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Potential of a native araticum species as the rootstock of atemoya cultivars propagated by grafting methods

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ABSTRACT. Technical and secure information on the production of quality atemoya seedlings (*Annona squamosa* L. x *Annona cherimola* Mill.) are of paramount importance due to the growing interest in this crop. This study aimed to assess the formation of atemoya seedlings propagated by grafting methods and cultivars using araticum (*Annona sylvatica*) rootstocks. The experiment was carried out in a screened environment at the Western Paraná State University (Unioeste), *Campus* of Marechal Cândido Rondon, Paraná State, Brazil. Atemoya grafts (Thompson, African Pride, and Gefner) were collected from plants from the CATI seedling nursery, located in São Bento do Sapucaí, São Paulo State, Brazil. Grafting was performed using 1-year-old araticum rootstocks with 8–10 mm in diameter at a height of 15 cm from the ground and grafts measuring, on average, 8.5 cm in length, 10 mm in diameter, and three buds. The experimental design consisted of randomized blocks in a 3 × 3 factorial arrangement (three grafting methods: cleft graft, whip and tongue graft, and chip budding × three cultivars: African Pride, Thompson, and Gefner), with four replications and 10 seedlings per replication, totaling 40 seedlings per treatment. The percentage of graft success (%), percentage of sprouting (%), number of sprouts, number of leaves, and longest sprout length (cm) were assessed at 60 days after grafting. The cleft graft method is efficient for producing atemoya seedlings. The cultivar African Pride can be grafted onto rootstocks by the cleft graft and whip and tongue graft methods, as they promote a higher percentage of graft success. The *Annona sylvatica* rootstock has potential for the production of atemoya seedlings, but chip budding is not viable.

Keywords: *Annona sylvatica* (A. St.-Hil) Mart.; *A. squamosa* L. x *A. cherimola* Mill.; native species; assexual propagation.

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Introduction

The commercial production of Annonaceae in Brazil stands out in the global scenario (São José, Pires, Freitas, Ribeiro, & Perez, 2014). The atemoya tree (*Annona squamosa* L. x *A. cherimola* Mill.) is an interspecific hybrid originated from the cross between the cherimoya and the sugar-apple (Leite, Mendonça, Mendonça, Dantas, & Cunha, 2013). The crossing between species can occur naturally, however, the hybrid atemoya was the result of an intentional crossing, which aimed to obtain fruits with the quality presented by their parents and that presented better adaptation to the tropical climate (Rabêlo, Costa, Libório, & Almeida, 2014).

There are some cultivars of atemoya trees available on the market, among them are: 'Thompson', 'Gefner', 'African Pride' and 'PR3' (Vieira, 2020). Some cultivars such as 'Gefner' and 'Thompson' are the most cultivated in the São Paulo State, due to their better adaptation to regional climatic conditions (Lemos, 2014).

With the growing interest in the atemoya culture, safe ways to produce seedlings are essential (Leite et al., 2013), since the onegrafted can take 3 to 4 years to produce, in addition to having considerable variation on fruit quality (Almeida, Alencar, & Yamanish, 2010). For Leite et al. (2013), the rootstock used in grafting is chosen due to its rusticity characteristics, availability of viable seeds, rapid development, compatibility with the graft, resistance to soil diseases and induction of good productivity.

Studies with native species of the family Annonaceae, such as *Annona glabra*, *A. mucosa*, *A. reticulata*, *A. sylvatica*, and *A. emarginata*, have shown their potential to be used as rootstock (Almeida et al., 2010; Leite et al., 2013; Silva et al., 2016; Baron, Gimenez, & Ferreira, 2018), moreover, few studies have been using different atemoya cultivars as a scion for these native species. The araticum [*Annona sylvatica* (A. St.-Hill.) Mart] is a native species distributed from Pernambuco to Rio Grande do Sul within the Atlantic Forest biome (Braga Sobrinho, 2014; Lorenzi, 2016) and presents economic potential in the Brazilian fruit growing sector (Arruda, Botrel, Fernandes, & Almeida, 2016).

Vegetative propagation is an option for producing quality seedlings, as it allows for their uniformity, in addition to obtaining seedlings identical to the parent plant, increasing production vigor (Hartmann, Kester, Davies Júnior, Geneve, & Wilson, 2018; Gomes & Krinski, 2018). Grafting is the recommended vegetative propagation technique for most fruit species (Beshir, Alemayehu, & Dessalegn, 2019).

An important aspect is the compatibility between scion and rootstock, as in many cases there may be incompatibility caused by a poor vascular union, vascular discontinuity, and phloem degeneration. A poor vascular union can interrupt the connection between scion and rootstock at the grafting point (Cassol et al., 2017; Oldoni, Nienow, Schons, & Mayer, 2019; Fadel et al., 2019).

The grafting technique to be used may influence the compatibility of the rootstock and scion and, consequently, the initial seedling formation. Cleft, bark, splice, and whip and tongue grafting, as well as T and chip budding, are among the most used techniques (Hartmann et al., 2018). Thus, this study aimed to assess the formation of atemoya seedlings propagated by grafting methods and cultivars using araticum (*Annona sylvatica*) rootstocks.

Material and methods

The experiment was carried out at the Experimental Station of Horticulture and Protected Cultivation, belonging to the Western Paraná State University (Unioeste), Marechal Cândido Rondon *Campus*, Paraná State, Brazil, under the geographic coordinates of 54°22’ W and 24°46’ S, and altitude of 420 m.

Ripe fruits were collected in March 2019 from four native araticum [*Annona sylvatica* (A. St.-Hill.) Mart] plants at Unioeste Experimental Farm. Immediately after collection, they were taken to the Laboratory of Seed Technology to remove the epicarp, placed in 5-L plastic buckets, and left to stand for 48h. Subsequently, the seeds were washed in running water over a sieve (06 mesh, 23 BWG wire, 65 cm hoop) until the total mucilage removal. Then, the seeds were placed to dry in a dry, shaded, ventilated place at an ambient temperature of 25±2°C.

The experiment was conducted in a screened environment covered with a 30% shading screen. Araticum rootstocks were produced from seeds submerged in gibberellic acid (GA_3) at a concentration of 1,000 mg L^{-1} for 24 hours and sown on a masonry bed filled with medium-textured, washed sand previously disinfected with a sodium hypochlorite solution. A sprinkler irrigation system was used with intermittent activations from 7:00 am to 6:00 pm every 30 min. and a duration of 2 min., with a flow rate of 1.17×10^{-8} m^3 s^{-1} each time. Manual hoeing was carried out whenever necessary.

Seedling transplanting was carried out at 150 days after sowing (8–10 cm in height and two pairs of definitive leaves, on average) to black polyethylene bags (25 cm high, 15 cm wide, and 0.02 mm thick) filled with a substrate composed of soil and cured manure (1:1, v:v). The chemical analysis of the substrate was carried out by the Laboratory of Environmental and Instrumental Chemistry of Unioeste. The following results of chemical attributes were obtained: pH in $CaCl_2$ = 6.43 mol L^{-1}, available P (Mehlich-1 extractor) = 253.62 mg dm^{-3}, available K = 2.02 $cmol_c$ dm^{-3}, Ca = 8.11 $cmol_c$ dm^{-3}, Mg = 3.62 $cmol_c$ dm^{-3}; Al = 0.0 $cmol_c$ dm^{-3}, H+Al = 2.95 $cmol_c$ dm^{-3}, CEC = 16.70 $cmol_c$ dm^{-3}, OM = 45.80 g dm^{-3}, SB = 13.75 $cmol_c$ dm^{-3}, and V = 82.34%.

Canopy branches of atemoya cultivars (‘Thompson’, ‘African Pride’, and ‘Gefner’) were collected on August 17, 2020 (end of the vegetative rest period), from plants in production with an average age of 12 years from the CATI seedling nursery, located in São Bento do Sapucaí, São Paulo State, Brazil. The scions were, on average, 50 cm long, 6-10 buds, and 10 mm in diameter. They were packed in black plastic bags soon after collection and wrapped with newspaper sheets for transportation to Unioeste.

Grafting was carried out in the morning on August 18 and 19, 2020, with scions measuring an average of 8.5 cm in length, 10 mm in diameter, and with three buds, being grafted onto 1-year-old araticum rootstocks with 8-10 mm in diameter at a height of 15 cm from the ground. The grafting procedure was performed using a clean and sterilized grafting knife, white polytetrafluoroethylene thread seal tape (12 mm and 25 m), and transparent polyethylene plastic bags with dimensions of 4 × 24 × 0.05 cm to cover the scions to avoid their desiccation (Kitamura, Ramos, & Lemos, 2004). Grafting and budding were performed according to the methodology proposed by Paiva et al. (2015).

After grafting, the seedlings remained in a screened environment covered with a 30% shading screen, and irrigations were carried out daily to maintain the substrate always moist. Manual weeding was carried out to control weeds. The removal of shoots below the grafting point was carried out at the time of their emergence and cultural management, such as fertilization. Fungicide and insecticide applications were not carried out. The plastic bags and tapes that fixed the scions were removed 30 days after grafting.

The experimental design was randomized blocks in a 3 × 3 factorial arrangement (three grafting methods: cleft graft, whip and tongue graft, and chip budding × three cultivars: African Pride, Thompson, and Gefner), with four replications and 10 seedlings per replication, totaling 40 seedlings per treatment.

The following assessments were carried out at 60 days after grafting: percentage of graft success (PGS), calculated from the number of successful rootstocks and scions relative to the total number of seedlings, consisting of their healing and sprouting, with results expressed as a percentage; percentage of sprouting (PS), calculated from the presence of sprouts in the successful grafts relative to the total number of seedlings, expressed as a percentage; number of shoots (NS), determined by directly counting the number of shoots emitted in the graft; number of leaves (NL), considering those with a length longer than 2 cm; and longest sprout length (LSL), measured using a millimeter ruler from the beginning of sprouting to the plant apex, with results expressed in centimeters.

The obtained data were subjected to the Shapiro-Wilk normality analysis ($p > 0.05$). Analysis of variance was performed when the model assumptions were met to verify the effects of isolated factors and their interaction. When significant ($p > 0.05$), the Tukey test at a 5% probability error was applied to compare the means, using the SISVAR statistical program (Ferreira, 2011).

Results and discussion

The analysis of variance (Table 1) showed a significant interaction between grafting methods and atemoya cultivars for the percentage of graft success and percentage of sprouting. The number of sprouts, number of leaves, and longest sprout length presented a significant difference for isolated factors.

Table 1. Summary of analysis of variance containing mean square values for set (VSET), percentage of sprouted grafts (SG), number of sprouts per graft (NSG), number of leaves (NF) and length of longest sprout (LLS) as a function of methods grafting (MG) and cultivar (C). Unioeste, *Campus* of Marechal Cândido Rondon, Paraná State, Brazil, 2021.

VF	DF	VSET (%)	SG (%)	NSG	NF	LLS (cm)
MG	1	600.00*	416.66*	1.17*	50.75*	27.62*
C	2	20216.66*	21066.66*	17.79*	690.63*	474.74*
MG x C	2	350.00*	416.66*	0.50^{ns}	4.73^{ns}	0.35^{ns}
Error	18	22.22	8.33	0.10	1.65	2.14
Total	23					
VC(%)		8.08	5.09	17.22	11.70	14.56

*Significant by F Test at 5% probability of error, ns not significant, VF = variation factor, DF= degrees of freedom, VC(%) = variation coefficient.

Tables 2 and 3 show that only the chip budding method presented on VSET, SG, NSG, NF, and LLS results because of the bud dryness in the three studied cultivars, leading to their death. According to Leite et al. (2013), the chip budding method presents survival difficulties before tissue union, as it has few reserves (carbohydrates), making it difficult the graft success and sprouting. These authors tested grafting methods on atemoya and two rootstocks (*Annona glabra* and *A. squamosa*) and concluded that the budding method was not efficient for the production of atemoya seedlings of the cultivar Gefner, a result that corroborates with the present study for all studied cultivars.

Table 2. Mean data of percentage of set (PES) and sprouting (SPRO) of grafts of atemoya seedlings (*Annona squamosa* L. x *A. cherimola* Mill.), as a function of grafting methods and cultivar. Unioeste, *Campus* of Marechal Cândido Rondon, Paraná State, Brazil, 2021.

	Percentage of set (PES) (%)		
Grafting methods	African Pride	Gefner	Thompson
Cleft graft	100 aA*	5 aC	85 aB
Whip and tongue graft	100 aA	0 aC	60 bB
Chip budding graft	-	-	-
CV(%)		8.08	
	Sprounting (SPRO) (%)		
Grafting methods	African Pride	Gefner	Thompson
Cleft graft	100 aA	0 aC	82 aB
Whip and tongue graft	100 aA	0 aC	57 bB
Chip budding graft	-	-	-
CV(%)		5.09	

*Averages followed by the same lowercase letter in the column and uppercase in the row, do not differ by Tukey test, at 5% probability of error. PES = percentage of graft set, SPRO = percentage of sprouting and CV(%) = coefficient of variation.

Studies with atemoya (*A. squamosa* L. x *A. cherimola* Mill.) (Leite et al., 2013), pomegranate (*Punica granatum*) (Paiva et al., 2015), mango (*Mangifera indica*), cashew (*Anacardium occidentale*) (Ferreira, Negrini Júnior, Valente, Távora, & Ferreira, 2016), and tamarind (*Tamarindus indica*) (Góes, Melo, Mendonça, Dantas, & Leite, 2016), support our results, with grafting methods (cleft graft and whip and tongue graft) being more viable for the atemoya seedling production than the budding method (chip budding).

On the other hand, the chip budding method has been effectively used to produce seedlings of other native species, such as brazilnut (*Bertholletia excelsa*) (Almeida et al., 2020), Paraná pine (*Araucaria angustifolia*) (Constantino & Zanette, 2015), and theobroma (*Theobroma grandiflorum*) (Souza & Souza, 2018), highlighting that the main factors in budding success are related to edaphoclimatic conditions and graft compatibility.

The cultivar African Pride showed a statistically equal PES between the cleft graft and whip and tongue graft, both superior to budding, with a higher percentage than the cultivars Thompson and Gefner (Table 2), propagated by the same method. The cultivar Thompson had a higher PES using the cleft graft. Lower percentages of graft success were also obtained for the cultivar Gefner, with values close to zero and with no significant difference between grafting methods.

This variation in the results may be related to the possible difference in the parenchymal tissues of the cultivars, which may interfere with the graft success when considering the several grafting methods (Hartmann et al., 2018). These authors also emphasize that the success of the cambium union requires a high capacity of the parenchymal cells to produce callosity. The cultivar African Pride was statistically superior (100%) regarding the cleft graft than the cultivars Thompson (85%) and Gefner (5%). Also, the cultivar African Pride stood out statistically with a higher value for the whip and tongue graft (100%) than the cultivars Thompson (60%) and Gefner (0%) (Table 2).

The compatibility between scion and rootstock is essential for graft success and, consequently, for producing quality seedlings (Campos, Marinho, Portella, Amaral, & Carvalho, 2017). The success or failure of the grafting technique is deeply related to the healing process of the graft union (Zeist, Resende, Giacobbo, Faria, & Dias, 2017).

The cultivar African Pride showed statistically equal results for sprouting (Table 2) regardless of the grafting method (cleft graft and whip and tongue graft), but with higher percentages than the cultivars Thompson and Gefner. The cultivar Thompson showed the highest percentages of sprouting for the cleft graft method. The cultivar Gefner had the lowest percentages of sprouting (0%), regardless of the used grafting method.

This variation in sprouting between species is possibly due to morphological disagreements between the rootstocks and the plants used as scions (Citadin, Scariotto, Wagner Júnior, & Sachet, 2012), as they developed under different edaphoclimatic conditions before the grafting was carried out, even with the parent plants that donated the scions having developed at the same location.

The cultivar African Pride was statistically superior (100%) regarding the cleft graft method than the cultivars Thompson (82%) and Gefner (0%). The cultivar African Pride also stood out statistically with a higher value (100%) for the whip and tongue graft method than the cultivars Thompson (57%) and Gefner (0%) (Table 2). Leite et al. (2013) verified that atemoya grafted onto pond apple (*Annona glabra*) presented higher sprouting compared to the atemoya grafted onto sugar apple (*A. squamosa*).

Table 3 shows the variable NS as a function of the grafting method. The cleft graft method (2.1) showed the highest sprouting value compared to the whip and tongue graft method (1.6). Possibly, the branch length did not influence the number of sprouts, with the cleft graft method presenting higher values regarding the number of sprouts and longest branch length than the whip and tongue graft method. In contrast, Citadin et al. (2012) observed that the higher number of sprouts increases the competition for carbohydrates and/or photoassimilates, thus reducing the sprout length. NS as a function of cultivars did not differ statistically from each other. The cultivars African Pride (2.9) and Thompson (2.6) showed better results than the cultivar Gefner (0.2).

According to Góes et al. (2016), sprout development is related to an intense cambium activity and the availability of reserves in the propagated material. Barreto, Cavallari, Venturini, Andrade, and Martins (2015) and Malagi, Citadin, Scariotto, Wagner Júnior, and Sachet (2012) pointed out that the relationship between the number of sprouts per plant and length is inversely proportional, which was not observed in the present study. Celant et al. (2010) worked with cold storage of bud-bearing branches and grafting methods of Chinese-quince (*Chaenomeles sinensis*) cultivars and observed a higher number of sprouts with the double cleft graft method, with an increase of 71.8% in the sprouting of grafts compared to the budding method, as it had few reserves.

Table 3. Mean data of number of shoots (NS), number of leaves (NL) e longest sprout length (LSL) of grafts of atemoya seedlings (*Annona squamosa* L. x *A. cherimola* Mill.), as a function of grafting methods and cultivar. Unioeste, *Campus* of Marechal Cândido Rondon, Paraná State, Brazil, 2021.

Grafting methods	NS	NL	LSL (cm)
Cleft graft	2.1 a*	12.5 a	11.1 a
Whip and tongue graft	1.6 b	9.5 b	9.0 b
Chip budding graft	-	-	-
Cultivar	NS	NL	LSL (cm)
African Pride	2.9 a	18.9 a	15.3 a
Thompson	2.6 a	13.9 b	13.7 a
Gefner	0.2 b	0.8 c	1.2 b
CV(%)	17.22	11.70	14.56

*Averages followed by the same lowercase letter in the column and uppercase in the row, do not differ by Tukey test, at 5% probability of error. NS = number of shoots, NL = number of leaves, LSL = longest sprout length and CV(%) = coefficient of variation.

The cleft graft method (12.5) showed a significant difference for NL as a function of the grafting method (Table 3), being statistically superior when compared to the whip and tongue graft method (9.5). The cultivar African Pride (18.9) had the highest number of leaves, differing statistically from the other cultivars, followed by the cultivars Thompson (13.9) and Gefner (0.8).

The results of the present study corroborate with Góes et al. (2016), who studied tamarind (*Tamarindus indica*) grafting methods and observed that the highest average number of leaves was verified in the cleft, whip and tongue, and splice graft methods, respectively. The number of leaves is important to assess the vigor induced by the rootstock depending on the plant stage (Leite et al., 2013). Similarly, Taiz, Zeiger, Moller, and Murphy (2017) stated that this characteristic is important for seedling development after graft success because the leaves are responsible for the synthesis of 90% of the carbon assimilated by the plants.

The cleft graft method was superior compared to the whip and tongue graft method regarding LSL, with values of 11.1 and 9.0 cm, respectively. LSL as a function of the cultivars did not differ statistically from each other for the cultivars African Pride (15.3 cm) and Thompson (13.7 cm), showing better results than the cultivar Gefner (1.2 cm).

Souza et al. (2010) found contrary results when working with different grafting methods on yellow mombin (*Spondias mombin*). The authors observed the longest bud length for the splice graft method and concluded that the cleft graft and side graft methods did not differ from each other. Keller, Mills, and Harbertson (2010) found that seedling vigor can be characterized by the speed and growth capacity of sprouts/branches of *Vitis vinifera* cultivars (Merlot, Syrah, and Chardonnay). Thus, seedlings that have sprouts/branches growing faster and reaching a longer length are considered more vigorous seedlings.

The present research demonstrated that the cleft graft method with the cultivar African Pride can be recommended for the atemoya seedling production when using *Annona sylvatica* rootstocks, considering that it presented better development at 60 days. However, few studies on atemoya seedling production are available in the literature. Thus, carrying out further studies using other grafting methods and cultivars that point to seedling development conditions in the field is of paramount importance, as rootstocks, grafting methods, and cultivars can better indicate the graft success and seedling development.

Conclusion

The cleft graft method is efficient for atemoya seedling production. The cultivar African Pride can be grafted onto rootstocks by the cleft graft and whip and tongue graft methods, as they promote a higher percentage of graft success. The *Annona sylvatica* rootstock has the potential for atemoya seedling production, but chip budding is not viable.

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