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 relation 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 commercially 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 flowering seasons and non-flowering 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-flowering 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-flowering 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.

Physiologically, the abscission of flowers on fruit trees 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, naphthalene 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 maintenance 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 acetoniitrile. 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 acetoniitrile 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 H₂O
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 H₂O
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 Na₂SO₄ + 12.5 g Na-K
tartrate + 10 g NaHCO₃ + 100 g Na₄P₂O₇, which was dissolved in
400 mL of water, while solution b contained 7.5 g CuSO₄.5H₂O +
1 drop of concentrated H₂SO₄, which was dissolved in 50 mL H₂O.
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 midle 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

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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

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

The numbers followed by the same letter in the same column indicates no significant different 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 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. Anjea 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 occurred 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

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

<table>
<thead>
<tr>
<th>Flowering season</th>
<th>IAA leaves (ng g⁻¹)</th>
<th>IAA flowers (ng g⁻¹)</th>
<th>Total sugar (%)</th>
<th>R-sugar (%)</th>
<th>Sucrose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>16.32 b</td>
<td>25.50 b</td>
<td>35.22 a</td>
<td>15.59 a</td>
<td>18.65 a</td>
</tr>
<tr>
<td>July</td>
<td>10.06 c</td>
<td>20.60 b</td>
<td>24.54 c</td>
<td>6.56 c</td>
<td>17.08 a</td>
</tr>
<tr>
<td>October</td>
<td>29.67 a</td>
<td>52.46 a</td>
<td>30.58 b</td>
<td>12.22 b</td>
<td>17.44 a</td>
</tr>
<tr>
<td>LSD 5%</td>
<td>5.82</td>
<td>6.52</td>
<td>4.23</td>
<td>2.00</td>
<td>3.10</td>
</tr>
</tbody>
</table>

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.

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References


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