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Increasing off-season snake fruit (*Salacca zalacca*) yield by preventing fruit-set failure with different exogenous auxin spray

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Abstract

Snake fruit blooms every three months, but only one to two of the flowering seasons result in fruit production. Failure of fruit-set causes the fruit harvest seasonally. Therefore, the aim of this research was to increase off-season production by preventing the failure of fruit-set with various exogenous auxin spray. The research was conducted in the snake fruit orchard at Bebandem Sub-District, Karangasem Regency, Bali Province, Indonesia, from February to November 2019. The experiment was designed as a nested factorial with a randomized block design. The treatment consisted of three types of auxin with a specific concentration for each, IAA at 0, 25, 50 and 75 ppm, IBA at 0, 50, 100 and 150 ppm, and NAA at 0, 50, 100 and 150 ppm. The IAA, IBA, and NAA were sprayed on the flowers and leaves 3 times in March, April, and May. The results showed the highest percentage of fruit-set, fruit weight per tree, and weight per fruit obtained with IAA treatment were 59.80 %, 2,612.91 g, and 91.06 g, respectively. These results were not statistically different to those of the IBA and NAA. Furthermore, the IAA concentration of 25, 50, and 75 ppm produced a significantly higher percentage of fruit-set compared with the control. The increased fruit-set in the IAA, IBA and NAA treatments resulted in better yield than the control. The novelty of this result is that spraying snake fruit with IAA, IBA and NAA can prevent the failure of the fruit-set. Therefore, IAA, IBA, and NAA are recommended to be applied to increase off-season fruit production of snake fruit.

Key words: Auxin, fruit-set, off-season, *Salacca zalacca*, snake fruit

Introduction

Snake fruit or salak is Indonesia's most important tropical fruit in Bali Province. Its market availability is seasonal as it is abundant in the harvest season (on-season), *i.e.,* from January to February, making its selling price low. However, it is mainly rare during the off-season (outside the harvest season) and this makes its price quite high, making production profitable (Rai *et al*., 2010).

Rai *et al*. (2010) observed that snake fruit blooms four times in a single year, *i.e.,* in January-February (called Raya blooming season), April-May (called Sela I flowering season), July-August (called Gadu flowering season) and October-November (called Sela II flowering season). However, the blossoms that can yield fruit only in July-August are picked in next 6 months (January-February). Fruit-set failure in three flowering seasons in snake fruit is associated with low IAA and leaf and flowers sugar content (Rai *et al*., 2016).

Generally, the development of flowers into fruits depends on the floral induction, a phase when vegetative meristem becomes cell prolific (Burondkar *et al*., 2013; Din *et al*., 2019). Snake fruit has the problem of fruit-set failure instead of floral induction (Rai *et al*., 2014). According to Mogea (1990), snake fruit belongs to the palm family that flowers all year round as they are not impacted by induction, like other palm trees. Therefore, efforts are needed to ensure that each seasonal flower develops into fruit. Some researchers have reported that the flowering and fruiting of fruit crops is mainly influenced by environmental and endogenous crop factors such as the content of carbohydrates (Boldingh *et al*., 2016, Hussain *et al*., 2016), growth hormone (Yan *et al*., 2019; Narayanswamy *et al*., 2019), internal water conditions (Blanco *et al*., 2020) and nutritional status (Khamis *et al*., 2018; Lobo *et al*., 2019).

Physiologically, the abscission of flowers is determined by the adequacy of photosynthate supply (Anuradha *et al*., 2017) and hormonal regulation (Kumari *et al*., 2018; Sebastian *et al*., 2019), in particular by the adequacy and balance of endogenous hormones such as IAA (Liu, 2019; Gundesly *et al*., 2020). Bangerth (2000) suggested that due to photosynthetic stimulation, increasing IAA content in flowers will boost flowers' ability to attract assimilates. However, due to restricted output and insufficient allocation, inadequate supply of assimilate might cause blossoms to fall off.

Auxin plays a crucial role in cell elongation, cell division, vascular tissue, differentiation, root initiation, apical dominance, leaf and fruit abscission, blooming and fruit-setting (Gallavotti, 2013; Gundesly *et al*., 2020). Indole-3-acetic acids (IAA), indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) are auxin-group phytohormones. They regulate practically all fruit plant development processes, including blooming, fruit-setting and fruit growth. Growth and yield of fruit crops are promoted in response to varying auxin levels (Bermejo *et al*., 2017; Liu 2019).

Auxins are generated in meristems and young leaves, transported polarly to other regions of the plant (Pattison *et al*., 2013;

Blakeslee *et al*., 2019). Moreover, natural and artificial auxins provided exogenously to horticultural plants stimulate flower induction and development as well as fruit-setting and growth, so these hormones can be employed to increase fruit-setting (Pandolfini, 2009; Liu *et al*., 2019). Exogenous auxins are capable of increasing assimilates production because auxin enhances the quality of the "source" (leaves) in the process of photosynthesis and also increases the ability of "sink" (flowers and fruits) to attract photosynthesis, hence minimising abscission and thinning of immature fruit (Bangerth, 2000). Molecular analyses recently verified the major involvement of auxin signalling in triggering and coordinating the transition from flower to fruit (Leyser, 2018) and fruit development (Robert, 2019). Furthermore, the studies reported so far demonstrate that auxin is involved in flowering and fruiting (Sebastian *et al*., 2019; Zhou *et al*., 2019). Hence, this work was undertaken to boost off-season fruit production of snake fruit by preventing fruit-set failure using various exogenous auxin spray.

Materials and methods

The field experiment was conducted at the production center of "Gula Pasir" snake fruit (*Zalacca salacca* var. Gula Pasir) orchards at Bebandem Sub-District, Karangasem Regency, Bali Province, from February to November 2019. This fruit has some superior characteristic such as sweet flavour before reaching full maturity, less sour and sandy taste, and thick fruit flesh separated from the seed.

Fifteen years old snake fruit trees with relatively similar canopy plant size and growth vigor were selected. The trees used were maintained routinely by fertilizing with cow manure at a dose of 5 $ton.ha^{-1}$, weeding, and pruning of the old-dried midrib leaves which are embedded around the plants as an organic fertilizer and maintaining the proper soil moisture. In this research, irrigation was only obtained from rain fall.

The experiment was designed as a nested factorial with a randomized block design. Furthermore, the treatment consisted of three types of auxin with specific concentration, IAA/Indole-3-Acetic Acid (I) with four concentration levels of 0 ppm/control (I_0), 25 ppm (I_1), 50 ppm (I_2), and 75 ppm (I_3); IBA/Indole-3 Butyric Acid (B) with 0 ppm/control (B_0), 50 ppm (B_1), 100 ppm (B_2), and 150 ppm (B_3); and NAA/Naphthalene Acetic Acid (N) with 0 ppm/control (N_0), 50 ppm (N_1), 100 ppm (N_2), and 150 ppm (N_3). Each treatment was repeated three times to make 36 sample plants and the IAA, IBA and NAA were sprayed on the flowers and leaves three times in March, April, and May.

Percentage of fruit-set: It was calculated by dividing the number of flowers developed into fruits with the number of flowers during the off-season period and multiplied by 100. The number of flowers were counted from April to May (Sela I flowering season) while the number of fruits, *i.e.,* the fruits developed from Sela I flowering season, were counted from July to November.

Leaf chlorophyll content: This was measured by using Chlorophyll Meter SPAD-502 once in April, June, and August making three times, and the results were averaged.

Total sugar, R-sugar, and sucrose. The total sugar was analyzed through the use of Anthrone, R-sugar by Nelson-Somogyi method, while the content of sucrose was calculated by subtracting the figure obtained for total sugar content from the value of R-sugar, and the result multiplied by 0.95. Analysis of total sugar by the Anthrone method and R-sugar by the Nelson-Somogyi method was conducted as Rai *et al*. (2016).

Relative water content (RWC). RWC was measured from matured leaves in the Laboratory of Agronomy and Horticulture, Faculty of Agriculture, Udayana University and carried out according to the method of Rai *et al*. (2016).

Leaf nutrient content of N and P. The N and P content were analyzed at the Soil Laboratory, Faculty of Agriculture, Udayana University using leaf samples taken in August 2019. N nutrient content (N total) was analyzed by the Kjeldahl method and P-available by Olsen method.

Fruit number, fruit weight per tree and weight per fruit. The number of fruits harvested and the weight of fruit per tree were calculated from October to November and cumulatively at the end of the study, while the weight per fruit was calculated by dividing the total weight of fruit per tree by the number of fruits harvested per tree.

The data obtained were analyzed using the analysis of variance (ANOVA) and in a situation where the F test showed a significant difference among treatments, the Least Significant Differences (LSD) test was performed.

Result and discussion

The IAA-treated sample had the highest percentage of fruit-set, fruit weight per tree, and number of fruits harvested per tree. However, the values obtained on the sample treated with IBA were not statistically different. As shown in Table 1, the IAA treated plants had the highest weight per fruit, which was significantly different from IBA. Higher leaf RWC of 88.65 percent, chlorophyll content of 75.42 SPAD, N content of 2.12 percent, and P content of 0.13 percent were positively correlated with higher fruit-set values observed in IAA treatments. As shown in Tables 1 and 2, these were not statistically significantly different from those treated with IBA and NAA. These findings revealed that IAA had a greater impact on fruit-set than IBA and NAA, but the difference was not statistically significant. However, the findings differed from those of Staswick (2009), who found that while the IAA- and NAA-induced fruits were similar in size, NAA-induced fruit-set was higher due to its longer-lasting effect due to the more stable synthetic hormone compared to IAA with unstable natural hormone.

The auxin treatments were further discovered to have no significantly different effects on total sugar, R-sugar, and sucrose of the leaf. However, IAA tended to increase the activity of photosynthetic process of the snake fruit tree, proved by the higher value of total sugar, R-sugar and sucrose content of leaf than those of IBA and NAA as presented in Table 2. Higher value of total sugar, R-sugar and sucrose content of leaf in IAA treatments resulted in a lower fruit-set failure, so that it increased the harvested fruits number and weight of fruit per tree in the off-season snake fruit. Mariotti *et al*. (2011) revealed that fruit-set to be the initial step of fruit development and that IAA plays a major role in its regulation to increase fruit-set

Furthermore, from the analysis conducted on different levels of concentration of IAA, 0 ppm/control (I_0) was found to be significantly lower than 25 ppm (I_1), 50 ppm (I_2) and 75 ppm (I_3) with the three concentrations not showing any substantial difference as shown in Table 1. Moreover, a higher percentage of fruit-set in IAA treated plants caused a significant increase in the weight of fruit per tree, number of fruits per tree and weight per fruit compared to control. The highest values for weight and number of fruits per tree were obtained at 50 ppm and found to be significantly higher than those of 0 ppm. However, there were no significant difference for those values among 25, 50 and 75 ppm. Similarly, the highest weight per fruit was obtained at 25 ppm and it was observed to be significantly higher than that in 0 ppm as shown in Table 1.

The data confirmed that spraying snake fruit trees with IAA positively affected the development of flowers into fruits by increasing the percentage of fruit-set, the weight of fruit per tree, number of fruits per tree and weight per fruit. Rai *et al*. (2016) found that the low percentage of fruit-set on snake fruit caused by the low content of auxin needed to increase the number of flowers into fruits (Fruit-set). In another study, Tiwari *et al*. (2012) reported the spraying of IAA (2×10^{-5} M) and NAA (2×10^{-5} M) on *Capsicum annuum* L. prevented flower and fruit abscission, balancing cell division and cell expansion during fruit growth in order to increase the quantity and quality of yield.

Similar to IAA concentration treatment, the percentage of fruit-set on control plant (B_0) was significantly lower than those treated with IBA 50 ppm (B_1), 100 ppm (B_2) and 150 ppm (B_3). In addition, the effects of all the treatments were found to be similar though in different concentrations. These data indicate the possibilities of using IBA and NAA to prevent the failure of fruit-set and, consequently, to increase the off-season fruit production of snake fruit. Furthermore, the favourable influence of auxins has been reported in minimizing fruit drop and improving the yield and fruit quality of date palm (Ahmed *et al*., 2013), increasing pollination and causing 100% parthenocarpy of fruits of oil palm (Somyong *et al.,* 2018), and increasing growth and yield of cowpea (Ullah *et al*., 2007). Moreover, Hifny *et al*. (2017) stated that spraying Washington Navel orange trees with NAA at 25 ppm decreased fruit drop percentage and increased significantly fruit weight, fruit size, fruit length, fruit diameter and fruit juice in comparison to control. Bhaure *et al*. (2014) also reported IBA 100 $mg.l^{-1}$ have promoted the yield of Winter Cherry (*Withania somnifera* (L.) Dunal.) during late rainy seasons by increasing floral initiation, fruit number, and enhancing nutrient uptake.

Furthermore, the fruit number and weight of fruit per tree which were found to be higher in the plant treated with IAA, IBA, and NAA compared to control were observed possibly due to the higher content of leaf RWC and chlorophyll as shown in Table 1. The data suggested the IAA, IBA, and NAA increased the water status and physiological process of the plant. This is in agreement with Ahmed *et al*. (2016) who found the pre-harvest anti-transpiration chitosan (polysaccharide biopolymers [$(C_8H_{13}NO_5)$ n] with a straight chain composed of 2000-3000 monomers) application on Navel orange to have a significant improvement on most of the studied fruit characters and leaf chemical constituents such as chlorophyll content, sugars, endogenous plant hormones (IAA, ABA and GA_3), as well as leaf nutritional status of N, P, K, Zn, Ca, B and Si. Therefore, it had positive effects on improving fruit weight and quality.

Table 2 shows that there was no significant difference in the effects of different concentration levels of IAA, IBA and NAA

Table 1. Effect of type and concentration of auxin on fruit-set, the relative water content of leaves, chlorophyll content of leaves, fruit weight per tree, fruit number per tree, and weight per fruit.

Treatment	Fruit-set (%)	Relative water content of leaves (%)	Chlorophyll content of leaves (SPAD)	Weight of fruit per tree (g)	Number of fruits per tree (unit)	Weight per fruit (g)
Auxin Type (A)						
IAA	59.80 a	88.65 a	75.42 a	2612.91 a	27.67 a	91.06 a
IBA	56.85 a	84.04 a	74.86 a	1214.98 a	28.25 a	40.54 b
NAA	56.48 a	82.25 a	73.43 a	1012.89 a	23.67 a	47.26 ab
LSD 5 %	34.64	27.47	17.64	1614.68	10.33	44.74
IAA Concentration						
0 ppm (I_0)	25.21 b	68.67 b	68.22 b	426.94 d	7.67 c	47.36 c
25 ppm (I_1)	72.51 a	81.00 a	75.08 a	2188.32 c	21.67 b	114.85 a
50 ppm (I_2)	69.16 a	81.83 a	78.50 a	4629.64 a	42.00 a	102.13 ab
75 ppm (I_3)	72.31 a	83.50 a	79.87 a	3206.76 b	39.33 a	99.91 b
LSD 5 %	15.66	6.39	5.57	517.82	7.82	12.81
IBA Concentration						
0 ppm (B_0)	30.05 b	69.83 b	67.96 b	299.36 c	13.00 c	26.07 b
50 ppm (B_1)	63.54 a	88.67 a	77.98 a	1473.52 ab	32.33 b	47.48 a
100 ppm (B_2)	70.93 a	88.33 a	76.17 a	1075.61 b	34.00 b	42.73 a
150 ppm (B_3)	62.89 a	89.33 a	77.32 a	2011.42 a	43.67 a	45.86 a
LSD 5 %	15.66	6.39	5.57	517.82	7.82	12.81
NAA Concentration						
0 ppm (N_0)	22.27 b	68.67 b	66.78 b	368.65 b	13.33 b	28.06 c
50 ppm (N_1)	62.74 a	84.33 a	80.94 a	1212.30 a	26.67 a	47.77 b
100 ppm (N_2)	67.15 a	87.83 a	79.47 a	1238.46 a	25.67 a	47.25 b
150 ppm (N_3)	73.77 a	88.17 a	78.54 a	1232.16 a	29.00 a	65.95 a
LSD 5 %	15.66	6.39	5.57	517.82	7.82	12.81

In a column, the numbers followed by the same letter for the treatment of auxin type and concentration of each type of auxin showed no significant effect based on LSD at P=0.05.

treatments on total sugar, R-sugar and sucrose of the leaf. However, those variables were higher in treated plants compared to those used as control. Similarly, the results revealed the ability of snake fruit plants to absorb N, P, and water needed to improve chlorophyll formation increased after it was sprayed with the auxins. This was associated with the treatments' ability to encourage the photosynthetic process as evidenced by the increased content of total sugar, R-sugar, and sucrose at all levels of concentration compared to control. This also led to the increment in photosynthate allocation to flower and fruit which consequently increased the percentage of flowers developed into fruit (fruit-set) as well as the number and weight of fruit per plant. The higher of chlorophyll and sugar contents in IAA, IBA and NAA treated plants compared to control indicated that exogenous auxin spraying has a good role in increasing the quality of the "source" (leaves) resulting in increased off season fruit production of snake fruit. Furthermore, it has been discovered from previous researches that the application of IAA or synthetic auxins to plants causes profound changes in growth and development (Liu, 2019) and influences physiological processes (Shin *et al*., 2015). This is possible because auxin is a prominent plant hormone functioning to regulate several aspects of growth and development by transmitting intrinsic and environmental signals into physiological processes (Simon and Petrasek, 2011; Kumari *et al.,* 2018). Action in plants has also been reported to depend on the cellular auxin level determined by the coordination of auxin transport and metabolism (Gallavotti, 2013; Gundesly *et al.,* 2020). In this study, auxins in the form of IAA concentration of 25, 50 and 75 ppm increased the fruit-set by 187.62, 174.34 and 186.83%, respectively, compared to 0 ppm (control). This was in accordance with Pandolfini (2009) and Kumar *et al*. (2014) findings who stated that exogenous application of auxin increases fruit-set in fruit crops.

Table 2. Effect of type and concentration of auxin to total sugar content, R-sugar content, sucrose content, and N and P content of leaves

Treatment	Total sugar content of leaves (mg/g)	R-sugar content of leaves (mg/g)	Sucrose content of leaves (mg/g)	N content of leaves (%)	P content of leaves (%)
Auxin type (A)					
IAA	0.64 a	0.46 a	0.27 a	2.12 a	0.13 a
IBA	0.57 a	0.43 a	0.24 a	2.07 a	0.12 a
NAA	0.55 a	0.36 a	0.18 a	2.05 a	0.11 a
LSD 5 %	0.13	0.21	0.21	0.33	0.05
IAA Concentration					
0 ppm (I_0)	0.48 a	0.29 a	0.16 a	1.93 a	0.09a
25 ppm (I_1)	0.52 a	0.43 a	0.18 a	2.14 a	0.10 a
50 ppm (I_2)	0.52 a	0.40 a	0.19 a	2.31 a	0.10 a
75 ppm (I_3)	0.57 a	0.36 a	0.20 a	2.10 a	0.12 a
LSD 5 %	0.16	0.16	0.26	0.27	0.03
IBA Concentration					
0 ppm (B_0)	0.59 a	0.32 a	0.25 a	2.00 a	0.12 a
50 ppm (B_1)	0.74 a	0.44 a	0.28 a	2.10 a	0.13 a
100 ppm (B_2)	0.65 a	0.43 a	0.32 a	2.10 a	0.13 a
150 ppm (B_3)	0.74 a	0.42 a	0.30 a	2.07 a	0.13 a
LSD 5 %	0.16	0.16	0.26	0.27	0.03
NAA Concentration					
0 ppm (N_0)	0.46 b	0.29 a	0.16 a	1.96 a	0.10 a
50 ppm (N_1)	0.49 ab	0.37 a	0.17 a	2.07 a	0.11 a
100 ppm (N_2)	0.64 a	0.40 a	0.23 a	2.11 a	0.12 a
150 ppm (N_3)	0.62 ab	0.39 a	0.22 a	2.07 a	0.11 a
LSD 5 %	0.16	0.16	0.26	0.27	0.03

In a column, the numbers followed by the same letter for the treatment of auxin type and concentration of each type of auxin showed no significant effect based on LSD at P=0.05.

Based on Tables 1 and 2, the difference in auxin types (IAA, IBA and NAA) did not have a significant effect on all observed variables, except for the weight per fruit, while for each type of auxin, IAA at concentrations of 25, 50, and 75 ppm and IBA and NAA at concentrations of 50, 100, and 150 ppm gave higher fruit-set than control and led to a significant increase in the weight and number of fruits harvested per tree and fruit weight per fruit. This result is very useful and important for the snake fruit growers where auxin in the form of IAA, IBA, and NAA can be applied to prevent fruit-set failure so that snake fruit can be harvested all year around.

Auxin in the form of IAA, IBA, and NAA significantly prevented the failure of fruit-set of snake fruit. The higher fruit-set in the IAA concentration of 25, 50 and 75 ppm led to a significant increase in the weight and number of fruits harvested per tree and fruit weight per fruit compared with control. The same results were observed in IBA and NAA, where the concentrations of 50, 100, and 150 ppm resulted in better yield than control. Therefore, IAA, IBA, and NAA are recommended to be applied for increasing the off-season fruit production of snake fruit.

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Conflict of Interest

All contributing authors declare no conflict of interests.

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