



## Gibberellic acid combined with hand pollination increases 'Red' and 'Lessard Thai' sugar apple fruit quality and produced parthenocarpic 'Gefner' atemoya fruits

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**ABSTRACT:** *The present research aimed to evaluate the effect of applying gibberellic acid ( $GA_3$ ) with hand (HP) or natural pollination (NP) on fruit set and the fruit quality of 'Red' and 'Lessard Thai' sugar apple and 'Gefner' atemoya fruits. This study was performed in an experimental orchard located in Homestead, Florida, USA. The experimental design included randomized blocks, with eight treatments, four replicates and four plants per plot. Treatments included: (1) HP; (2) HP + 10 mg L<sup>-1</sup>  $GA_3$ ; (3) HP + 100 mg L<sup>-1</sup>  $GA_3$ ; (4) HP + 1,000 mg L<sup>-1</sup>  $GA_3$ ; (5) NP; (6) NP + 10 mg L<sup>-1</sup>  $GA_3$ ; (7) NP + 100 mg L<sup>-1</sup>  $GA_3$  and (8) NP + 1,000 mg L<sup>-1</sup>  $GA_3$ . The HP plus 1,000 mg L<sup>-1</sup>  $GA_3$  promoted fruit setting above 90% over the 14 weeks for all genotypes evaluated. Significant increments for length and total fruit weight were observed. 'Red' sugar apple and atemoya had a reduced number of seeds per fruit. The NP plus  $GA_3$  (1,000 mg L<sup>-1</sup>) was effective in producing high quality seedless 'Gefner' atemoya fruits. This investigation demonstrated that  $GA_3$  plus hand pollination produced high quality sugar apple and atemoya seeded fruits and in association with natural pollination promoted seedless 'Gefner' atemoya fruits.*

**Key words:** *Annona squamosa, A. cherimola x A. squamosa, fruit set, parthenocarp, plant growth regulator, seedless fruits.*

### Ácido giberélico associado com a polinização manual incrementa a qualidade dos frutos em anonáceas e produz frutos partenocárpicos em atemoieira 'Gefner'

**RESUMO:** *O presente trabalho teve como objetivo avaliar o efeito da aplicação de ácido giberélico ( $GA_3$ ) associado com e sem polinização artificial, no pagamento e na qualidade de frutos das pinheiras 'Red' e 'Lessard Thai' e da atemoieira 'Gefner'. O experimento foi realizado em pomar localizado em Homestead, EUA. O delineamento experimental foi em blocos casualizados, com oito tratamentos, quatro repetições e quatro plantas por parcela. Os tratamentos utilizados neste experimento foram: 1) Polinização Artificial (PA); 2) PA + 10 mg L<sup>-1</sup>  $GA_3$ ; 3) PA + 100 mg L<sup>-1</sup>  $GA_3$ ; 4) PA + 1000 mg L<sup>-1</sup>  $GA_3$ ; 5) Polinização natural (PN); 6) PN + 10 mg L<sup>-1</sup>  $GA_3$ ; 7) PN + 100 mg L<sup>-1</sup>  $GA_3$ ; e 8) PN + 1000 mg L<sup>-1</sup>  $GA_3$ . A PA combinada com a concentração de 1000 mg L<sup>-1</sup> de  $GA_3$  promoveu o estabelecimento de frutos acima de 90% durante as 14 semanas de avaliação para todos os genótipos avaliados. Incrementos significativos para o comprimento e peso dos frutos também foram observados a variedade de pinha "Red" e a atemoieira, em que apresentaram redução no número total de sementes. A PN em associação com  $GA_3$  (1000 mg L<sup>-1</sup>) foi eficaz na produção de frutos sem sementes de alta qualidade em atemoieira 'Gefner'. Estes dados mostram que o  $GA_3$  combinado com a polinização manual, produziu frutos com semente de alta qualidade e, em associação com a polinização natural, promoveu frutos sem sementes em atemoieira 'Gefner'.*

**Palavras-chave:** *Annona squamosa, A. cherimola x A. squamosa, pagamento de frutos, partenocarpia, frutos sem sementes.*

Among the various management techniques for sugar apple and atemoya, hand pollination (HP) is unequivocally one of the most important since self-fertilization is negligible for this genus and natural pollination is not commercially effective. The HP ensures the formation of fruit with excellent commercial quality; its disadvantages include intensive labor requirements and a significant increase in the average number of seeds per fruit (PEREIRA et al., 2014a; RODRIGUES et al., 2016).

The development of technology for production of seedless fruits by applying plant growth-regulators in Annonaceae has been investigated

recently (FERRARA et al., 2014; PEREIRA et al., 2014a; SANTOS et al., 2016; TANG et al., 2015; MAHMOOD et al., 2016). Some studies with cherimoya (*Annona cherimola*) and the atemoya (*A. cherimola* x *A. squamosa*) suggested the possibility of using this technique to increase fruit set, produce parthenocarpic fruit and improve the physical and chemical qualities of the fruit (PEREIRA et al., 2014a; SANTOS et al., 2016).

Among such regulators, auxins and gibberellins are the most commonly used, with several studies reporting significant variations in plant growth regulator concentrations, combinations of regulators

and the number and times of applications. In general, atemoya is very responsive to plant growth-regulator applications; however, for sugar apple there is little to no information described in the literature.

The objective of this investigation was to determine the capacity of GA<sub>3</sub> to produce parthenocarpic fruits in sugar apple and atemoya. This research also aimed to verify the effect of gibberellic acid at different concentrations combined with hand pollination, on fruit set and the quality of 'Red' and 'Lessard Thai' sugar apple and 'Gefner' atemoya fruits, under South Florida conditions.

The study was conducted in an experimental orchard composed of three *Annona* varieties: 'Red' sugar apple (14-years-old), 'Lessard Thai' green sugar apple (11-years-old) and 'Gefner' atemoya (12-years-old). Plants were selected from a field plot at the University of Florida's Tropical Research and Educational Center (TREC) (25°50'N and 80°50'W, 3.8 m above sea level), in Homestead, FL, USA. The experimental design included randomized blocks, with eight treatments, four replicates and four plants per plot. The present research was planned considering two formerly published manuscripts (PEREIRA et al., 2014a; SANTOS et al., 2016). Three independent experiments were conducted with each variety. For each plant, eight flowers were selected, with a different treatment applied to each flower. The treatments were: (1) Hand pollination (HP); (2) HP + 10 mg L<sup>-1</sup> GA<sub>3</sub>; (3) HP + 100 mg L<sup>-1</sup> GA<sub>3</sub>; (4) HP + 1,000 mg L<sup>-1</sup> GA<sub>3</sub>; (5) Natural pollination (NP); (6) NP + 10 mg L<sup>-1</sup> GA<sub>3</sub>; (7) NP + 100 mg L<sup>-1</sup> GA<sub>3</sub> and (8) NP + 1,000 mg L<sup>-1</sup> GA<sub>3</sub>. In treatments with HP, GA<sub>3</sub> was applied at weeks 1, 3 and 5 after anthesis. In treatments with NP, GA<sub>3</sub> applications started at anthesis (female stage) in treatments 6, 7 and 8 and were repeated at weeks 1, 3 and 5 after anthesis.

The commercial product used was 10% GA<sub>3</sub> in the form of a soluble powder (Abbott Laboratories, São Paulo, SP, Brazil). Plant growth-regulator solutions contained 250 mL of non sterile, non-distilled water supplemented with 0.1% nonionic spreader sticker (mean pH of 8.17) were prepared on the day of each treatment application. First application of the solution targeted the female flowers (functionally pistillate). Each flower received the equivalent of 3 mL of solution using a spray bottle. HP was performed when the flowers reached the female stage by using pollen grains collected from *A. squamosa* and pollination was performed using a number 2 watercolor paintbrush, from 8:00 am to 9:00 am (TANG et al., 2015). The percentage of fruit set, physical characteristics and total soluble solids of

the fruits were evaluated. Results were subjected to analysis of variance, and the means were compared using Tukey's test, at a 5% probability.

The HP treatment alone exhibited 95%, 100% and 75% fruit set for the 'Red' and 'Lessard Thai' sugar apple varieties and 'Gefner' atemoya, respectively (Figure 1A, 1B and 1C). The use of NP alone and the combination of NP plus 10 and 100 mg L<sup>-1</sup> GA<sub>3</sub> did not result in enough set fruit for fruit quality evaluations. The combination of NP plus 1,000 mg L<sup>-1</sup> GA<sub>3</sub> resulted in mean fruit set of 5%, 25% and 90% for the 'Lessard Thai', 'Red' and 'Gefner', respectively (Figure 1D, 1E and 1F). Studies have shown that fruit set of many species is a complex trait and associated with the capacity to produce gibberellins, auxins and cytokinins by fruits and seeds (ZHANG et al., 2007; DE JONG et al. 2009). The differences observed in the present research pointed to a wide genetic divergence among the varieties evaluated.

HP plus 1,000 mg L<sup>-1</sup> GA<sub>3</sub> promoted a significant increase in fruit weight of 'Red' sugar apple and greater length, diameter and fruit pulp weight (Table 1). The HP plus 1,000 mg L<sup>-1</sup> GA<sub>3</sub> significantly increased fruit size of 'Lessard Thai' sugar apple and the 'Gefner' atemoya but fruit quality was similar (Table 1). 'Gefner' atemoya and 'Red' sugar apple fruits treated with NP plus 1,000 mg L<sup>-1</sup> GA<sub>3</sub> resulted in significantly lower fruit length, diameter, and weight when compared with HP plus 1,000 mg L<sup>-1</sup> GA<sub>3</sub>.

The NP plus 1,000 mg L<sup>-1</sup> GA<sub>3</sub> produced seedless 'Gefner' atemoya fruits and this same GA<sub>3</sub> concentration with HP resulted in a smaller number of seeds per 100 g of fruit and pulp without affecting fruit quality (Table 1). A previous study reported the efficiency of GA<sub>3</sub> at 1,000 to 1,500 mg L<sup>-1</sup> to produce seedless atemoya fruits (PEREIRA et al., 2014b). The authors described flowers exclusively treated with GA<sub>3</sub> and no hand pollinated had 65% to 80% fruit set. STIKIĆ et al. (2015) reported that gibberellins, when applied exogenously in plants, promotes parthenocarpy, and GA<sub>3</sub> is most commonly used for producing fruits quite similar to those produced in normal conditions. Our hypothesis is that the fruit set and the growth of non-pollinated GA<sub>3</sub>-induced atemoya ovaries is similar to seedy atemoya fruits; therefore, GA<sub>3</sub> triggers and maintains cell division in the ovary walls promoting fruit set and subsequent growth and development of 'Gefner' fruits in non-pollinated flowers.

'Red' sugar apple treated with HP plus 1,000 mg L<sup>-1</sup> GA<sub>3</sub> significantly increased pulp weight

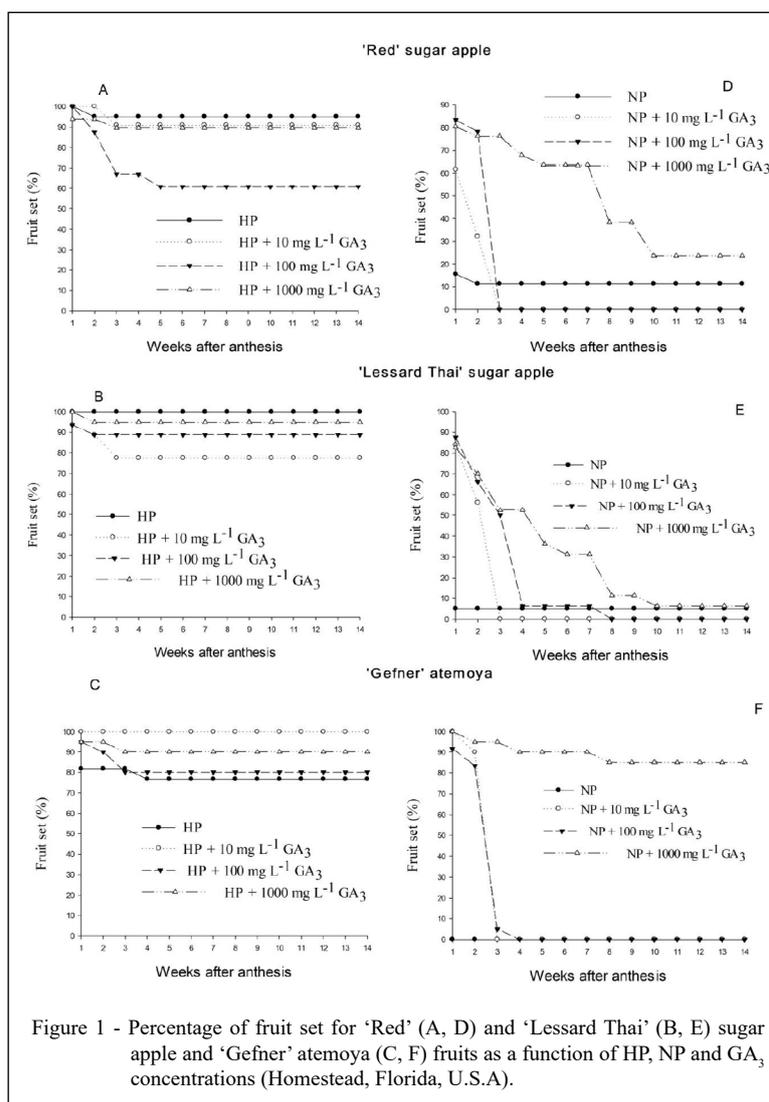


Figure 1 - Percentage of fruit set for 'Red' (A, D) and 'Lessard Thai' (B, E) sugar apple and 'Gefner' atemoya (C, F) fruits as a function of HP, NP and GA<sub>3</sub> concentrations (Homestead, Florida, U.S.A).

and decreased seed weight and number of seeds per 100 g of fruit pulp (Table 1). As expected, NP plus 1,000 mg L<sup>-1</sup>GA<sub>3</sub> treated 'Red' sugar apple had significantly smaller fruit and lower °Brix and percent pulp (~25% compared to ~35% for HP plus 1,000 mg L<sup>-1</sup>GA<sub>3</sub>). 'Lessard Thai' sugar apple treated with HP plus 1,000 mg L<sup>-1</sup>GA<sub>3</sub> had similar °Brix and significantly larger fruit and fewer seeds per 100 g of pulp than all other treatments (Table 1).

The HP plus 1,000 mg L<sup>-1</sup> GA<sub>3</sub> resulted in 90%, 90%, and 95% fruit set of 'Gefner' atemoya and 'Red' and 'Lessard Thai' sugar apple fruits, respectively (Table 1). This treatment also resulted in the largest fruit when compared to all other treatments. HP plus 1,000 mg L<sup>-1</sup> GA<sub>3</sub> applied to 'Gefner' atemoya may be an alternative for the production

of high-quality seeded fruits. Results of the present research is in agreement with a previous investigation conducted with the same hybrid but under Brazilian semi-arid conditions (SANTOS et al., 2016). This region is characterized by two main seasons (dry and rainy) with an average precipitation of 830 mm, mean annual temperature of 24 °C, average maximum of 30 °C, and minimum of 20 °C. The present research was established in Homestead and the climate is classified as marine subtropical climate. Average precipitation is 1473 mm per year, with mean annual temperature of 24 °C, average maximum of 29 °C, and minimum of 19 °C. Despite of these climatic differences atemoya was very responsive in both regions. These studies suggested that split applications of GA<sub>3</sub> concentrations between 500 and 1,000 ppm after

Table 1 - Hand and natural pollination associated to GA<sub>3</sub> in sugar apple and atemoya fruits on fruit diameter (DIA), fruit length (LGT), total fruit weight (TFW), pulp weight (PWE), total seed weight (TSW), number of seeds per fruit (NSF), number of seeds per 100 g pulp (NPU) and total soluble solids (SS) in Homestead, Florida, USA.

TREATMENT	-----'Red' sugar apple*-----							
	DIA (cm)	LGT (cm)	TFW (g)	PWE (g)	TSW (g)	NSF	NPU	SS (°Brix)
HP	92.97ab	92.78ab	346.10b	146.53ab	31.09a	89.66a	62.83a	23.15 a
HP +10 mg L <sup>-1</sup> GA <sub>3</sub>	91.92ab	91.92b	333.84bc	136.49b	29.24ab	84.43ab	64.24a	23.17 a
HP +100 mg L <sup>-1</sup> GA <sub>3</sub>	86.14b	90.42b	368.24bc	91.73c	21.93b	63.91c	71.08a	23.32 a
HP +1000 mg L <sup>-1</sup> GA <sub>3</sub>	104.36a	108.89a	475.10a	171.68a	23.85bc	76.93b	47.1b	22.84 a
NP +1000 mg L <sup>-1</sup> GA <sub>3</sub>	85.92b	89.13b	233.30c	59.30c	0.00d	0.00d	0.00c	16.10 b
CV (%)	7.1	7.6	14.0	12.7	13.0	8.4	11.8	5.1
	-----'Lessard Thai' sugar apple*-----							
HP	89.90b	91.52b	307.70b	121.91a	28.73a	76.79a	65.87ab	21.41 a
HP +10 mg L <sup>-1</sup> GA <sub>3</sub>	89.03b	94.44b	318.71b	116.46a	30.11a	80.25a	71.60a	22.80 a
HP +100 mg L <sup>-1</sup> GA <sub>3</sub>	92.96ab	94.80b	340.20b	130.43a	27.78ab	76.75a	62.16ab	20.80 a
HP +1000 mg L <sup>-1</sup> GA <sub>3</sub>	99.01a	105.0a	413.63a	150.48a	24.21b	72.35a	50.81b	20.97 a
CV (%)	3.1	3.3	8.7	12.2	6.8	8.1	13.9	5.0
	-----'Gefner' atemoya*-----							
HP	85.55b	103.06bc	352.70bc	163.63bc	35.74a	76.79a	48.63a	24.24 a
HP +10 mg L <sup>-1</sup> GA <sub>3</sub>	93.17b	110.13b	407.28b	184.48b	40.83a	90.78a	50.47a	24.74 a
HP +100 mg L <sup>-1</sup> GA <sub>3</sub>	95.83b	110.64b	420.28b	180.63b	37.42a	88.27a	49.27a	24.50 a
HP +1000 mg L <sup>-1</sup> GA <sub>3</sub>	114.20a	129.90a	664.42a	302.13a	40.97a	97.82a	33.29b	25.01 a
NP +1000 mg L <sup>-1</sup> GA <sub>3</sub>	86.42b	99.62c	256.53c	111.77c	0.01b	0.16b	0.14c	23.55 a
CV (%)	5.1	3.8	12.1	14.2	13.9	13.6	7.7	3.4

Data followed by the same letter in the column do not differ statistically at the 5% level of significance according to Tukey's test. CV: coefficient of variation.

\*The use of NP alone and the combination of NP with concentrations of 10 and 100 mg L<sup>-1</sup> GA<sub>3</sub> did not result in a sufficient number of set fruit for conducting the analyses planned for the three varieties evaluated.

anthesis promoted a favorable increase in all physical characteristics of the fruit without the loss of sensory and post-harvest quality (PEREIRA et al., 2014b; SANTOS et al., 2016).

The *Annonaceae* is characterized by producing fruits with a large number of seeds per fruit and seed weight is an important component, corresponding among 10 to 12% of total fruit weight. Another relevant result was the production of seedless 'Gefner' atemoya and the reduced seed weight per fruit in 'Red' sugar apple. Many investigations have reported that in general seedless fruits have lower weight, length and diameter compared to seeded fruits. Seedless fruit are highly desirable if fruit quality is not adversely affected. However, in many cases fruit size, shape and/or organoleptic characteristics are negatively affected by a lack of seeds.

Considering the findings of the present study and previous investigations, GA<sub>3</sub> plus HP can be considered as a new practice in order to improve fruit quality in sugar apple ('Red' and 'Lessard Thai')

and 'Gefner' atemoya. The next step is to develop new studies to adjust this methodology to the field conditions. Some questions must be elucidated: (1) How to apply the GA solution efficiently in all flowers? (2) At which floral development stage will treatments be most efficient? (3) How many flowers must be treated in order to achieve an economic return? Although, methodological adjustments are still needed and more studies will be necessary in order to elucidate and understand the mechanism of the parthenocarpy in atemoya, today it is can be considered a potential practice for producing seedless fruits in 'Gefner' atemoya.

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## DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

## AUTHORS' CONTRIBUTION

MCTP, JHC and SN conceived and designed research. MCTP and WM conducted experiments. MCTP, CLS and JSR analyzed data. SN, MCTP, JHC and CLS wrote the manuscript. All authors read and approved the manuscript.

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