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Gibberellic acid induces parthenocarpy and increases fruit size in the 'Gefner' custard apple (*Annona cherimola* x *Annona squamosa*)

Rayane Carneiro dos Santos, Marlon Cristian Toledo Pereira, Débora Souza Mendes, Raquel Rodrigues Soares Sobral, Silvia Nietsche, Gisele Polete Mizobutsi, Bruna Hanielle Carneiro dos Santos

Departamento Ciências Agrárias da Universidade Estadual de Montes Claros, 39440-000, Janaúba, Minas Gerais, Brazil

***Corresponding author: marlon.pereira@unimontes.br**

Abstract

The biological function of fruiting is to produce and disseminate seeds. However, seedless fruits have some advantages, including the ease of consumption and the increased added value of the final product and fruit quality. The present study aimed to evaluate the combined use of gibberellic acid (GA_3) and hand pollination with *Annona squamosa* pollen grains on fruit set and the physical and chemical parameters of 'Gefner' custard apple fruits. The experiment was performed in a commercial custard apple orchard in a randomized design, with 11 treatments, four replications and five plants per parcel. The treatments applied were as follows: (1) hand pollination with the pollen of sugar apple (HPPSA) (control); (2) HPPSA + 500 ppm GA_3 applied on the 1st week after anthesis (WAA); (3) HPPSA + 500 ppm GA_3 applied on the 1st and 3rd WAA; (4) HPPSA + 500 ppm GA_3 applied on the 1st, 3rd and 5th WAA; (5) HPPSA + 1,000 ppm GA_3 applied on the 1st WAA; (6) HPPSA + 1,000 ppm GA_3 applied on the 1st and 3rd WAA; (7) HPPSA + 1,000 ppm GA_3 applied on the 1st, 3rd and 5th WAA; (8) 1,000 ppm GA_3 applied at flowering and on the 1st and 3rd WAA; (9) 1,000 ppm GA_3 applied at flowering and on the 1st, 3rd and 5th WAA; (10) 1,500 ppm GA_3 applied at flowering and on the 1st and 3rd WAA, and (11) 1,500 ppm GA_3 applied at flowering and on the 1st, 3rd and 5th WAA. A total of 20 plants were used. The harvest was performed at the 18th WAA. The fruit set rate, number of seeds per fruit, fruit length, fruit diameter, fruit mass, pulp mass, peel mass and fruit chemical characteristics were evaluated. The results were submitted to an analysis of variance, and the means were compared by the Tukey test ($P > 0.05$). A significant difference among the treatments was not observed for fruit set, presenting an average of 77% at the 18th WAA. Three applications of 500 ppm GA_3 plus hand pollination induced the production of fruits with increased length, diameter and mass. Fruits produced by hand pollination alone or combined with GA3 had a lower percentage of fruit peel. The use of 1,500 ppm of gibberellic acid is efficient for producing seedless fruits in custard apple with high physical and chemical parameters.

Keywords: *Annona squamosa x Annona cherimola* Mill., gibberellic acid, plant-growth regulator, seedless fruits, fruit set.
Abbreviations: GA_3_gibberellic acid, HP_hand pollination, WAA_week after anthesis, HPPSA_hand pollination with pollen of the sugar apple.

Introduction

Custard apple fruit production and quality may be affected by several factors. However, the factors involving the pollination process are undoubtedly the most important. The difficulty involving natural pollination is due to the protogynous dichogamy phenomenon; that is, although custard apple trees have hermaphrodite flowers, self-pollination is nearly impossible because the stigma becomes receptive or viable long before the pollen is released (Campbell and Phillips, 1994). This botanical phenomenon is a factor associated with a low degree of pollination and fertilization, affecting not only the quantity but also the quality of the fruits produced (Pereira et al., 2011). Custard apple fruits develop normally with the uniformity desired by the consumer market when they are pollinated artificially. However, these fruits have a large number of seeds and high costs associated with labor (Janick and Paul, 2006). Studies have shown that the fruit setting of many species may be promoted through the application of gibberellins, auxins and cytokinins, without requiring pollination. Studies conducted on tomato plants show that the exogenous application of auxins in nonpollinated flowers induces fruit setting and growth (Dorcey et al., 2009). Similarly, gibberellins play a key role in the fruit setting and growth process. Studies of species belonging to the *Annonaceae* family have reported an effect of gibberellins on fruit setting, increased fruit size and the production of seedless fruits (Saavedra, 1979, Yonemoto et al., 2004; Pereira et al., 2014). Saavedra (1979) published one of the first reports on the use of gibberellic acid (GA_3) in Annonaceae. The use of this plant-growth regulator promoted the production of seedless fruits and increased fruit length and diameter in the Concha Lisa cherimoya variety. Pino (2008) showed that the combination of gibberellins and cytokines was also efficient in the generation of seedless fruits in cherimoya. Yonemoto et al. (2004), working with 'Big Sister' and 'Suiho' cherimoya cultivars, succeeded in growing seedless fruits with the same quality as the pollinated fruits. Recent studies also showed that applying 1,000 ppm GA_3 without hand pollination enabled the production of parthenocarpic custard apple fruits (Pereira et al., 2014). Despite these significant advances, seedless custard apple fruits still do not have the desired standard commercial grade, indicating the need for more detailed studies in order to achieve this goal. Considering the findings to date, the present study aimed to assess the effect of applying GA_3 in different doses and numbers of applications, with and without HP, on the setting and quality of 'Gefner' custard apple fruits with and without seeds, under irrigated conditions in the Brazilian semiarid region.

Results and Discussion

Fruit set

No significant differences were observed ($P > 0.05$) for the fruit set rate among the 11 treatments during the 18 weeks after anthesis (WAA) (Table 2). The HP (control) presented an average of 80% fruit set (Table 2). Similar results were obtained by Melo et al. (2002) in the State of São Paulo, Brazil. The 'Gefner' custard apple hand pollinated with sugar apple pollen grains presented an average of 80.5% fruit set. The use of HP combined with two applications of 500 ppm GA_3 after anthesis provided an average rate of 90% fruit set (Table 2). The fruit set rate of flowers exclusively treated with GA_3 without hand pollination varied from 65% to 80% (Table 2). This result shows that applications of 500 ppm GA_3 at anthesis and the 1st and 3rd WAA are able to promote 'Gefner' custard apple fruit setting and can be used as an alternative to hand pollination. Similar behavior was observed in Homestead, South Florida. 'Gefner' custard apple flowers treated with 1,000 ppm GA_3 at anthesis and at the 1st, 3rd and 5th WAA without hand pollination (HP) presented an average of 80% fruit set by fruit harvest at the 19th WAA (Pereira et al., 2014). Another study also showed that the number of applications of plant-growth regulators affects the fruit set. Six periods of applications of gibberellin or a mixture of gibberellin and cytokinin provided up to an 84% fruit set of cherimoya (Pino, 2008). Fruit fall was observed in two periods. The first fall occurred from the 4th WAA and the second from the 9th WAA, stabilizing thereafter until the harvest (Table 2).

Fruit growth

The growth curves followed a sigmoidal pattern, and the logistic model was adjusted to fruit length and fruit diameter. The expressed fruit length and diameter growth rate showed an uninterrupted increase in all treatments, until reaching the peak, observed at the 5th and 6th WAA (Figs 1 and 2). Treatments with HP and with two or three applications of GA_3 (500 or 1,000 ppm) showed the highest growth rates in fruit length (14 and 15 mm week^{-1}) and fruit diameter (12 and 13 mm week^{-1}), respectively (Figs 1 and 2). Hand pollination alone or combined with one application of GA_3 (500 or 1,000 ppm), with 1,000 ppm GA_3 in four applications and/or with 1,500 ppm in three or four applications showed a peak growth rate ranging from 11 to 12 mm week^{-1} for fruit length and 9 to 10 mm week^{-1} for fruit diameter. Three applications of 1,000 ppm GA_3 and no hand pollination resulted in the lowest peak growth rate for both parameters: 8 mm week^{-1} for fruit length and 6 mm week^{-1} for fruit diameter. An uninterrupted decrease in the growth rate for fruit length and for fruit diameter from the 5th and 6th WAA until fruit ripening was observed for all treatments (Figs 1 and 2).

In general, fruits derived from treatments with HP and in combination with GA_3 showed higher growth rates than those that originated from non-hand pollinated flowers during all fruit growth phases, most likely resulting from the supply of plant hormones produced by the embryo (Varga and Bruinsma, 1976). Seeded fruits have four main growth phases. Phase I is characterized by floral development, pollination, fertilization and fruit setting. Phase II is characterized by intense cell division, and most fruit cells develop in this phase. Phase III comprises cell expansion, whereas phase IV involves fruit growth slowdown and ripening (Srivastava and Handa, 2005). Two peaks of gibberellin accumulation occur during fruit growth, coinciding with cell division activation at the beginning of the second phase and with cell expansion in the third phase (Gillaspy et al., 1993). Gibberellin increments in the phase III occur during the maximum fruit growth, and according to the authors, the accumulation of this plant growth hormone during phase III in parthenocarpic fruits is less pronounced than in fruits with seeds, which could explain the reduced growth rate of seedless fruits in the present study. Significant differences among the treatments for fruit length and fruit diameter were observed (Table 3). The combined use of hand pollination and the dose of 500 ppm of GA_3 applied in the 1st, 3rd and 5th WAA promoted fruits with greater length and diameter (Table 3). This result is probably due to increased gibberellin levels (endogenous) provided by the pollination and fertilization processes, in addition to the exogenous application of this plant-growth regulator, which most likely operated synergistically and induced increased fruit growth and diameter. Serrani et al. (2007) report the importance of fruit induction through the combined application of gibberellins and auxins. The authors support the hypothesis that cell elongation and cell division activities are coordinated and driven by a delicate balance between these plant hormones. Otherwise, the use of 1,000 ppm of GA_3 applied at anthesis and at the 1st and 3rd WAA significantly reduced the length and diameter of the custard apple fruits (Table 3). Flowers treated only with GA_3, regardless of the dose or number of applications, produced fruits with small patterns compared to those fruits originating from a combination of hand pollination and GA3. However, Pino (2008) obtained cherimoya fruits with similar size and shape to fruits hand pollinated by combining the use of two growth regulators (gibberellins and cytokinin) and six periods of application.

Physical and chemical characteristics of the fruits

Three doses of GA3 (500 ppm or 1,000 ppm) combined with hand pollination, produced fruits with superior physical and chemical parameters (Tables 4 and 5). Of all treatments evaluated, T4 and T7 promoted significant increases in fruit mass, peel + stem mass and pulp mass. Compared to T1 (control), the observed increases in fruit mass were 66% and 69 %, respectively (Table 4). The growth of plant organs promoted by gibberellins is due primarily to the increased size of pre-existing or newly divided cells, which may be accompanied by an increase in cell number (Métraux, 1988). The effect of gibberellins is also associated with the transport of expansin proteins from the cytoplasm to the cell wall, which break the hydrogen bonds between hemicellulose molecules and cellulose microfibrils, causing distention of the cellulosic framework and thus promoting cell elongation (Taiz and Zeiger, 2010). All fruits treated with gibberellin alone showed no seeds (Table 4; Fig 3). The average production of parthenocarpic fruits was 75% and 70% for flowers and fruits treated with three applications of GA3 after anthesis at doses of 1,500 ppm and 1,000 ppm, respectively.

Table 1. Description of treatments applied on 'Gefner' custard apple flowers and fruits in Janaúba, Brazil, 2013.

Treatments	Hand Pollination	GA_3 Dose	Period of GA_3 Application
T1 (control)	Yes	0	0
T2	Yes	500 ppm	1st WAA
T3	Yes	500 ppm	1st and 3rd WAA
T4	Yes	500 ppm	1st, 3rd and 5th WAA
T5	Yes	1,000 ppm	1st WAA
T6	Yes	1,000 ppm	1st and 3rd WAA
T7	Yes	1,000 ppm	1st, 3rd and 5th WAA
T8	No	1,000 ppm	Anthesis, 1st and 3rd WAA
T9	No	1,000 ppm	Anthesis, 1st, 3rd and 5th WAA
T10	No	1,500 ppm	Anthesis, 1st and 3rd WAA
T11	No	1,500 ppm	Anthesis, 1st, 3rd and 5th WAA

WAA: weeks after anthesis; GA_3: gibberellic acid; ppm: parts per million.

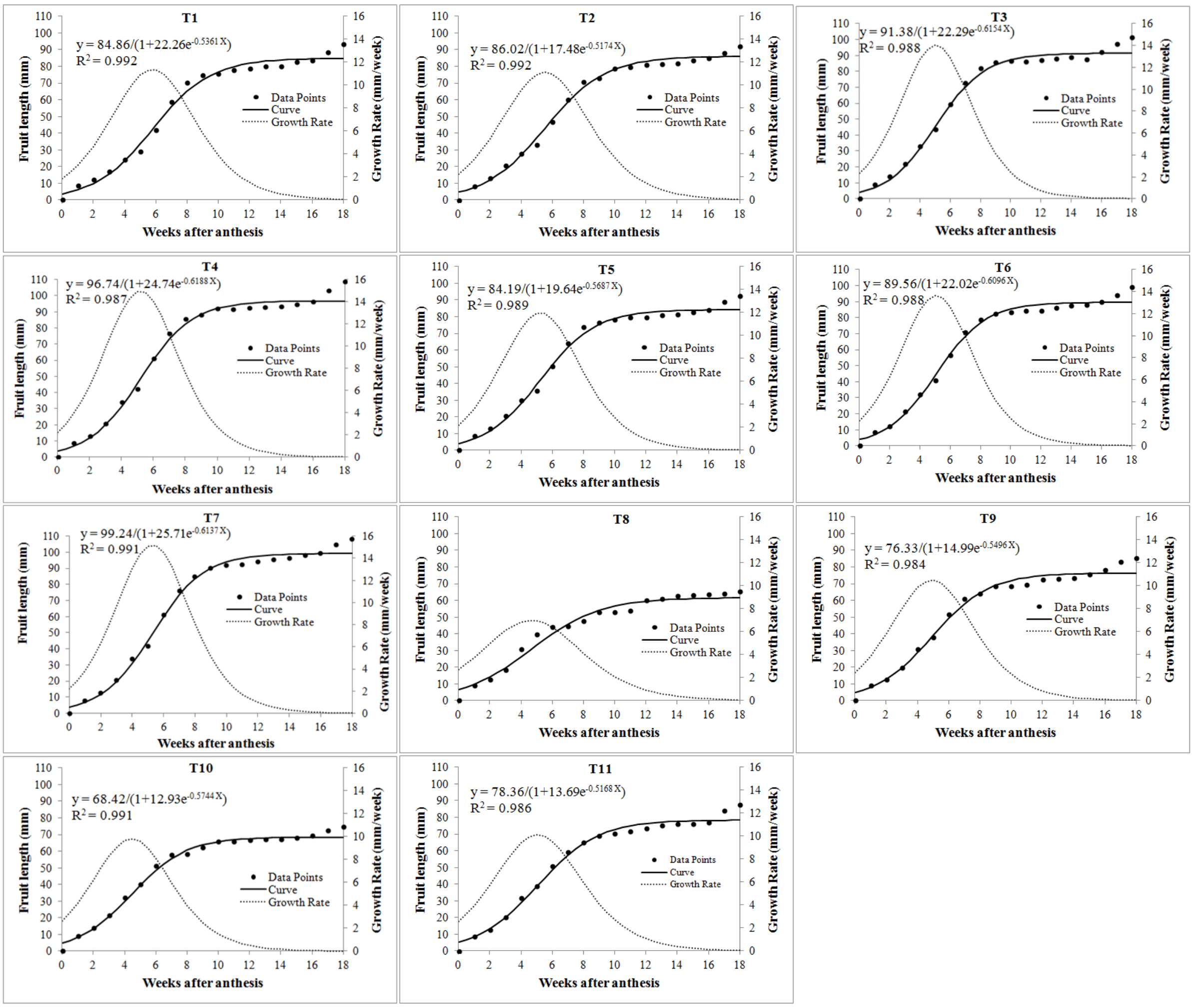

Fig 1. Fruit length and growth rate of 'Gefner' custard apple submitted to hand pollination (HP) and gibberellic acid (GA_3) at the 18th WAA, Janaúba, Brazil, 2013.

Similar results were observed by Saavedra (1979), Yonemoto et al. (2004) and Pino (2008), who also found high rates of parthenocarpic fruits in cherimoya. According to Pereira et al. (2014), the use of 1,000 ppm GA_3 resulted in 80 % seedless fruits in the 'Gefner' custard apple, and when applied to pollinated fruits, this same dose resulted in the lowest seed number/100 g of fruit and pulp ratio. No significant differences were observed among the treatments for the percentage of fruit pulp, ranging from 64.2 to 73.9 % (Fig 4). In general, fruits originated by hand pollination alone or combined with GA3 had a lower percentage of fruit peel.

The peel rates varied from 21.6 to 31.5 % (Fig 4). Fruits artificially pollinated and treated with three and two GA_3 applications after anthesis showed a remarkable reduction in seed ratios, with no dose effect (Fig 4). Variations in the custard apple pulp, peel and seed rates have already been reported in studies evaluating the efficiency of hand pollination. Reports have shown variations of 60 to 63 % in pulp, 25 to 28.1 % in peel and 11.6 to 12 % in seeds (Neves and Yuhara, 2003). The present study reports higher pulp rate values than those reported in the previous studies. The increments observed in the present study are probably due to

Table 2. Fruit set percentage of 'Gefner' custard apple submitted to hand pollination (HP) and gibberellic acid (GA_3) treatments at the 18th WAA, Janaúba, Brazil, 2013.

Week after anthesis	Treatments^{ns}										
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11
1	100	100	95	95	100	95	100	100	100	100	95
2	95	100	95	95	100	85	100	100	85	100	95
3	95	100	95	95	100	85	100	95	85	100	95
4	95	100	95	95	100	85	95	80	75	95	90
5	95	100	95	95	100	85	95	80	75	95	90
6	95	100	95	95	100	85	95	80	75	95	90
7	80	95	95	95	95	85	95	80	75	95	90
8	80	90	95	90	90	85	95	80	75	95	90
9	80	85	95	95	95	80	95	65	75	95	80
10	80	95	95	95	95	85	95	65	70	95	80
11	80	95	95	95	95	85	95	65	70	95	80
12	80	95	95	95	95	85	95	65	70	95	80
13	80	95	95	90	90	85	95	65	70	70	80
14	80	95	95	90	90	85	95	65	70	70	80
15	80	95	95	90	90	85	95	65	70	70	80
16	80	95	95	90	90	85	95	65	70	70	80
17	80	95	95	90	90	85	95	65	70	70	80
18	80	85	90	85	75	85	85	65	70	70	80
CV(%)						16.12					

ns: not significant.

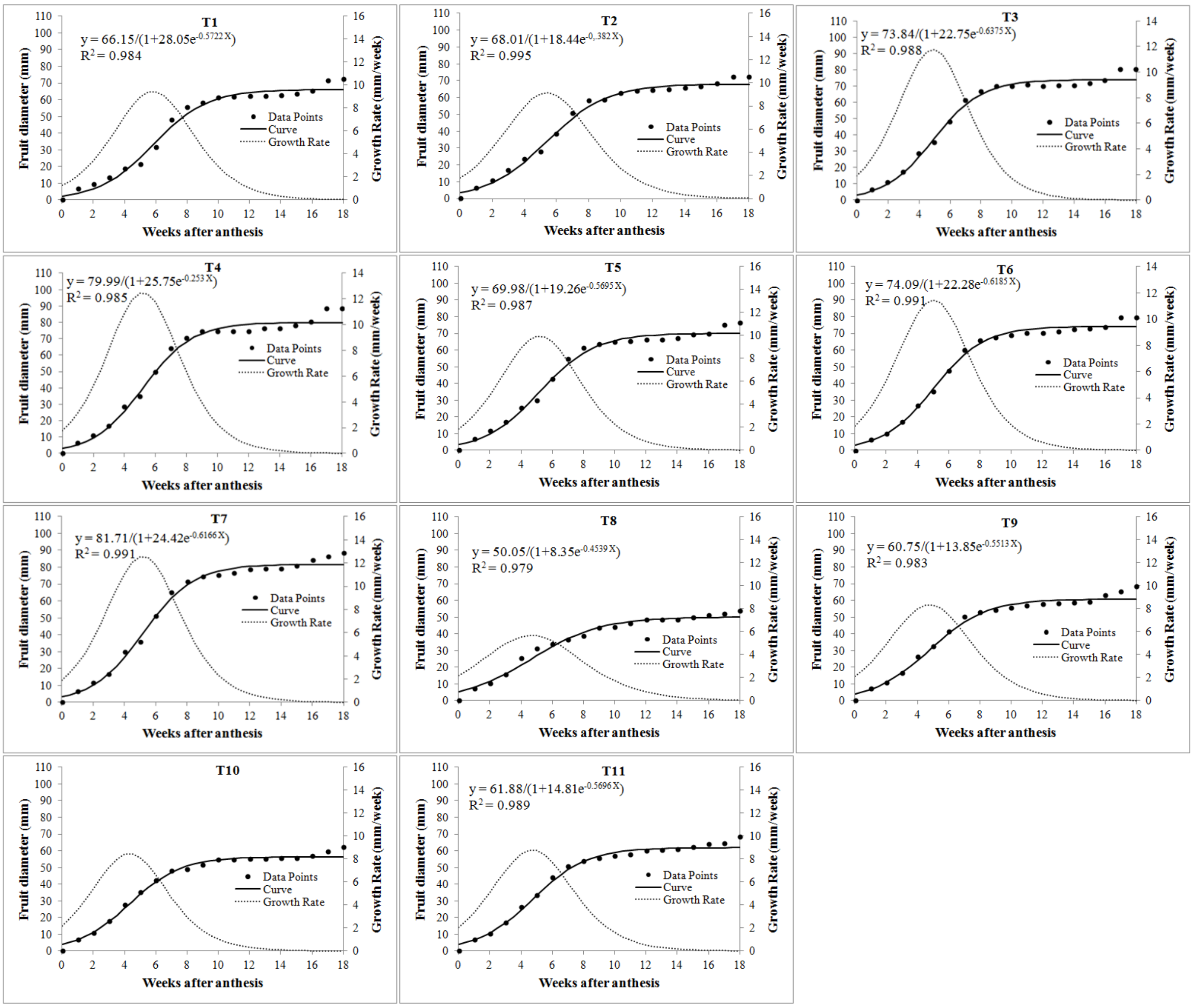

Fig 2. Fruit diameter and growth rate of 'Gefner' custard apple subjected to hand pollination (HP) and gibberellic acid (GA_3) at the 18th WAA, Janaúba, Brazil, 2013.

Table 3. Fruit length and fruit diameter of 'Gefner' custard apple submitted to hand pollination (HP) and gibberellic acid (GA_3) at the 18th WAA, Janaúba, Brazil, 2013.

Treatments	Length (cm)	Diameter (cm)
T1	9.3 bcd	7.3 bcd
T2	9.2 bcd	7.3 cd
T3	10.1 ab	8.1 abc
T4	10.8 a	8.8 a
T5	9.2 bcd	7.6 c
T6	9.9 abc	8.0 b
T7	10.8 a	8.6 ab
T8	6.4 f	4.8 g
T9	8.2 de	6.5 de
T10	7.4 ef	5.9 e
T11	8.7 cd	6.4 e
CV (%)	5.23	4.59

Means in the same column followed by the same letter are not different from each other according to the Tukey test at 5% probability.

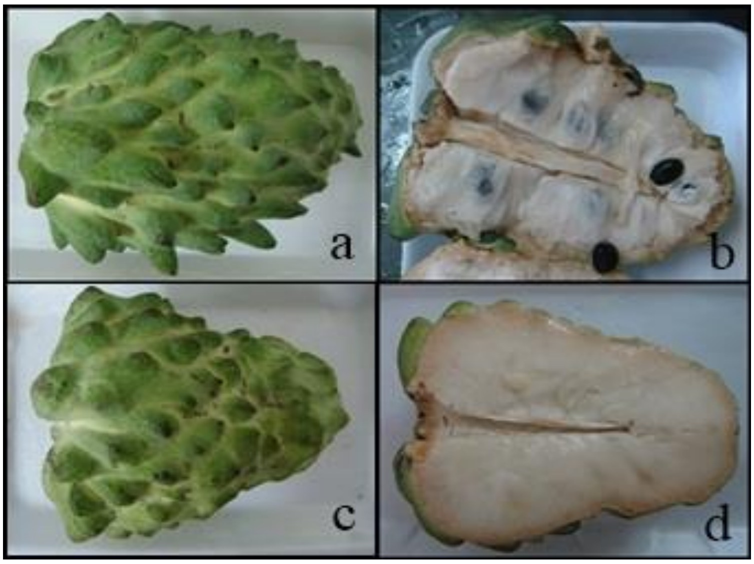

Fig 3. 'Gefner' custard apple fruits, a and b: fruit hand pollinated; c and d: fruit treated with gibberellic acid (GA_3) and no hand pollination (seedless), Janaúba, Brazil, 2013.

the exogenous application of GA_3, combined with hand pollination, which stimulated both cell division and increased volume in the newly divided cells (Métraux, 1988). The pH and titratable acidity showed no significant differences ($P > 0.05$, F test) between the treatments evaluated (Table 5). However, a correlation between the seed number and titratable acidity has been observed in grapes and apples (Coombe and Hale, 1989; Buccheri and Di Vaio, 2004). The pulp levels of the soluble solids of the pollinated fruits were higher than in the fruits treated only with gibberellin (Table 5). The significant reduction in total soluble solids of the fruits treated with exogenous gibberellin may be explained by the direct effect of cell expansion promoted by this plant growth regulator. Increased enzymatic activity of xyloglucan endotransglycosylase/hydroxylase (XTH) occurs upon exogenous GA_3 application, which facilitates the entry of expansins in the cell wall, preventing the development of wall pressure, enabling water to enter the cell, thereby decreasing the concentration of soluble solids in the cytoplasm (Taiz and Zeiger, 2010). However, Yonemoto et al. (2004), working with 'Big Sister' and 'Suiho' cherimoya cultivars, produced fruits with the same quality of pollinated fruits using 1,600 ppm GA_3, with no significant differences in soluble solids. Importantly, the levels of soluble solids are controlled by several cellular components and, therefore, represent a quantitative trait strongly affected by the environment. Increased pulp firmness was observed in hand-pollinated fruits with or without GA_3 application (Table 5). Compared to the control (T1), the fruits originated by use of GA_3, independently of dose and number of application, showed significant reduction for this parameter (Table 5). The reduced firmness of fruits that originated from treatments exclusively involving GA_3 application may be associated with the reduced lignin concentration resulting from the absence of seeds. Lignin provides a matrix for cellulose and hemicellulose polymerization within secondary cell walls, which collectively contribute to tissue rigidity and tensile strength (Dardick and Callahan, 2014). According to Cai et al. (2006), fruit firmness increased during loquat (*Eriobotrya japonica*) ripening, showing a positive correlation with lignin accumulation in the mesocarp.

Materials and Methods

Plant selection and treatment application

The experiment was performed in the semiarid region of the State of Minas Gerais, Janaúba, Brazil (15°52'07" latitude, 43°19'42" longitude), from February to July 2013. A total of 20 plants of custard apple (*A. cherimola* x *A. squamosa*) were selected and properly identified. The plants were six years old and were grown in 4.0 m x 2.5 m spacing. Plants presenting uniform traits, including size, vigor and health, were selected. The experimental design consisted of randomized

Table 4. Fruit mass (FRM), peel + stem mass (PSM), pulp mass (PUM), total seed mass (TSM), and number of seeds per fruit (NSF) of 'Gefner' custard apple submitted to hand pollination (HP) and gibberellic acid (GA_3), Janaúba, Brazil, 2013.

Treatments	FRM (g)	PSM (g)	PUM (g)	TSM (g)	NSF
T1	252.77 c	54.25 cd	169.17 c	27.26 b	64.00 b
T2	268.32 c	70.24 bcd	170.48 c	25.80 b	63.00 b
T3	343.10 b	81.42 b	233.07 ab	26.80 b	65.00 b
T4	421.95 a	122.76 a	271.35 a	25.42 b	65.00 b
T5	267.26 c	60.35 bcd	178.61 bc	26.41 b	60.00 b
T6	335.16 b	77.74 bc	231.65 ab	23.96 b	62.00 b
T7	429.18 a	117.34 a	286.48 a	25.42 b	59.00 b
T8	61.28 f	16.27 e	44.14 e	0.00 a	0.00 a
T9	185.14 de	57.72 bcd	127.03 cd	0.00 a	0.00 a
T10	147.04 e	45.53 d	101.04 d	0.00 a	0.00 a
T11	236.59 cd	60.39 bcd	163.73 c	0.00 a	0.00 a
CV (%)	9.83	15.77	12.49	11.66	9.91

Means in the same column followed by the same letter are not different from each other according to the Tukey test at 5% probability.

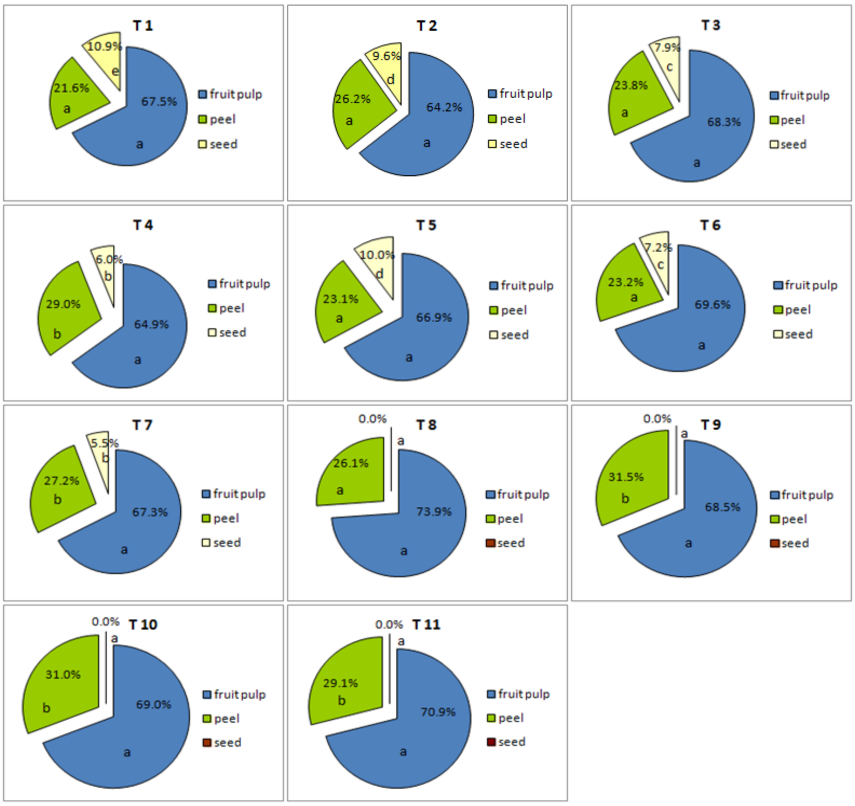

Fig 4. Fruit pulp, peel + stem and seed mass rates of 'Gefner' custard apple submitted to hand pollination (HP) and gibberellic acid (GA_3), Janaúba, Brazil, 2013. Means followed by the same letter are not different from each other according to the Tukey test at 5% probability.

Table 5. Firmness (FIR), soluble solids (SS), titratable acidity (ACI) and pH of the fruit pulp (pH) of 'Gefner' custard apple submitted to hand pollination (HP) and gibberellic acid (GA_3), Janaúba, Brazil, 2013.

Treatments	FIR (N)	SS (% Brix)	ACI	pH
T1	7.91 ab	28.97 abcd	0.41	4.41
T2	8.16 a	29.62 abc	0.35	4.34
T3	8.23 a	29.72 abc	0.34	4.26
T4	6.12 b	29.57 abc	0.40	4.18
T5	8.13 a	30.17 ab	0.30	4.24
T6	6.93 ab	30.30 a	0.37	3.97
T7	7.91 ab	28.97 abcd	0.39	4.31
T8	2.73 c	28.12 bcd	0.33	4.54
T9	2.72 c	27.80 cd	0.35	4.44
T10	2.94 c	27.40 d	0.31	4.40
T11	2.72 c	27.67 cd	0.31	4.40
CV (%)	5.86	2.99	7.43^{ns}	4.25^{ns}

Means in the same column followed by the same letter are not different from each other according to the Tukey test at 5% probability.
NS: not significant.

blocks with 11 treatments and four replicates, with five plants per plot and 11 flowers per plant. All plants received the treatments described in Table 1.The commercial product Pro-Gibb®, consisting of 10% gibberellic acid as a soluble powder, was used (Abbott Laboratories, São Paulo, SP, Brazil). Hand pollination (treatment 1) was performed in the morning (7:00 and 9:00 am) by using a fine brush with soft bristles. Pollen grains from sugar-apple plants (*Annona squamosa* L.) were collected from functionally pistillate flowers and were deposited on the stigmas of functionally staminate custard apple flowers (Pereira et al., 2011). HP using sugar-apple pollen was performed on the same day and time of the first application of the plant growth regulator.

Plant growth-regulator solutions containing 500 ml of nonsterile and nondistilled water supplemented with 0.1% nonionic spreader sticker (mean pH of 8.17) were prepared on the day of each treatment application. The experiment was installed on February 21, 2013. Eleven flowers of the functionally staminate flowers (anthesis) were selected per plant, corresponding to each treatment. The flowers were properly tagged, and the plant growth-regulator solution was applied by using a spray bottle. The solution was applied directly to the stigmas and anthers of the flowers. Each flower received, on average, 1.5 ml of plant growth-regulator solution. The solutions were applied weekly within the respective time intervals of each treatment described in Table 1.

Traits assessment

The variables of fruit set, fruit length and fruit diameter were assessed weekly. The fruit length and fruit diameter were measured with a 0.01 mm precision digital caliper. The fruit harvest was performed on June 27, 2013, at the 18^{th} WAA, when the fruits showed physiological maturity, observed by separation of the carpels, yellow-green intercarpelar tissue and light green peel (Kavati, 1992). The fruits were maintained in the laboratory at a constant temperature of ± 25°C until fully ripened. The fresh mass, peel + stem mass, pulp mass and seed mass of the fruits were determined with a 0.1 g precision balance. The number of seeds per fruit were also assessed. A Brookfield CT3 10-kg texturometer was used to assess the fruit firmness, which was measured by the penetration force expressed as Newton (N), using a 3.5-cm long and 4-mm wide tip in the intercarpelar tissue of the fruit peel. A fruit pulp aliquot was used for the analysis of the soluble solid levels by refractometry using a benchtop N1 ATAGO refractometer, reading in the range from 0 to 95 % Brix. The pH was measured by using a DM20 Digimed pH meter. The titratable acidity analysis was performed by standardizing the samples to a concentration of 10% fruit pulp diluted in distilled water and was assessed using 0.1 mol L^{-1} NaOH standardized solution and a phenolphthalein indicator. The results were expressed as mg of citric acid per 100 g of sample (AOAC, 1992).

Statistical analyses

The traits were submitted to an analysis of variance, and the means were compared using the Tukey test at 5% probability. The statistical analyses were conducted using SISVAR statistical software. The analyses of fruit length and fruit diameter were adjusted to the logistic equation (y = a / (1 + be^cx)), and the growth rates were assessed using the first derivative of the adjusted equation (Richards, 1969).

Conclusions

The application of GA_3 alone provides the same fruit set rate as hand pollination in 'Gefner' custard apple. Three applications of 500 ppm GA_3 combined with HP in the ‘Gefner’ custard apple provides a 66% fruit mass increase compared to the mass of fruits from plants that were only hand pollinated. Four applications of 1,500 ppm GA_3 in flowers and fruits of ‘Gefner’ custard apple is efficient in producing seedless fruits with high physical and chemical parameters.

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