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Physiological changes modulated by rootstocks in atemoya (*Annona* x *atemoya* Mabb.): gas exchange, growth and ion concentration

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Abstract Incompatibility reactions of grafted plants are triggered at the moment of grafting and/or at the early stages of re-establishment vascular connections plants; moreover, incompatibility may occur after years in orchard. The objective of this study was to evaluate the gas exchange, growth and ion concentrations on early stage after grafting to atemoya scion (*Annona* x *atemoya* Mabb.), an interspecific hybrid between cherimoya (*Annona cherimola* Mill.) and sweetsop (*Annona squamosa* L.), commonly used in food industry and for '*in natura*' consumption, grafted onto *biribá* rootstock [*Annona mucosa* (Bail.) H. Rainer] and *Annona emarginata* (Schltdl.) H. Rainer var. *terra-fria* and *mirim* rootstocks to elucidate the physiological mechanisms between grafted plants. Different types of rootstocks evaluated caused variations on scion gas exchange 60 days after grafting (DAG), and these variations reflected in changes in growth at 90 DAG, which was also influenced by differential ion accumulation for each rootstock. The *biribá* rootstock demonstrated an increase in net carbon assimilation related to carboxylation efficiency and transpiration; however, this rootstock did not result in increased water-use efficiency.

The rootstocks exhibited differences in scion leaf ion accumulation, particularly phosphorus and potassium, in *biribá* and *araticum-de-terra-fria*, respectively. We concluded that *biribá* rootstocks increased gas exchange and ion concentration in the atemoya scion, which were reflected directly in the vegetative growth of the scion, indicating that the physiological changes caused by this rootstock to scion do not trigger early incompatibility.

Keywords Annonaceae · Photosynthesis · Plant propagation · Reestablishment post-grafting

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1 Introduction

The Annonaceae family contains approximately 110 genera with 2400 species (Chatrou et al. 2012). Of these species, the atemoya (*Annona* x *atemoya* Mabb.), a hybrid between *Annona cherimola* Mill. and *Annona squamosa* L., is used in food (e.g. ice cream, candy, jam, juice, liqueur) and consumed *in natura*. For the propagation of atemoya, vegetative methods, particularly grafting, are utilised to ensure the perpetuation of clones with high fruit quality and productivity (Heenkenda et al. 2009). Although Almeida et al. (2010) described wild species as potential rootstocks, in Brazil, as *biribá* and *araticuns*, the identification of rootstocks that are resistant to pathogens and do not exhibit incompatibility problems remains very difficult (Kavati 2013). The incompatibility symptoms usually occur in the early stages of re-establishing plants after grafting, when the vascular connections are formed (Martínez-Ballesta et al. 2010), and it can be triggered in the moment of grafting, including mortality, caused by *biribá* [*Annona mucosa* (Bail.) H. Rainer] (Almeida et al. 2010) or

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could arise after few days after grafting (Santos et al. 2005).

According to literature, the rootstocks major used to atemoya scion are atemoya itself, *Annona emarginata* (Schltdl.) H. Rainer var. *terra-fria* and var. *mirim* (araticuns). Atemoya grafted onto *araticum-de-terra-fria* rootstock results in further development of the scion and tolerance to cave nematodes, stem borers and water stress; moreover, the *araticum-mirim* species reaches more adapted to conditions of higher temperature and relative humidity and, probably, does not present adaptation in cold conditions, in this case, *araticum-de-terra-fria* plants should be indicated as rootstock plants to atemoya scion.

Furthermore, Baron et al. (2016) reported that to early stage post-grafting, atemoya scion grafted onto *araticum-de-terra-fria* rootstock shows increased cell wall gene expression of tissue vascular compared to atemoya scion grafted onto *aracticum-mirim*, indicates a rapid formation and union of grafted plants vascular tissues. Kavati (2013) suggested that atemoya scion grafted onto *araticum-mirim* induces dwarfing in plants and that the resulting plants do not survive longer than 4 years in orchards due to growth restrictions in the grafted stem tissue, and this response is considered to reflect incompatibility in the expression or repression of genes between the plants.

Grafting incompatibility between plants can induce decrease in the growth of the scion and in water transportation, which may cause the death of the plant and can also manifest in response to alterations to gas exchange (Magalhães Filho et al. 2008). Furthermore, rootstock effects on photosynthesis can alter scion development and ionic composition of grafted plants (Huang et al. 2010) and can increase the absorption efficiency of essential elements (Ruiz et al. 1997).

Therefore, it is extremely critical to assess the ability of different rootstocks, *araticum-de-terra-fria*, *araticum-mirim* and *biribá*, modulating gas exchange, growth and changes in ion concentrations to atemoya scion. In order to elucidate the physiological mechanisms on the stage early after grafting, that would be a prognosis to early verify graft-incompatible combinations and avoid unnecessary costs on planting non-productive combinations.

2 Materials and methods

The experiment was conducted under greenhouse conditions at Universidade Estadual Paulista (Unesp), Botucatu *campus*. Seeds of *araticum-de-terra-fria*, *araticum-mirim*, *biribá* and atemoya were sown in polystyrene trays filled with vermiculite until emergence, and the seedlings were transplanted into plastic pots filled with a commercial mixture of fertile soil + texture vermiculite + coconut fibre (2:1:1 v/v). The plants were irrigated with water (400 mL per pot/day, or as necessary) and supplemented, via soil, with Hoagland and Arnon no. 2 nutrient solution, diluted to 50% of its ionic strength, with electrical conductivity (E.C.) range 1.0 to 1.5 miliSiemens cm^{-1} and calcium nitrate, E.C. range 0.20 to 0.25 miliSiemens cm^{-1}, recommended for grown up annonaceous plants, from seedlings until young plants, according to Baron et al. (2015).

The atemoya self-grafted and grafted onto rootstocks: *araticum-de-terra-fria*, *araticum-mirim*, *biribá* and atemoya using the 'whip and tongue grafting', whereas ungrafted atemoya was used as a control plant. The experiment was conducted using a randomised block design, with five replicates, and the physiological evaluations (gas exchanges, plant growth and ion concentration) were measured immediately prior to grafting (0 days after grating, DAG) in ungrafted plants and at 60 and 90 DAG in grafted plants.

Gas exchange levels were measured with an infrared CO_2 and water vapor analyzer (LI-6400, Li-Cor Inc., Lincoln, NE, USA), between 09:00 a.m. and 11:00 a.m. This period was selected according to the response curve of the daily gas exchange previously performed. The net CO_2 assimilation rates (A_{net}), transpiration rates (E), stomatal conductance (g_s) and intercellular CO_2 concentrations (C_i) were evaluated. The water-use efficiency (WUE) was determined by the relationship between the net assimilation rate and the transpiration rate (A_{net}/E), and the apparent carboxylation efficiency (A_{net}/C_i) was determined by the relationship between the net CO_2 assimilation rate and the intercellular CO_2 concentration. During the evaluation were recorded: photosynthetic photon flux density (PPFD) (462.7 ± 111.6 $\mu mol\ m^{-2}\ s^{-1}$), relative humidity ($54.6 \pm 13.3\%$) and temperature (26.4 ± 0.3 °C). The PPFD was standardised through the use of a light-emitting diode that was coupled to a photosynthesis chamber and emitted 1500 $\mu mol\ m^{-2}\ s^{-1}$. The reference CO_2 concentration that was used during the evaluation was the ambient value, which ranged from 380 to 400 $\mu mol\ CO_2\ mol^{-1}$.

The stem diameter of the ungrafted and grafted plants was measured using a digital calliper and taken in the graft union; plant height was measured from the neck to the last fully expanded leaf. The number of fully expanded leaves was counted 0, 60 and 90 DAG; the number of shoots on the grafted scions was measured at 60 and 90 DAG, which are the initial reestablishment post-grafting period according to Baron et al. (2016).

The concentrations of essential mineral analyses were performed: nitrogen (N-total, micro Kjeldahl method); phosphorus (ammonium metavanadate, NH_4VO_3 method); potassium, calcium and magnesium (atomic absorption spectrophotometry, AAS–method) determined in leaf and

root tissues of ungrafted plants prior to grafting and in grafted plants at 60 DAG, according to Malavolta et al. (1997).

Data were subjected to an analysis of variance (ANOVA), and the means were compared by Tukey's test ($P \leq 0.05$).

3 Results and discussion

Before grafting, no differences in gas exchange were noted among the species (Table 1). However, at 60 DAG, alterations in gas exchange in the scion atemoya were observed; these differences were not apparent at 90 DAG (Table 1). The high A_{net} observed when we used *biribá* rootstock was attributed to carboxylation efficiency because the use of C_i for the synthesis of organic compounds caused variation in the carbon chemical gradient, resulting in flux into the leaf (Table 1). Op de Beeck et al. (2010) reported similar observations, confirming that when a treatment provides conditions of high g_s, the A_{net} tends to be high, depending on the use of C_i for the synthesis of organic compounds. Thus, the CO_2 chemical gradient is maintained, ensuring its entry into the leaf, as noted with *biribá* rootstock.

In this study, g_s was not a limiting factor that reduced input CO_2, as there was no difference among the rootstocks; however, C_i did differ, as observed with atemoya grafted onto *araticum-mirim*. In this case, the data indicate that a smaller amount of CO_2 was utilised by the photosynthetic apparatus of this graft combination. Although Ojeda et al. (2004) reported that atemoya grafted onto *A. squamosa* (sweetsop) and *A. glabra* (pond apple) exhibited variations in A_{net} and not E, the *biribá* rootstock in the present study exhibited an increased A_{net} compared to atemoya ungrafted, *araticum-mirim* and *araticum-de-terra-fria* rootstock and increased scion E compared to atemoya ungrafted. The rootstocks used did not resulted in increased WUE compared to self-grafted plants, though the *araticum-mirim* and *araticum-de-terra-fria* rootstocks did promote a reduction in this variable compared with

Table 1 Gas exchange in atemoya, *araticum-de-terra-fria*, *araticum-mirim* and *biribá* ungrafted plants before grafting (0 days after grafting–DAG) and grafted plants at 60 and 90 DAG

Before grafting (0 DAG)	Ungrafted atemoya	Ungrafted *araticum-de-terra-fria*	Ungrafted *araticum-mirim*	Ungrafted *biribá*	F value
A_{net} (µmol CO_2 m^{-2} s^{-1})	4.80 ± 0.45^a	5.40 ± 0.7^a	6.40 ± 0.81^a	5.30 ± 0.73^a	1.44^{ns}
E (mol H_2O m^{-2} s^{-1})	0.87 ± 0.1^a	1.11 ± 0.3^a	1.01 ± 0.2^a	0.83 ± 0.1^a	0.59^{ns}
g_s (mol H_2O m^{-2} s^{-1})	0.04 ± 0.01^a	0.05 ± 0.01^a	0.04 ± 0.01^a	0.04 ± 0.01^a	1.00^{ns}
WUE (µmol CO_2 (mol $H_2O)^{-1}$)	7.5 ± 1.4^a	5.63 ± 0.9^a	8.29 ± 1.2^a	5.88 ± 0.3^a	1.57^{ns}
A_{net}/C_i	0.03 ± 0.003^a	0.03 ± 0.004^a	0.05 ± 0.001^a	0.04 ± 0.001^a	3.25^{ns}

60 DAG	Ungrafted atemoya	Atemoya/atemoya	Atemoya/araticum-de-terra-fria	Atemoya/*araticum-mirim*	Atemoya/*biribá*	F value
A_{net} (µmol CO_2 m^{-2} s^{-1})	5.70 ± 0.7^b	6.10 ± 0.6^b	5.40 ± 0.7^b	5.40 ± 0.5^b	8.40 ± 0.9^a	4.24^*
E (mol H_2O m^{-2} s^{-1})	1.05 ± 0.12^b	1.31 ± 0.09^{ab}	1.47 ± 0.12^{ab}	1.51 ± 0.13^{ab}	1.80 ± 0.20^a	4.83^*
g_s (mol H_2O m^{-2} s^{-1})	0.12 ± 0.01^a	0.11 ± 0.01^a	0.12 ± 0.02^a	0.13 ± 0.01^a	0.15 ± 0.01^a	2.22^{ns}
WUE (µmol CO_2 (mol $H_2O)^{-1}$)	5.38 ± 0.31^a	4.73 ± 0.27^{abc}	3.66 ± 0.30^{bc}	3.57 ± 0.11^c	4.76 ± 0.59^{ab}	8.03^*
A_{net}/C_i	0.02 ± 0.003^{ab}	0.02 ± 0.007^{ab}	0.02 ± 0.003^b	0.02 ± 0.002^{ab}	0.03 ± 0.003^a	3.56^*

90 DAG	Ungrafted atemoya	Atemoya/atemoya	Atemoya/araticum-de-terra-fria	Atemoya/*araticum-mirim*	Atemoya/*biribá*	F value
A_{net} (µmol CO_2 m^{-2} s^{-1})	3.82 ± 0.5^a	3.77 ± 0.5^a	4.71 ± 0.5^a	4.88 ± 0.5^a	5.14 ± 0.6^a	1.76^{ns}
E (mol H_2O m^{-2} s^{-1})	1.54 ± 0.1^a	2.14 ± 0.1^a	1.71 ± 0.2^a	2.05 ± 0.05^a	2.11 ± 0.3^a	3.25^{ns}
g_s (mol H_2O m^{-2} s^{-1})	0.11 ± 0.01^b	0.15 ± 0.01^a	0.12 ± 0.02^{ab}	0.14 ± 0.01^{ab}	0.14 ± 0.02^{ab}	3.38^*
WUE (µmol CO_2 (mol $H_2O)^{-1}$)	2.49 ± 0.4^a	1.76 ± 0.3^a	2.90 ± 0.5^a	2.40 ± 0.1^a	2.51 ± 0.4^a	1.98^{ns}
A_{net}/C_i	0.01 ± 0.002^a	0.01 ± 0.002^a	0.02 ± 0.002^a	0.02 ± 0.001^a	0.02 ± 0.002^a	2.70^{ns}

Means followed by the same letter in a line do not differ by Tukey's test at 5% probability ($P < 0.05$) (ns = not significant; $n = 5$, $\pm$ standard error)

Table 2 Variables of plant growth in atemoya, *araticum-de-terra-fria*, *araticum-mirim* and *biribá* ungrafted plants prior to grafting (stem diameter, plant height and number of leaves) and grafted plants at 60 and 90 days after grafting (stem diameter, plant height and number of leaves and number of shoots)

Before grafting	Stem diameter (cm)	Plant height (cm)	Number of leaves	
Ungrafted atemoya	0.9 ± 0.3^a	159.3 ± 15.7^a	45.3 ± 19.5^b	
Ungrafted *araticum-de-terra-fria*	1.2 ± 0.2^a	121.0 ± 11.3^a	107.3 ± 18.7^{ab}	
Ungrafted *araticum-mirim*	1.4 ± 0.3^a	148.3 ± 5.8^a	136.5 ± 18.6^a	
Ungrafted *biribá*	1.1 ± 0.2^a	121.3 ± 12.6^a	74.3 ± 17.8^{ab}	
F value	1.06^{ns}	2.65^{ns}	4.51^*	
60 days after grafting	**Stem diameter (cm)**	**Plant height (cm)**	**Number of leaves**	**Number of shoots**
Atemoya/atemoya	1.4 ± 0.2^a	24.8 ± 1.4^b	17.2 ± 3.3^a	2.2 ± 0.42^a
Atemoya/*araticum-de-terra-fria*	1.5 ± 0.2^a	29.4 ± 1.4^{ab}	15.8 ± 2.7^a	2.2 ± 0.22^a
Atemoya/*araticum-mirim*	1.5 ± 0.1^a	25.6 ± 4.9^b	13.8 ± 1.1^a	2.6 ± 0.27^a
Atemoya/*biribá*	1.4 ± 0.1^a	37.2 ± 2.1^a	17.8 ± 2.6^a	2.8 ± 0.22^a
F value	0.02^{ns}	4.96^*	0.6^{ns}	1.29^{ns}
90 days after grafting	**Stem diameter (cm)**	**Plant height (cm)**	**Number of leaves**	**Number of shoots**
Atemoya/atemoya	1.5 ± 0.14^a	29.6 ± 4.72^b	14.4 ± 3.62^b	2.0 ± 0.0^a
Atemoya/*araticum-de-terra-fria*	1.6 ± 0.12^a	66 ± 5.65^a	21 ± 2.32^{ab}	2 ± 0.0^a
Atemoya/*araticum-mirim*	1.5 ± 0.11^a	33.6 ± 7.29^b	14.2 ± 2.68^b	1.2 ± 0.22^a
Atemoya/*biribá*	1.7 ± 0.08^a	70.6 ± 6.65^a	27.6 ± 3.47^a	2.0 ± 0.0^a
F value	0.91^{ns}	15.02^*	5.38^*	16^{ns}

Means followed by the same letter in a column do not differ by Tukey's test at 5% probability ($P < 0.05$). (ns = not significant; $n = 5$, $\pm$ standard error)

ungrafted atemoya and the *biribá* rootstock caused high E, yet WUE did not decrease. Similar to gas exchange, differences were not observed in vegetative development before grafting, demonstrating uniformity between the species, except for the *araticum-mirim* rootstock that displayed an increased number of leaves compared with atemoya (Table 2).

In relation to ion accumulation, it is interesting that the various rootstocks caused increases in scion absorption and accumulation that differed from that obtained when atemoya was grafted onto itself. This finding may be largely attributed to the genetic characteristics of the root system of the rootstock, which are generally more vigorous than those of cultivated varieties (Martínez-Ballesta et al. 2010). No restrictions in ion flux were observed in the grafted plants, demonstrating that the rootstock plays an important role in such responses. San Bautista et al. (2011) also observed increased N, P, K and Ca accumulation in grafted plants compared with non-grafted plants. Nitrogen (N) concentrations in the atemoya scion were equal among all combinations, and this result has also been reported for Cucurbitaceae and Solanaceae family grafted plants (Yetisir and Erhan 2013).

Before grafting, the ion concentration was higher in leaf tissue compared with root tissue in ungrafted plants, with

the exception of P (Table 3). At 60 DAG, mineral elements were not accumulated in the root system of any rootstock. Prior to grafting, *biribá* exhibited an increased concentration of N in the leaf, when compared to atemoya (Table 3); however, after grafting, the increased concentration of N was not observed to the atemoya scion when grafted onto biribá (Table 3). Regarding P, *araticum-de-terra-fria* and *biribá* ungrafted plants (0 DAG) exhibited increased concentrations of this mineral element in the roots, compared with atemoya (Table 3). After grafting, *araticum-de-terra-fria*, at 60 DAG, continued to exhibit the highest P concentration in the roots; however, only small quantities were translocated to the scion, resulting in lower concentrations of P in atemoya scion leaves. The P is crucial for the maintenance of phosphorylation reactions during carbon assimilation and is also beneficial for other photosynthetic parameters, such as g_s and WUE (Bruck et al. 2000). In this study, the high A_{net} as well as the A_{net}/C_i observed when *biribá* was used as the rootstock could be attributed to increased P accumulation in the scion.

Among the combinations studied, *araticum-de-terra-fria* rootstock caused increased K accumulation leaf in the atemoya scion, compared to atemoya self-grafted and also exhibited the highest root concentration. However, this accumulation was not different compared to *biribá*

Table 3 Ion concentrations (g.kg dry matter) from tissue leaf and root in atemoya, *araticum-de-terra-fria*, *araticum-mirim* and *biribá* plants prior to grafting (0 DAG) and tissue leaf and root scion atemoya and rootstocks atemoya, *araticum-de-terra-fria*, *araticum-mirim* and *biribá* at 60 days after grafting (60 DAG)

0 DAG	Atemoya	*Araticum-de-terra-fria*	*Araticum-mirim*	*Biribá*
Nitrogen (N)				
Leaf	27.4 ± 2.1^{aB}	30.7 ± 1.0^{aAB}	32.2 ± 1.7^{aAB}	34.3 ± 1.1^{aA}
Root	19.5 ± 2.8^{Ba}	23.3 ± 1.8^{bA}	24.0 ± 1.3^{aA}	17.4 ± 1.7^{bA}
Factors: plant organ (PO) = 54.51*; ungrafted (UG) = 2.19^{ns}; PO × UG = 2.8*				
Phosphorus (P)				
Leaf	1.7 ± 0.17^{aB}	2.1 ± 0.1^{aAB}	2.8 ± 0.3^{aA}	2.7 ± 0.1^{aA}
Root	1.3 ± 0.23^{aB}	2.5 ± 0.5^{aA}	2.2 ± 0.3^{aAB}	2.6 ± 0.5^{aA}
Factors: plant organ (PO) = 1.27^{ns}; ungrafted (UG) = 12.93*; PO × UG = 2.2*				
Potassium (K)				
Leaf	22.3 ± 1.1^{aA}	23.4 ± 0.4^{aA}	21.5 ± 2.3^{aA}	22.5 ± 0.9^{aA}
Root	16.4 ± 2.8^{aB}	18.3 ± 1.4^{bAB}	24.5 ± 3.9^{aAB}	28.5 ± 4.5^{aA}
Factors: plant organ (PO) = 0.13^{ns}; ungrafted (UG) = 3.86^{ns}; PO × UG = 4.77*				
Calcium (Ca)				
Leaf	11.1 ± 0.3^{aA}	11.7 ± 0.6^{aA}	12.2 ± 1.0^{aA}	11.4 ± 0.9^{aA}
Root	3.0 ± 0.3^{bB}	5.3 ± 1.3^{bAB}	6.9 ± 0.6^{bA}	5.9 ± 0.9^{bAB}
Factors: plant organ (PO) = 161.15*; ungrafted (UG) = 4.31*; PO × UG = 1.59*				
Magnesium (Mg)				
Leaf	5.4 ± 0.20^{aAB}	4.6 ± 4.7^{aB}	5.8 ± 0.4^{aA}	5.0 ± 0.2^{bAB}
Root	2.8 ± 0.80^{bB}	3.6 ± 0.6^{bB}	3.3 ± 0.3^{bB}	7.5 ± 1.4^{aA}
Factors: plant organ (PO) = 11.58*; ungrafted (UG) = 14*; PO × UG = 18.68*				

60 DAG	Atemoya/atemoya	Atemoya/*araticum-de-terra-fria*	Atemoya/*araticum-mirim*	atemoya/*biribá*
Nitrogen (N)				
Leaf (scion)	42.9 ± 1.6^{aA}	43.1 ± 1.5^{aA}	41.0 ± 0.7^{aA}	40.3 ± 0.3^{aA}
Root (rootstock)	25.2 ± 1.8^{bA}	17.4 ± 2.6^{bB}	14.6 ± 0.9^{bB}	15.0 ± 1.6^{bB}
Factors: plant organ (PO) = 10.62*; rootstock (RS) = 16.76*; PO × RS = 7.84*				
Phosphorus (P)				
Leaf (scion)	3.37 ± 0.10^{aC}	3.67 ± 0.10^{aBC}	3.97 ± 0.20^{aAB}	4.35 ± 0.10^{aA}
Root (rootstock)	1.67 ± 0.30^{bB}	2.70 ± 0.05^{bA}	1.90 ± 0.20^{bB}	1.86 ± 0.20^{bB}
Factors: plant organ (PO) = 7.55*; rootstock (RS) = 13.2*; PO × RS = 14.37*				
Potassium (K)				
Leaf (scion)	23.5 ± 1.9^{aB}	32.8 ± 2.2^{aA}	27.5 ± 2.7^{aAB}	22.5 ± 0.9^{aB}
Root (rootstock)	14.4 ± 1.2^{bB}	23.9 ± 0.3^{bA}	15.9 ± 2.5^{bBC}	21.0 ± 1.3^{bAB}
Factors: plant organ (PO) = 46.25*; rootstock (RS) = 12.1*; PO × RS = 3.68*				
Calcium (Ca)				
Leaf (scion)	4.6 ± 0.3^{aB}	10.5 ± 2.0^{aA}	8.4 ± 0.8^{aAB}	9.7 ± 0.2^{aA}
Root (rootstock)	4.3 ± 0.4^{aB}	5.6 ± 0.6^{bA}	4.9 ± 0.3^{bAB}	4.9 ± 0.5^{bAB}
Factors: plant organ (PO) = 47.87*; rootstock (RS) = 10.04*; PO × RS = 4.76*				
Magnesium (Mg)				
Leaf (scion)	4.8 ± 0.5^{aAB}	4.5 ± 0.6^{aB}	4.9 ± 0.3^{aAB}	6.5 ± 0.4^{aA}
Root (rootstock)	2.8 ± 0.3^{bB}	3.6 ± 0.9^{aAB}	2.6 ± 0.2^{bB}	4.5 ± 0.5^{bA}
Factors: plant organ (PO) = 49.02*; rootstock (RS) = 10.18*; PO × RS = 1.34*				

Means followed by the same uppercase letter in the line and lowercase letter in the column are not significantly different at 5% probability according to Tukey's test ($P < 0.05$) (ns = not significant; $n = 5$, $\pm$ standard error)

reflected in gas exchange, mainly with regard to stomatal regulation, as proposed by Maathuis (2009). The increased root systems could improve all nutrient uptake, not only K uptake, and this nutrient accumulation are potentially associated with the vigorous root system of this species, which is native and adapted to soils with low nutrient availability (Kavati 2013).

Despite the fact that the ungrafted species exhibited increased Ca concentrations in leaves compared with roots, no differences were noted among the leaves of the rootstock species (Table 3). However, at 60 DAG, *araticum-de-terra-fria* and *biribá* exhibited significant increases in Ca concentrations in scion leaves compared to atemoya rootstock (Table 3). Furthermore, atemoya roots also exhibited the lowest Ca concentrations compared with other rootstocks, which did not differ from the leaves. Similar to N and Ca, the ungrafted plants exhibited increased Mg concentrations in leaves compared with roots (Table 3), nevertheless, *biribá* roots exhibited increased Mg concentrations compared with other *araticum-de-terra-fria* rootstocks and exhibited similar root Mg concentrations compared with other species (Table 3). The atemoya scion exhibited reduced Ca and Mg concentrations compared with ungrafted atemoya, and this feature was also observed in grafted Cucurbitaceae (Ruiz et al. 1997).

According to literature, for example to tomato plants, Ca and Mg levels decreased when this species were grafted onto pepper and vice versa compared with tomato grafted onto tomato or pepper grafted onto pepper; this response was due to a reduced hydraulic conductivity of the rootstock xylem in relation to the scion utilised (Kawaguchi et al. 2008). Moreover, grafted melon plants exhibited no differences in Ca concentrations; however, Mg concentrations in leaf tissues were significantly influenced by the combination (Colla et al. 2010).

In general, increased photosynthetic rates and changes in ionic accumulation were reflected directly in the vegetative growth of the scion, exhibiting higher rates for atemoya grafted onto *araticum-de-terra-fria* and *biribá*. This effect was evident at the end of the evaluation period. The effects of the rootstocks on plant growth were observed at 90 DAG, namely the number of leaves and plant height; however, no differences in stem diameter were observed (Table 2). In atemoya grafted onto *araticum-mirim* and atemoya, plant height was lowest compared with other combinations, confirming that *araticum-mirim* rootstock confers dwarf characteristics to grafted plants (Kavati 2013). Although the rootstock *biribá* presents incompatibility immediately after grafting (Almeida et al. 2010), all rootstocks promoted a greater number of leaves on the scion in the present study; the latter is considered by growers and commercial nurseries to be more compatible for atemoya scion. A visual symptom of incompatibility is

the swelling of plant tissue immediately below the union of tissues; however, this symptom was not observed in this study at 90 DAG.

In summary, the present study offers advances in elucidation about physiological mechanisms involved in grafted *Annona* plants, and we observed that rootstocks modulate atemoya biochemical responses, such as photosynthetic and plant development. We concluded that *biribá* rootstocks increased gas exchange and ion concentration in the atemoya scion, indicating that the physiological changes caused by this rootstock to scion do not trigger early incompatibility.

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