BIOSCIENCE JOURNAL

ARTIFICIAL POLLINATION AND USE OF GROWTH REGULATORS IN ATEMOYA 'Gefner' FRUITS

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How to cite: CHAGAS, P.C., et al. Artificial pollination and use of growth regulators in atemoya 'Gefner' fruits. *Bioscience Journal*. 2023, **39**, e39040. https://doi.org/10.14393/BJ-v39n0a2023-65135

Abstract

The objective was to study the effectiveness of the growth regulator (ANA + GA_3) associated or not to the application of adjuvant and artificial pollination in 'Gefner' atemoya. The experiment was conducted in the experimental orchard at Florida's Tropical Research and Education Center. The experimental design was in a randomized block, with 14 treatments, 10 repetitions and 3 flowers per plot. The highest percentages of fixed fruits were obtained with hand pollination – HP and 450 NAA + 1250 GA_3 mg L^{-1} + adjuvant and HP. The use of hand pollination for 'Gefner' atemoya tree proved to be the most efficient method so far. Applying growth regulator without artificial pollination produces parthenocarpic fruits, however with high rate of abortions, and small fruits. Growth regulators together with hand pollination produces small and uneven fruits, and cause reduction in the fruits' titratable acidity. The use of adjuvant caused low fixation and toxicity to fruits, and its use is not recommended.

Keywords: *Annona cherimola* x *Annona squamosa*. Fruit fixation or fruit set. GA_3. NAAaa. Parthenocarpy. Plant growth regulators.

1. Introduction

Atemoya tree is an interspecific hybrid created with the crossing of sugar-apple and cherimoya (*Annona squamosa* L. x *Annona cherimola* Mill.). The hybrid yields fruits with qualitative characters of both species, presenting, however, as strong characteristic, cherimoya sweet taste, much appreciated by consumers (Santos et al. 2019).

The annonaceae flowers are hermaphrodite, however they present protogynous dichogamy, that is, the female organ (gynoecium) occurs before the male organ (androecium), thus preventing self-fertilization with the pollen grain from the same flower (Araújo et al. 2021). This phenomenon, associated to the small opening of the flowers' petals during the female phase, hinders natural pollination (Kishore et al. 2012). The natural pollination is performed by small beetles and in commercial plantations, the variation of formed fruits are small, varying according to the number of visits and the pollinator species, from 10% (one visit) to 25% (*C. domidiatus*) or 35% (*C. Hemipterous*) with five visits, depending on the

pollinator (Kishore et al. 2012). However, in a tropical climate it is necessary that visits to flowers exceed 16% for effective fruiting (Jenkins et al. 2013).

Among the management techniques for the culture or crop, hand pollination is definitely one of the most important, and is often used due to its high fixation rate, size and evenness of fruits. However, this technique disadvantage is that it generates a large number of seeds in the fruits and presents high costs associated to work (Chagas et al. 2022).

The use of plant growth regulators in species of the Annonaceae family has been studied as an alternative to reduce costs, increase yield and generate fruits with commercial quality. Gibberellins and auxins are the most used plant growth regulators. These studies have demonstrated success in the production of seedless fruits, by parthenocarpy (Prado Verotti et al. 2019; Santos et al. 2019).

However, only 8% of fixation of 'Gefner' atemoya fruits was observed where 450 mg L^{-1} of ANA was applied 148 days after the first application (Mota Filho et al. 2012), while (Pereira et al. 2014b) observed 87% of fixation of fruits using the same dose, also in 'Gefner' atemoya. This controversial effect may have occurred due to the date when the treatment with NAA was made, or even the inhibition of the pollen grain germination, interrupting the pollen tube growth (Reig et al. 2014). Therefore, complementary studies are necessary to clarify the effect of plant growth regulators in annonaceae species.

Thus, considering the fact that a profitable production is based on productivity and fruits quality, and information in the literature still have to be clarified; the objective of the present study was to assess 'Gefner' atemoya trees or flowers response when subject to the use of growth regulators (GA_3 and NAA) associated or not to the application of adjuvant and artificial pollination.

2. Material and Methods

Experimental conditions and experiment location

The experiment was conducted in experimental 'Gefner' atemoya (*A cherimola* x *A. squamosa*) orchard, variety 'Lessard Thai', Homestead, Florida, USA, at the Florida Tropical Research and Education Center (TREC/UF), (25° 30' 40,809'' N, 80° 30' 3.983'' W), altitude 3.8 m, subtropical climate. The location mean annual precipitation is 1,490 mm, based on meteorological data collected in TREC by the Florida Automated Weather Network (http://fawn.ifas.ufl.edu/), with most rainfalls (70%) concentrated in late May until early November (Ali et al. 2000). The soil is clayey (Noble et al. 1996), well drained, with overlapping of limestone layer and presence of rocks. During the experimental period, meteorological data were collected: rainfall, relative humidity, maximum, average, and minimum temperature (Figure 1).

Plant material and experimental design

The experiment was installed on February 10, 2018. Ten plants of 'Gefner' atemoya were selected, considering uniformity, force and soundness. The plants were 18 years old and were cultivated in a 6 m x 4 m spacing. They were mechanically and manually pruned in the second fortnight of February 2018. The irrigation system used was conventional spraying twice a week, four hours a day. Invasive plants were controlled between rows with mechanical trimmer at each two months, and in the plantation line, canopy projections, chemical control was made. Fertilization was conducted according to soil analysis and culture demand, after production pruning.

The experimental design was in a randomized blocks with 14 treatments and 10 repetitions, with 3 flowers per portion or plot, and to each plant of each cultivar, treatments described in Table 1 were applied.

Hand pollination (HP) was performed according to the following procedures: pollen grains were collected from flowers in male stage, in the morning, at 6:00 am. and placed in in Eppendorf type flasks T-25 (3 to 7 mL). By using a brush number 6, the pollen grains were deposited on the stigma of flowers in female stage, as described by Chagas et al. (2022).

The following commercial products were used: PoMaxa (Valente BioSciences Corporation Technology Way, Libertyville, IL, USA) with 3.1% of 1-Naphthalene Acetic Acid (NAA); ProGibb LV Plus

(Valente BioSciences Corporation Technology Way, Libertyville, IL, USA) with 5.7% of Gibberellic acid (GA_3) and Adjuvant LI 700 (Loveland Products, Greeley, CO, USA).

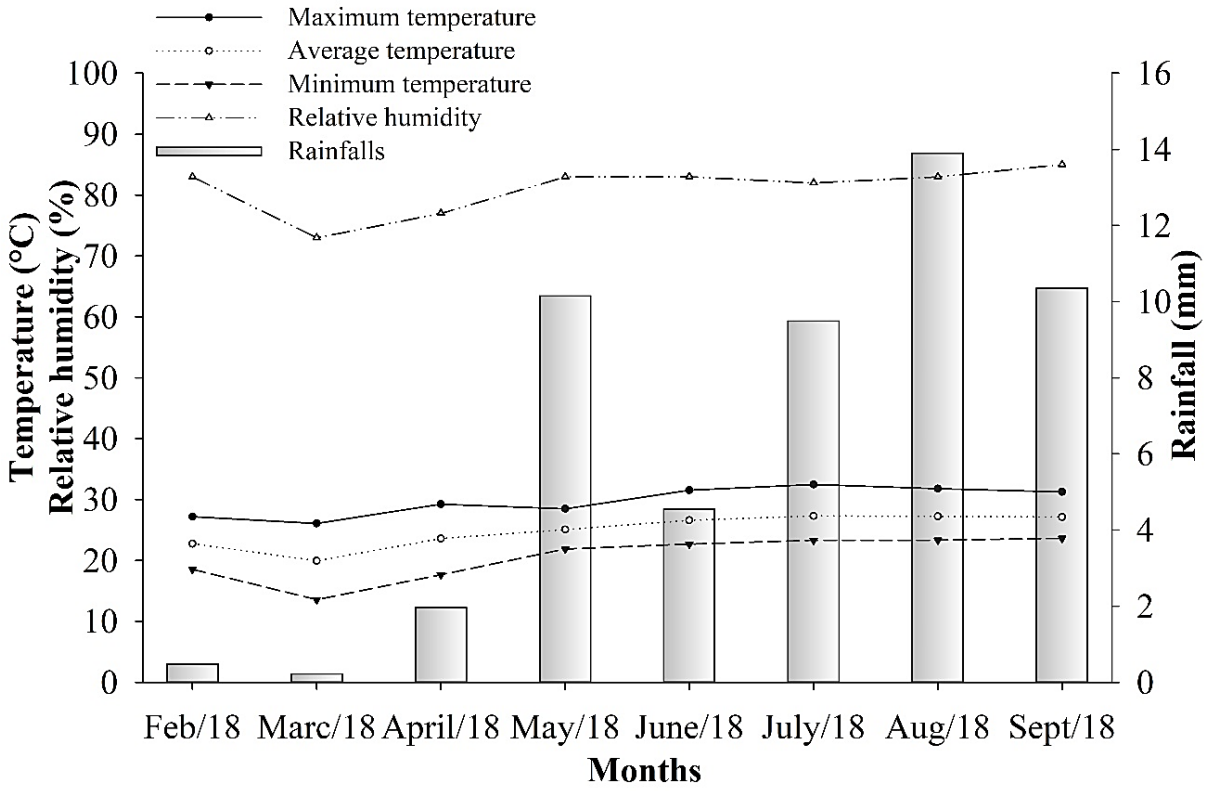

Figure 1. Rainfall (mm), relative humidity (%) and maximum (° C), average (° C) and minimum (° C) temperatures for the months of the Homestead - Florida-USA evaluation year, 2018.

Table 1. Treatments applied to flowers at anthesis and after anthesis of the 'Gefner' atemoya.

Treat	NAA[z]	GA_3[z]	LI 700 Adjuvantz	Hand Pollination	Period of application of the solutions
T1	0	0	No	No	0
T2	0	0	No	Yes	0
T3	0	0	Yes	No	Female stage
T4	0	0	Yes	Yes	Female stage
T5	450	750	Yes	Yes	Female stage, 1 and 3 WAA
T6	450	1000	Yes	Yes	Female stage, 1 and 3 WAA
T7	450	1250	Yes	Yes	Female stage, 1 and 3 WAA
T8	450	1500	Yes	Yes	Female stage, 1 and 3 WAA
T9	450	750	Yes	No	0
T10	450	1000	Yes	No	0
T11	450	1250	Yes	No	0
T12	450	1500	Yes	No	0
T13	450	1000	No	No	0
T14	450	1000	No	Yes	Female stage, 1 and 3 WAA

z, WAA, weeks after anthesis; NAA, naphthalene acetic acid; GA_3, gibberellic acid; ppm, parts per million; LI 700 surfactant at 1.5% of the commercial rate.

The solutions were prepared with dilution of the commercial products in distilled water, using 150 mL volumetric flask. In treatments with adjuvant 1.5% concentration of the commercial product was used. Later they were stored in “spray” type flasks at a temperature of 5 ºC, in refrigerator, during 24 hours.

On May 09, 2018, during the flowering period, closed flowers (pre-anthesis stage) were selected, tagged according to each treatment and protected from pollinators with white paper bags.

In the following morning, the solutions were applied, directing them to the flowers during anthesis (flowers in female stage) and each flower received approximately 0.5 mL (2 sprinkles) on the stigma (Pereira et al. 2014a). In treatments without hand pollination, the flowers were once again protected with

white paper bags, thus avoiding natural pollination. All treatments were applied on the same day. For treatments with plant regulators, two additional applications were made 7 and 21 days after the anthesis.

Determination of the evaluated characteristics

Fruit fixation was assessed one week after the anthesis and then at each two weeks. The harvest of fruits started in the 13th week after anthesis (92 days) and was made when they presented distance from the peel carpels and light pink color of tissues between carpels, as described by Moura et al. (2021).

After harvest, the following characteristics were assessed: Fruit mass (FM); Peel + stalk mass (PSM); and Seed mass (SM), by using a digital scale (±0.01 g), and results were expressed in grams (g). Fruit length (FL) and Fruit diameter (FD) were assessed with digital caliper (±0.01 g). Number of seeds per fruit (NS) was assessed when found with manual counting. Shape of fruits was assessed according to classification proposed by Pereira et al. (2014b), where fully irregular fruits obtained grade 25%; partially irregular, grade 50%; regular, with little deformation, grade 75%; and fully regular (perfect round fruit), grade 100%.

By the time of ripening, nine fruits of each treatment were assessed, with three 3 repetitions with three fruits by repetition. The fruits were assessed as to: Pulp yield – calculated by subtracting PSM and SM from FM, expressed in percentage (%). Soluble solids (SS), determined in digital refractometer, with values expressed in °Brix (AOAC 2012); pH (hydrogen-ion potential) and titratable acidity (TA), determined by titrimetry with sodium hydroxide solution (0.1N) using Compact Titrator G20S (Mettler Toledo®) and results were expressed in grams of citric acid, 100 g^{-1} of pulp (AOAC 2012). The ratio was calculated by the relation between SS/TA contents.

Statistical analysis

Data were submitted to normality (shapiro-Wilk) and homogeneity (Bartlett) tests ($p<0.05$). Two-way analysis of variance was performed by the F test ($p<0.05$) followed by the Tukey test ($p<0.05$). The relationship between the parameters evaluated were estimated considering the Pearson correlation coefficient ($p <0.05$). Multivariate analysis of data was performed by principal component analysis (PCA). All statistical analyses were performed in R software (R Core Team 2020). For values outside normality and homogeneity standards, data were transformed into $(x+1)^{0.5}$.

3. Results

The percentage of 'Gefner' atemoya fruits' fixation reduced over the 11 weeks after the anthesis (WAA) for all treatments. After this period there was no longer fall of fruits until harvest. However, until the 1st WAA, all treatments with growth regulator (NAA and GA_3) presented excellent percentages, ranging from 93 to 100%. The highest percentages of fixed fruits were obtained with hand pollination – HP (T2) and 450 NAA + 1250 GA_3 mg L^{-1}+ adjuvant and HP (T7), with 40% of fruit fixation (Table 2).

Treatments without hand pollination, fruit fixation percents were very low, ranging from 0 to 17%, (Table 2). The use of the adjuvant proved to be ineffective in the increase of fruit fixation, and 82.5% (T4) less fruit set was observed when the adjuvant was applied in treatments without growth regulator.

For treatments T1, T3, T4, T9, T10, T11, T12 and T13, the physical and physical-chemical quality assessment was not made due to the absence or insufficient number of fruits for statistical analysis.

The growth regulators increased the number of irregular fruits, and the highest level of fruit deformation was observed in treatments with adjuvant ($p<0.05$). The fruits that received growth regulators (450 NAA+ 1000 GA_3 mg L^{-1}) without adjuvant + PM (T14), presented lower deformity level, which did not statistically differ from the fruits that received only hand pollination (T2) ($p>0.05$).

The fruits produced by the plants that received only hand pollination (T2) presented superior physical quality compared to those fruits originated by growth regulators applications (Table 3). The largest fruits were considered by observing larger mass of fruits, number of seeds and pulp of fruits, which were significantly superior ($p<0.05$). Therefore, new studies must be conducted testing different concentrations

and methods for application of growth regulators in order to obtain increase in fruits' size, and with that, more profitability to farmers.

Table 2. The percentage of fruit set of the 'Gefner' atemoya after after treatment applications with or without growth regulators (NAA and GA_3) and LI 700 adjuvant and with or without hand pollination.

Treatments	NAA	GA_3	LI 700 Adjuvant	Hand Pollination	Weeks after anthesis (WAA) Fruit Set (%)					
					1	3	5	7	9	11
1	0	0	No	No	13	0	0	0	0	0
2	0	0	No	Yes	57	40	40	40	40	40
3	0	0	Yes	No	13	0	0	0	0	0
4	0	0	Yes	Yes	23	13	10	10	7	7
5	450	750	Yes	Yes	100	47	33	23	23	23
6	450	1000	Yes	Yes	97	73	57	37	37	37
7	450	1250	Yes	Yes	100	77	63	53	50	40
8	450	1500	Yes	Yes	93	77	60	37	37	37
9	450	750	Yes	No	97	60	23	7	3	3
10	450	1000	Yes	No	90	50	40	27	20	17
11	450	1250	Yes	No	97	57	47	27	20	17
12	450	1500	Yes	No	97	80	57	20	17	7
13	450	1000	No	No	100	60	30	10	10	10
14	450	1000	No	Yes	100	50	37	30	30	30

NAA: naphthalene acetic acid; GA_3: Gibberellic acid; ppm: parts per million, Adjuvant: 1.5% of the commercial product LI700.

For soluble solids and pH variables there was no statistical difference across treatments ($p>0.05$) (Table 3). For titratable acidity (TA), the fruits with hand pollination alone (T2) presented the highest contents (0.39 g citric acid.100 g $pulp^{-1}$), while the fruits treated with 450 NAA + 1000 GA_3 mg L^{-1}, with adjuvant and HP obtained the lowest content (0.26 g citric acid.100 g $pulp^{-1}$) of TA (Table 3). It was observed that treatments with lower number of seeds, T6 and T8, presented the lower average contents of TA.

With regard to SS/TA ratio, the fruits treated with 450 NAA, 1000 GA_3 mg L^{-1}, adjuvant and HP (T6) obtained the highest means (115.90), while the fruits treated with hand pollination (T2) presented the lower contents of SS/TA (80.21) (Table 3). These results directly reflect the TA contents, producing sweeter fruits in treatments with low TA contents.

Table 3. Physicochemical characteristics of 'Gefner' atemoya fruits: fruit shape (%), fruit length – FL (mm), fruit diameter – FD (mm), fruit mass - FM (g), peel + stem mass - PSM (g), seed mass - SM (g), number of seeds per fruit – NS, percent of the pulp (%), soluble solids - SS (°Brix), hydrogen potential (pH), titratable acidity - TA (g citric acid 100 g $pulp^{-1}$), Ratio - SSTA after application of different treatments with or without growth regulators (NAA and GA_3) and adjuvant, and with hand pollination.

*Treat	2	5	6	7	8	14	C.V. (%)
Shape	91.66^{a}	52.77^{b}	58.33^{b}	58.33^{b}	55.55^{b}	72.22^{ab}	10,99
FL	121.12^{a}	103.30^{ab}	80.31^{c}	93.56^{bc}	90.18^{bc}	90.44^{bc}	33,85
FD	105.21^{a}	78.43^{b}	61.85^{c}	77.17^{bc}	69.58^{bc}	74.71^{bc}	7,26
FM	591.27^{a}	328.48^{b}	154.67^{c}	277.94^{bc}	226.18^{bc}	257.02^{bc}	20,08
PSM	112.05^{a}	75.34^{bc}	51.16^{c}	76.42^{bc}	66.46^{bc}	83.80^{b}	12,69
SM	24.01^{a}	8.95^{b}	2.42^{c}	5.73^{bc}	4.37^{bc}	5.41^{bc}	27,71
NS	51.44^{a}	22.66^{b}	6.66^{c}	15.44^{bc}	12.00^{bc}	13.00^{bc}	29,87
Pulp	76.97^{a}	70.43a^{b}	63.40^{b}	67.25^{ab}	67.06^{ab}	62.04^{b}	6,32
SS	31.37^{a}	31.26^{a}	30.25^{a}	30.55^{a}	30.86^{a}	32.31^{a}	3
pH	4.57^{a}	4.54^{a}	4.64^{a}	4.63^{a}	4.74^{a}	4.64^{a}	1,3
TA	0.39^{a}	0.34^{ab}	0.26^{c}	0.33^{abc}	0.31^{bc}	0.32^{abc}	1,9
SSTA	80.21^{b}	92.41^{ab}	115.90^{a}	95.26^{ab}	99.91^{ab}	102.90^{a}	8,4

Means followed by the same letter in the line do not differ from each other by the Tukey test at the 5% probability level. * T2: 0ppm de NAA, 0ppm de GA_3, sem adjuvante, com polinização manual; T5: 450ppm de NAA, 750ppm de GA_3, com adjuvante, com polinização manual; T6: 450ppm de NAA, 1000 ppm de GA_3, com adjuvante, com polinização manual; T7: 450ppm de NAA, 1250ppm de GA_3, com adjuvante, com polinização manual; T8: 450ppm de NAA, 1500ppm de GA_3, com adjuvante, com polinização manual; T14: 450ppm de NAA, 1000ppm de GA_3, com adjuvante, com polinização manual. NAA: naphthalene acetic acid; GA_3: Gibberellic acid; ppm: parts per million, Adjuvant: 1.5% of the commercial product LI700.

The multivariate analysis of data conducted through the principal components analysis (PCA) confirmed the results previously found in the univariate analysis. PCA presented 89.03% of variance, of which 79.16% was obtained in PCA1 and only 9.87% in PCA2 (Figure 2).

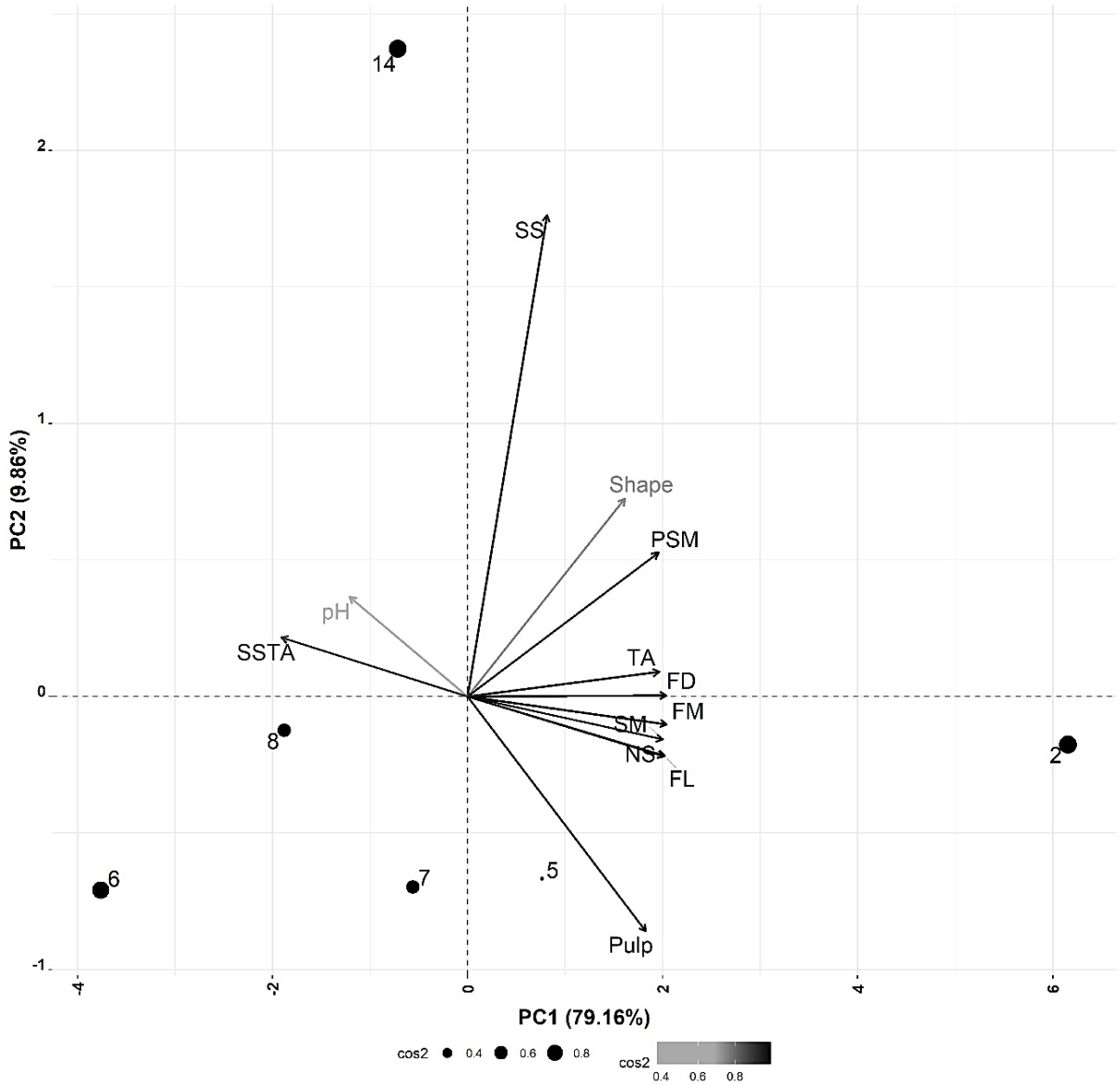

Figure 2. Principal component analysis (CP) performed in different treatments on the physical and physical-chemical quality characteristics. Treatments – 2 (with HP and without adjuvant), 4 (with HP and with adjuvant), 5 (450 NAA + 750 GA_3 with HP and with adjuvant), 6 (450 NAA + 1000 GA_3 with HP and with adjuvant), 7 (450 NAA + 1250 GA_3 with HP and with adjuvant), 8 (450 NAA + 1500 GA_3 with HP and with adjuvant), 14 (450 NAA + 1000 GA_3 with HP and with adjuvant).

Treatments with hand pollination (T2) and 450 NAA + 1000 GA_3 mg L^{-1} + adjuvant + PM (T6) were those that contributed most to PCA1, with 66,52% (r = 0.98, p<0.001) and 24.83% (r = 0.88, p<0.001), respectively. For PCA2, only the treatment with 450 NAA + 1000 GA_3 mg L^{-1} + adjuvant + PM contributed significantly, with 79.18% (r= 0.90; p<0.001).

The variables that presented higher correlations with PCA1 were FL (r=0.99; p=0.00001), FD (r= 0.99; p=0.00003), FL (r=0.96; p=0.0002), NS (r=0.98; p=0.0004), SM (r=0.98; p=0.0008), TA (r=0.96; p=0.002), PSM (r=0.96; p=0.02), Pulp (r=0.89; p=0.01) and SS/TA (r= -0.93; p=0.006). These variables showed higher correlation with treatment T2, confirming the results previously reported with regard to fruits size and sweetness.

For PCA2, only SS presented correlation (r=0.86; p= 0.02), showing negative correlations with treatments that received adjuvant (T5, T6, T7 and T8), confirming that the use of these products contributed to lower contents of SS.

4. Discussion

The low fixation rate of atemoya tree fruits may have occurred due to incompatibility mechanisms that act in the reproduction process of species from the annonaceae family. Moreover, it was observed that environmental conditions may cause severe damages to the reproduction process of several species, causing variations in fruits' fixation rate (Urbanowicz et al. 2018; Araújo et al. 2021). These variations in the environment may cause drying of pollen grains, thus contributing to low feasibility (Hinojosa et al. 2019; Martin et al. 2019; Pacini and Dolferus 2019).

The low rates of fruit fixation when NAA was applied become more evident when observe the treatments that did not received hand pollination, where fixation percents were lower. NAA harmful effect acts by inhibiting the germination of pollen grains and/or interrupting the pollen tube growth (Reig et al. 2014).

There are currently no reports of studies assessing *in vitro* this response to for NAA annona species, these responses were already confirmed in several cultures (Reig et al. 2014), and *in vivo,* variations are observed in fixation percents in treatments with NAA for atemoya, ranging from 8% of fixation (Mota Filho et al. 2012) to 87% of fruit fixation (Pereira et al. 2014b) The application period is one of the factors that can most influence the fixation percentage (Reig et al. 2014; Khalate et al. 2018).

The irregularly shaped fruits were due to the absence of seeds, caused by the non-fertilization of multiple ovaries, is due to the poor deposition of pollen grains or optimization of the stigmatic exudate (Lau et al. 2017; Meade and Parnell 2018; Santos et al. 2019).Treatments with application of chemicals (adjuvant and/or growth regulators), The spraying of the solution carried part of the pollen grains deposited on the stigma, causing malformation of the fruits.

The present work pointed a low effectiveness of the plant growth regulators to the growth of 'Gefner' atemoya tree fruits, however, it enabled the production of fruits with lower amounts of seeds (Table 3). On the other hand, several authors report the contribution of growth regulators (GA_3) in the production of fruits. Likewise, Pereira et al. 2014a, while testing doses of GA_3 in 'Gefner' atemoya observed that the fruit mass increased according to the dose increase, obtaining in the 1000 GA_3 mg L^{-1} dose the maximum value of 196.49 g. Therefore, we can observe that the isolated use of GA_3 provides improvements in the fruits' size, a fact that was not observed when used in combination with NAA.

Though statistical differences were verified for the fruits physical-chemical variables, causing changes in the fruits quality content, all treatments presented excellent quality, with pleasant taste for consumption. Changes in the fruits' chemical contents with different concentrations of growth regulators were observed by Prado Verotti et al. (2019).

The lower TA content in treatments is correlated with the number of seeds in the fruits. The higher the number of seeds, the higher the contents of TA (r=0.90; p=0.001) and lower the contents of SS/TA ratio (r=-0.89; p=0.001). Similar responses were obtained by (Kumar et al. 2014) and (Galimba et al. 2019) in whose studies the application of growth regulators reduced NS and TA. One possible explanation is that auxin induces the production of ethylene, accelerating the ripening of fruits (Kumar et al. 2014).

5. Conclusions

The use of hand pollination for 'Gefner' atemoya tree proved to be the most efficient method, presenting good fixation of fruits and production of fruits with excellent commercial quality.

Applying growth regulator without artificial pollination produces parthenocarpic fruits, however, fruit fixation is low and the fruits are small. Growth regulators together with hand pollination produces fruits with pleasant taste; however, they are small and a level of unevenness in shape, and cause reduction in fruits' titratable acidity.

The application of ANA leads to high rates of fruit abortion, and so complementary studies are necessary to seek new methods and dosages in applications. The use of adjuvant led to low fixation and toxicity of fruits, and its use is not recommended.

Authors' Contributions: CHAGAS, P.C: conception and design, acquisition of data, analysis and interpretation of data and drafting the article; CRANE, J.H.: drafting the article; CHAGAS, E.A.: conception and design, acquisition of data, analysis and interpretation of data and drafting the article; VENDRAME, W.: drafting the article; COSTA, B.N.S.: acquisition of data and drafting the article; R. NETO, A.: acquisition of data and drafting the article; MOURA, E.A.: analysis and interpretation of data, drafting the article and critical review of important intellectual content. Al authors have read and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: Not applicable.

Acknowledgments: The authors are grateful to CAPES (Process POS-DOC - 88881.120512/2016-01) and CNPq for Pollyana C. Chagas and Edvan A.Chagas scholarships.

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Received: 21 April 2022 | **Accepted:** 11 October 2022 | **Published:** 10 March 2023