punch with a diameter of 1 cm, and then weighed (W1). Those leaf samples were immersed in water and at the same time irradiated with 40 watt fluorescent light at room temperature for 5 hours. Each pieces of leaf sample was carefully dried with paper towel, and weighed (W2), then the samples were oven dried at 70 °C for 24 hours, and weighed (W3). The value of RWC was calculated by the formula of (W1-W2)/(W3-W2) x 100%.

The data obtained were analyzed with analysis of variance. If the F test showed significantly different among treatments, the Least Significant Differences (LSD) test was performed.

Results and discussion

The highest percentage of fruit-set (70.10%) was obtained during October flowering season, which was significantly higher than

those of April (54.16%) and July (47.00%) (Table 1). The highest fruit-set in October was also indicated by the highest number of flower-clusters developed into fruit-clusters (4.11 units). The percentage of fruit-set was positively correlated to the relative water content (RWC) of leaves. Higher RWC of leaves was associated with higher fruit-set. Table 1 show that the lowest RWC of leaves was in July (67.80%), followed by the lowest percentage of fruit-set (47.00%), while the highest RWC of leaves in October (89.32%) was accompanied by the highest percentage of fruit-set (70.10%). The data indicated that water content of leaves played an important role in determining the development of flowers into fruits. This result was in line to the study on sunflower (Kowalska, 2008) and apple crop (Chauhan *et al*., 2006).

Table 1. The percentage of fruit-set, RWC of leaves and the number of fruit bunches per plant during flowering season in April, July and October

Flowering season	Fruit-set (%)	Leaf RWC (%)	Number of fruit bunches per plant
April	54.16 b	86.01 b	3.16 b
July	47.00 c	67.80 c	2.38 c
October	70.10 a	89.32 a	4.11 a
LSD 5%	6.21	1.74	0.52

The numbers followed by the same letter in the same column indicates no significant difference at the 5% LSD level.

The result shows that low percentage of fruit-set was closely associated with low content of IAA, both in leaves and flowers. The low IAA content in the leaves and flowers (10.06 ng g^{-1} and 20.60 ng g^{-1}, respectively) was found in July with low percentage of fruit-set (47.00%), whereas, in October flowering season, the IAA content was highest in the leaves and flowers (29.67 ng g^{-1} and 52.46 ng g^{-1}, respectively) with highest fruit-set (70.10%) (Table 2). This finding indicated that the content of IAA in snake fruit plants was very important and influenced the of fruit-set development. According to Bangerth (2000), high synthesis of auxin (IAA) increased the pollination success and fertilization of flower in fruit plants, but did not affect abscission. Aneja *et al*. (1999) reported that auxin was involved in stimulating the cocoa fruit-set, that could be from pollen after pollination and also be formed later in the ovary. Therefore, it was suggested that fruit-set can be induced by administration of exogenous auxin as a substitute for pollination. Other studies found that abscission of flowers and fruit on mangosteen occured due to decrease in IAA, but increase in ABA concentration (Rai *et al*., 2013). While Baker *et al*. (1997) reported, compatible pollinated flower in cocoa had a high concentration of endogenous IAA, so that the flowers did not easily fall off. However, unpollinated and pollinated flower with high concentration of incompatible endogenous hormone such as ABA and ethylene increased the flower plunge. Thus, low abscission in the pollinated flowers was affected by the low level of ABA and ethylene, but high IAA. The differences of the endogenous IAA content in the leaves and flowers of snake fruit harvested in July and April flowering seasons, and its effects on the differences of fruit-set, indicated that it is possible to prevent the failure of fruit-set with the application of synthetic IAA in order to improve the content of endogenous IAA.

The percentage of fruit-set in snake fruit was not only influenced by the IAA content in the leaves and flowers, but also determined by sugar content in the leaves. This can be seen that low percentage of fruit-set in July flowering season (47.00%) was correlated with low total sugar and R-sugar in leaves. On the other hand, higher total sugar and R-sugar in leaf during flowering season was observed in April and October corresponding with the high fruit-set formation at both flowering seasons, compared to that of July. Low total sugar and R-sugar content of leaves reduce the leaf's ability to support fruit development. Miller *et al.,* (2015) reported that insufficient supply of assimilates, as a result of limited production and/or low allocation of assimilates to fruit can cause fruit drop in apple. However, Bonghi *et al*. (2000) stated, assimilates insufficiency did not directly determine the abscission of flowers or fruits, because it was also determined by the level of competition among sink and closeness or proximity between the sink and the source.

Low fruit-set of snake fruit in July flowering season was most likely due to the high competition between various organs for photosynthates. This was indicated by a low carbohydrate content in leaves, which was determined by low content of total sugar, R-sugar, and sucrose in the leaf. Shu (1999) found that low fertilization in mangoes was caused by high competition among flowers and fruits. Snake fruit plants in July flowering season was also producing lot of fruits from April flowering season which increased the resource competition among them. Moreover, higher number of flowers which fail to develop into fruit-set that still growing on flower bunch without pruning and the presence of new growing flowers may have resulted in low total sugar content, R-sugar and sucrose concentration in the leaf of snake fruit. Luis *et al*. (1995) found that if young fruits of citrus were removed from the plant, it resulted in increase in starch levels, but reduced the sugar content in the leaves. They found that the highest levels of starch in the leaves occurred after 7 days of fruit removal, whereas in the branch, the highest starch levels was after 17 days of fruit removal. On the other hand, if fruits allowed to grow continuously until maturity, starch and sugar content of the leaves was low. Stoy (1972) stated, fruit under normal circumstances produced certain hormones that translocated in the leaves, resulted in increases of leaf photosynthetic rate. When the fruit development is interrupted, amount of hormones decrease, and the rate of translocation of photosynthates also decline.

In conclusion, fruit-set in snake fruit (*Z. salacca*) is closely related to the IAA content in the leaves and flowers, and is also related to the content of total sugar and reducing sugar in the leaves. The percentage of fruit-set is higher with the higher IAA content in the leaves and flowers, and with the higher content

Table 2. Concentration of IAA in leaves and flowers, total sugar, R-sugars and sucrose of snake fruit during flowering seasons in April, July and October

Flowering season	IAA leaves (ng.g^{-1})	IAA flowers (ng.g^{-1})	Total sugar (%)	R-sugar (%)	Sucrose (%)
April	16.32 b	25.50 b	35.22 a	15.59 a	18.65 a
July	10.06 c	20.60 b	24.54 c	6.56 c	17.08 a
October	29.67 a	52.46 a	30.58 b	12.22 b	17.44 a
LSD 5%	5.82	6.52	4.23	2.00	3.10

In the same column, the numbers followed by the same letter indicates no significant different at the 5% level of LSD.

of total sugars and reducing sugar in the leaves, and vice versa. A research concerning of the application of exogenous auxin application is required to improve fruit-set during off-season snake fruit production.

Acknowledgement

This work was funded by The Ministry of Research, Technology and Higher Education of Republic of Indonesia through the Project of National Competitive Grant 2015.

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Received: June, 2016; Revised: June, 2016; Accepted: June, 2016