

Leaf gas exchanges responses of atemoya scion grafted onto *Annona* rootstocks

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Abstract We examined the hypothesis that leaf gas exchange of scion is affected by different rootstocks in wood plants. We investigated daily courses of gas exchange and photosynthetic potential using the CO₂ assimilation rates as a function of photosynthetic photon flux density, and then assessed CO₂ response curves in atemoya scion (*Annona* × *atemoya* Mabb.) grafted onto *araticum-de-terra-fria* [*A. emarginata* (Schltdl.) H. Rainer var. *terra-fria*]: ATF, *araticum-mirim* [*A. emarginata* (Schltdl.) H. Rainer var. *mirim*]: ATM, *biribá* [*A. mucosa* (Bail.) H. Rainer]: ATB, atemoya (autograft): ATA, and in ungrafted atemoya plants: CTR. Throughout the entire evaluation period, the net assimilation rate (A_{net}) and stomatal conductance (g_s) of CTR plants remained practically constant, being lower than those of grafted plants between 08:00 a.m. and 12:00 a.m., regardless of the rootstock used. Moreover, ATM plants proved to be more efficient in keeping the stomata open, even during the hottest hours of the day, improving A_{net} and

carboxylation use efficiency. However, this occurred at the lowest maximum carboxylation rate of ribulose-1,5-bisphosphate ($V_{\text{cmáx}}$). Overall, ATF plants presented a low light saturation point and photosynthetic electron transport rates, though increased maximum quantum yield of photosynthesis was observed. Thus, we accept our hypothesis and conclude that grafting might affect the photosynthetic metabolism of the atemoya hybrid, regardless of the combination used, which promotes enhanced A_{net} and low $V_{\text{cmáx}}$ and light saturation points.

Keywords Annonaceae · Gas exchange · Grafted plants · Light curves

1 Introduction

From the earliest times, since the Old Testament of the Bible, Greek Civilization, and ancient China, food producers have connected the root part of a species to the aerial part of another species, forming a “new” plant through a process known as grafting (Melnik and Meyerowitz 2015; Melnik et al. 2015; Xu et al. 2016). The grafting technique and the combination of different graft and rootstock species has long been the target of investigations aimed at proposing solutions to problems in acclimation of commercial plants to numerous biotic and abiotic factors in the field, such as resistance to pathogens, the influence of

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temperature and shading conditions, as well as thermal, saline, and nutritional stresses (Colla et al. 2010; Goldschmidt 2014; Warschefsky et al. 2016).

In orchards, atemoya (*Annona* × *atemoya* Mabb.) is a hybrid fruit of economic importance and is grafted to ensure that the genetic characteristics of productive scions are maintained (Encina et al. 2014). Rootstocks most often used to graft atemoya include *araticum-de-terra-fria* [*Annona emarginata* (Schltdl.) H. Rainer “var. terra-fria”], *araticum-mirim* [*Annona emarginata* (Schltdl.) H. Rainer “var. mirim”], *biribá* [*Annona mucosa* (Bail.) H. Rainer], and atemoya (autograft) (Kavati 2013). However, the latter is susceptible to fungi present in the soil when used as a rootstock (Stenzel et al. 2003).

Atemoya scion grafted onto *araticum-de-terra-fria* (ATF) rootstock results in further development of the scion and tolerance to cave nematodes, stem borers, and water stress (Tokunaga 2005). However, *araticum-mirim* (ATM) rootstock causes dwarfism (Baron et al. 2017), which is considered beneficial, as it facilitates the management of commercial orchards (Prassinós et al. 2009). Additionally, *biribá* has been studied to avoid problems with nematodes and stem-borers, with improved adaptation to adverse conditions (de Almeida et al. 2010) and the facilitation of expanded adaptability in scion (Baron et al. 2017).

However, the physiology and biochemistry of grafted plants tend to differ from that of ungrafted plants due to graft–rootstock interactions. Most grafting studies suggest that changes in the scion are controlled by the rootstock through controlled uptake, synthesis, and translocation of water, minerals and plant hormones (Al-Harbi et al. 2018; Balal et al. 2017; dos Santos et al. 2017).

Moreover, several authors have reported that grafting improves net CO₂ assimilation rate, stomatal conductance, and transpiration, which results in higher plant growth and yields (Borgognone et al. 2013; He et al. 2009; Penella et al. 2017). As a result of this metabolic change, grafting affects photosynthetic metabolism by means of increased net CO₂ assimilation rate improvement and decreased maximum quantum yield of photosynthesis (Amaro et al. 2014; Covarrubias et al. 2016). Since all biomass production depends on photosynthetic activity, agricultural practice aims to maximize the photosynthetic efficiency of crops and improve the final crop yield in terms of productivity and quality.

Gas exchange in grafted plants are directly modified by rootstock, as this can alter the vigor and productivity of the scion (Colla et al. 2012; Karimi and Nowrozy 2017; Lima et al. 2017). For example, grafting affects the plant water relations, whereas sufficient vascular connection between the rootstock and scion increases the flow of nutrients and water, facilitating increased photosynthesis (Martínez-Ballesta et al. 2010; Salehi et al. 2010).

Several physiological responses of the plants to grafting in herbaceous plants are found in the literature. However, little is known about its effects on woody plants due to the difficulty of working with plants that require a longer periods of time to present stages suitable for grafting. Therefore, we examine the hypothesis that leaf gas exchange of scion is affected by different rootstocks in wood plants.

2 Material and methods

2.1 Plant material and treatments

The present study was conducted in a greenhouse. Seeds of three rootstocks species, including *araticum-de-terra-fria* [*Annona emarginata* (Schltdl.) H. Rainer ‘variety terra-fria’], *araticum-mirim* [*Annona emarginata* (Schltdl.) H. Rainer ‘variety mirim’], *biribá* [*Annona mucosa* (Bail.) H. Rainer], and atemoya (*Annona* × *atemoya* Mabb.) were sown in polystyrene trays containing vermiculite, according to the method of Baron et al. (2011).

When seedlings developed fully expanded leaves, they were transplanted to plastic pots (approximately 20 dm³) containing a mixture substrate with fertile soil, vermiculite, and 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. This solution was diluted to 50% of its ionic strength, with an electrical conductivity (EC) range of 1.0–1.5 mS cm^{−1} and calcium nitrate, EC range from 0.20 to 0.25 mS cm^{−1}, which is recommended for growing annonaceous plants from seedlings until young plants, according to Baron et al. (2017).

The whip and tongue grafting technique was performed according to technical bulletins on plant propagation for atemoya hybrid ‘Thompson’ (Tokunaga 2005). Rootstocks were prepared 18 months

after sowing, when the plants possessed stem diameters ranging from 8 to 15 mm and were 15 cm in height. The plants were prepared using stem segments (12 cm in length, 8–15 mm in diameter) from the same plant.

An evaluation of gas exchange was performed in each graft combination [atemoya scions grafted onto ATF, ATM, *biribá* (ATB), atemoya (ATA) rootstocks], and in ungrafted atemoya (CTR). Gas exchange was measured 12 months after grafting, when grafted plants exhibited complete post-grafting re-establishment using an infrared CO₂ and water vapor analyzer (LI-6400, Li-Cor, Inc., Lincoln, NE, USA) using the second fully expanded leaves from the apex.

2.2 Daily gas exchange

Daily gas exchange was performed every 2 h from 8:00 a.m. until 4:00 p.m. Net CO₂ assimilation rate (A_{net} , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration (E , $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), and vapor pressure deficit (VPD, kPa) were evaluated. Water use efficiency [WUE , $\mu\text{mol CO}_2 (\text{mmol H}_2\text{O})^{-1}$] was determined by the relationship between net assimilation rate and transpiration (A_{net}/E), and the carboxylation efficiency (A_{net}/C_i) was determined by the relationship between the CO₂ assimilation rate and the intercellular CO₂ concentration (C_i , $\mu\text{mol CO}_2 \text{ mol air}^{-1}$).

To ensure the consistency of experimental conditions, photosynthetic photon flux density (PPFD) was standardized through the use of a light-emitting diode coupled to a photosynthesis chamber. Moreover, all plants were placed under the same light conditions to ensure a consistent light environment during each experimental period (Table 1). The reference CO₂ concentration used during the evaluation was $380 \mu\text{mol mol}^{-1}$. Air temperature and relative humidity, as well as PPFD, were recorded by the LI-6400 during gas exchange evaluations in the greenhouse (Table 1).

Measurements were conducted by selecting 12 plants of each treatment (four of each grafting combination). Evaluations were performed over three consecutive days, each representing an experimental block.

2.3 Response curve of the CO₂ assimilation rate (A_{net} , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) as a function of photosynthetic photon flux density (PPFD)

The response curve for CO₂ assimilation rate (A_{net} , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) as a function of PPFD was obtained by decreasing PPFD from 2000 to $0 \mu\text{mol m}^{-2} \text{ s}^{-1}$ at intervals of $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$ until $200 \mu\text{mol m}^{-2} \text{ s}^{-1}$, and thereafter at 100, 50, and $0 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Measurements were then conducted by selecting three plants from each treatment (one from each experimental block).

The response curve was adjusted to the hyperbolic function $A = a + [(A_{\text{máx}} \times \text{PPFD})/(b + \text{PPFD})]$, where $A_{\text{máx}}$ is the maximum net CO₂ assimilation rate, and a and b are the parameters of the hyperbolic equation. This function allowed us to calculate respiration in the dark (a in the equation) and at the light compensation point (τ , corresponding to the value of PPFD where A is zero). The light saturation point was determined by fitting a straight line ($y = 1$) to the higher points of the curve. The hyperbolic function was then fitted using SAS 9.2 statistical software (SAS Institute, Inc., Cary, NC). The concentration of the reference CO₂ during the evaluation was $380 \mu\text{mol mol}^{-1}$.

2.4 CO₂ response curves (A_{net}/C_i)

CO₂ response curves (A_{net}/C_i) were performed using the light saturation point, previously determined by the light curves for each treatment. The CO₂ concentrations first ranged from 400 to $0 \mu\text{mol mol}^{-1}$ of CO₂ at intervals of $50 \mu\text{mol mol}^{-1}$ CO₂. Thereafter, CO₂ concentrations ranged from 400 to $2000 \mu\text{mol mol}^{-1}$ of CO₂ at intervals of $200 \mu\text{mol mol}^{-1}$ CO₂. Measurements were then performed by selecting three plants from each treatment (one from each experimental block).

Curves were fitted according to the Sharkey model (Sharkey et al. 2007), calculating the maximum carboxylation rate of ribulose-1,5-bisphosphate (RuBP, $V_{\text{cmáx}}$, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), photosynthetic electron transport rate (J , $\mu\text{mol electrons m}^{-2} \text{ s}^{-1}$), triose phosphate use (TPU), respiratory rate (Rd^* , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), and mesophyll conductance (g_m^* , $\mu\text{mol m}^{-2} \text{ s}^{-1}$).

Table 1 Photosynthetic photon flux density (PPFD, $\mu\text{mol m}^{-2} \text{s}^{-1}$), air temperature ($^{\circ}\text{C}$), and relative air humidity (%) in the experimental greenhouse from 8:00 a.m. to 4:00 p.m.

	PPFD ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Air temperature ($^{\circ}\text{C}$)	Relative humidity (%)
08:00 a.m.	831.72 \pm 18.85	29.06 \pm 0.38	40.78 \pm 1.25
10:00 a.m.	1357.57 \pm 24.60	30.60 \pm 0.32	40.78 \pm 0.93
12:00 a.m.	1536.50 \pm 16.89	34.99 \pm 0.20	32.76 \pm 0.97
02:00 p.m.	1206.72 \pm 39.61	34.07 \pm 0.15	32.58 \pm 0.63
04:00 p.m.	674.39 \pm 21.82	32.18 \pm 0.26	35.58 \pm 0.81

2.5 Experimental design and statistical analysis

The experimental design was conducted using a randomized block design consisting of five treatments involving three blocks with 12 plants each. To determine the homogeneity of treatment variances, Levene's test was performed using SAS 9.2 statistical software (SAS Institute, Inc., Cary, NC). The results were subjected to an analysis of variance test, and means were compared using the Tukey test ($p \leq 0.05$) using SAS 9.2 statistical software (SAS Institute, Inc., Cary, NC).

3 Results

Between 08:00 a.m. and 04:00 p.m., stomata of atemoya remained open (g_s) throughout the entire evaluation period (Fig. 1) in all grafting combinations (ATF, ATM, ATB and ATA) and in ungrafted plants (CTR). During this period, the A_{net} and g_s (Fig. 1) of CTR plants remained practically constant and smaller than those of grafted plants between 08:00 a.m. and 12:00 a.m., regardless of the rootstock used ($p < 0.05$).

Additionally, ATM and ATA plants exhibited larger A_{net} from 08:00 a.m. to 02:00 p.m. (Fig. 1; $p < 0.001$). The ATF plants presented higher A_{net} from 10:00 a.m. to 02:00 p.m. ($p < 0.001$), while ATB plants presented higher A_{net} from 08:00 a.m. to 12:00 a.m. ($p < 0.001$). At 10:00 a.m., PPFD (Table 1) exceeded the light saturation point of these plants (Table 2).

Furthermore, ATM plants reached higher values of A_{net} ($p < 0.0001$; Fig. 1) and E ($p < 0.01$; Fig. 2) than the other combinations at 02:00 p.m., when the

ambient temperature was between the highest and the lowest relative humidity (Table 1). This is due to g_s remaining high ($p < 0.001$) despite having reached greater A_{net}/C_i ($p < 0.01$; Fig. 2), which represents a similar response to that of ATA plants.

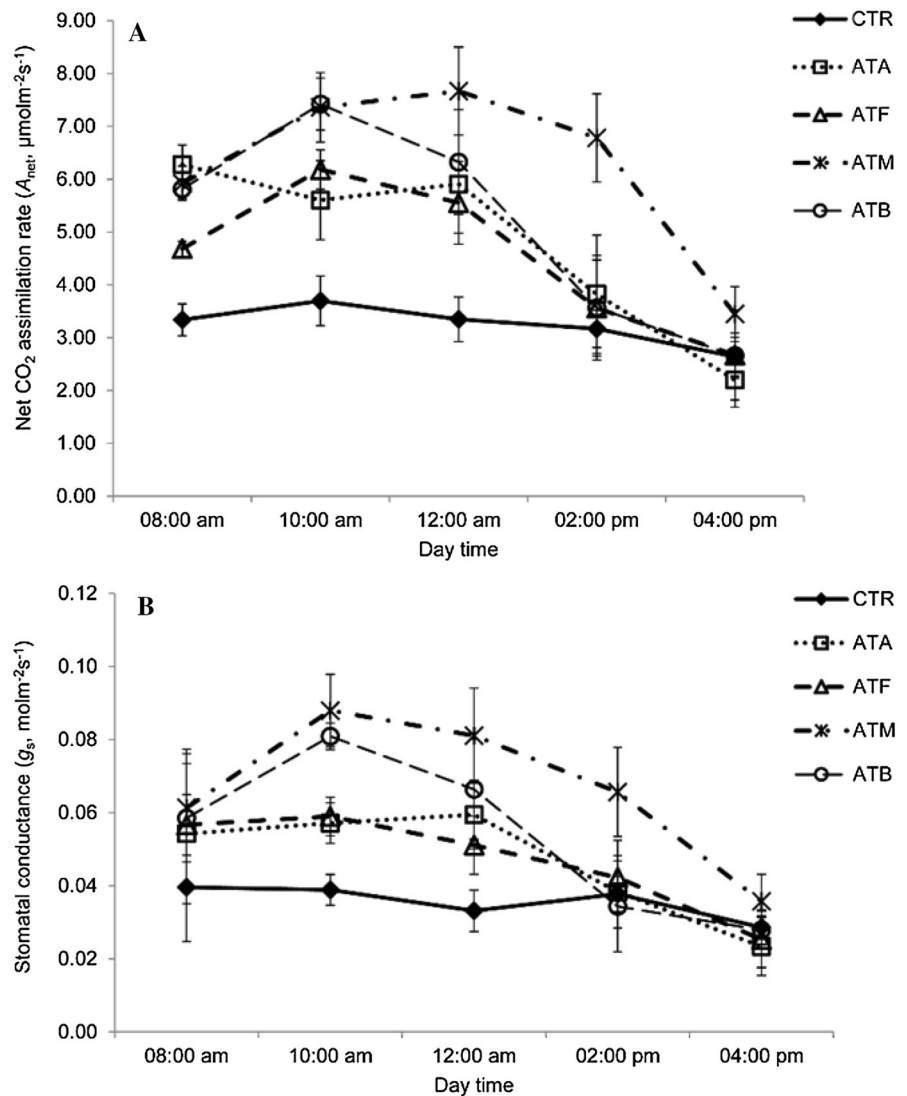
At 02:00 p.m., a decrease of g_s in ATF, ATB and ATA plants was observed, which led to a decrease of A_{net} , E and A_{net}/C_i , which were similar to each other and to CTR plants. At 04:00 p.m. ATM plants equalized their gas exchange rates to a greater extent than other evaluated plants ($p > 0.005$).

Leaf temperature (Fig. 3) and VPD (Fig. 4) were also similar between evaluated plants, and followed diurnal temperature variations (Table 1), exhibiting high values between 12:00 a.m. and 02:00 p.m. ($p < 0.0001$).

The graft–rootstock interaction also influenced the light and carboxylation curves. Atemoya-grafted plants, regardless of the combination used, presented a lower light saturation point and maximum carboxylation rate of RuBP ($V_{\text{cmáx}}$) (Table 2). The lowest $V_{\text{cmáx}}$ was observed in ATM plants. The greatest light compensation point (Table 2) was presented by ATA plants, with no differences between other types of plants. The maximum quantum yield of photosynthesis (Table 2) was higher in ATF plants, which also exhibited the lowest photosynthetic electron transport rate (J) (Table 2), while no differences were observed between the other rootstock combinations. TPU (Table 2) was higher in ATB plants and lowest in ATF plants.

No differences were observed between ATA, ATM, and CTR plants in relation to respiratory rate (Rd^*) (Table 2). ATF and ATA plants exhibited the lowest mesophyll conductance (g_m^*) (Table 2), though this was not statistically different from ATM plants.

Fig. 1 **a** Net assimilation rate (A_{net} , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and **b** stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) in atemoya scions grafted onto atemoya (ATA), *araticum-de-terra-fria* (ATF), *araticum-mirim* (ATM), and *biribá* (ATB) rootstocks, and ungrafted atemoya (CTR), from 8:00 a.m. to 4:00 p.m. Values are mean \pm SE ($n = 12$)



4 Discussion

The daily gas exchange analysis performed in this experiment demonstrated that grafting increased the photosynthetic efficiency of atemoya, regardless of the combination used, while also increasing A_{net} , E , A_{net}/C_i and g_s values. These increases also occurred in ATA plants, providing evidence for increased photosynthetic efficiency throughout the day being caused by the grafting itself, which differed according to the rootstock used. This result was also evident upon analyzing the maximum carboxylation rate of RuBP ($V_{\text{cmáx}}$)—which was lower in all grafted plants—indicating that grafting increases the efficiency of this

enzyme and resulted in an increased affinity with its substrate (CO_2), consequently enhancing the CO_2 assimilation.

In herbaceous plants belonging to Cucurbitaceae and Solanaceae families, the reestablishment of vascular connections in grafted plants is of fundamental importance for water flow (Martínez-Ballesta et al. 2010). When forming the callus at the scion/rootstock interface, grafted plants enable water flow from the rootstock to the scion and, when the vascular connection is successful, several authors have reported that the graft improves A_{net} , A/C_i , E and g_s , which results in increased growth and productivity (Amaro et al. 2014; He et al. 2009; Salehi et al. 2010; Yang et al. 2006).

Table 2 Light compensation point (τ , $\mu\text{mol m}^{-2} \text{s}^{-1}$), light saturation point ($\mu\text{mol m}^{-2} \text{s}^{-1}$), maximum quantum yield of photosynthesis ($\mu\text{mol CO}_2 \mu\text{mol photons}^{-1}$), maximum carboxylation rate of ribulose-1,5-diphosphate ($V_{\text{cmáx}}$, $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$), photosynthetic electron transport rate (J , $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$), triose phosphate use (TPU), respiratory rate (R_d^* , $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$), and mesophyll conductance (g_m^* , $\mu\text{mol m}^{-2} \text{s}^{-1}$) in atemoya scions grafted onto atemoya (ATA), *araticum-de-terra-fria* (ATF), *araticum-mirim* (ATM), and *biribá* (ATB) rootstocks, and ungrafted atemoya (CTR)

	Light compensation point	Light saturation point	Maximum quantum yield of photosynthesis	$V_{\text{cmáx}}$	J	TPU	R_d^*	g_m^*
CTR	15.82 ± 3.62b	1617.68 ± 142.54a	0.0130 ± 0.0029b	109.00 ± 2.12a	84.50 ± 2.12a	4.10 ± 0.14b	2.21 ± 0.39ab	23.79 ± 0.33ab
ATA	49.06 ± 12.79a	1012.83 ± 4.63bc	0.0158 ± 0.0002b	54.00 ± 0.70b	82.50 ± 2.48a	4.05 ± 0.88b	2.57 ± 0.16a	18.95 ± 0.25c
ATF	10.55 ± 3.55b	736.82 ± 97.41c	0.3807 ± 0.0043a	66.50 ± 8.84b	28.00 ± 0.10b	2.30 ± 0.07c	1.18 ± 0.08c	18.43 ± 0.62c
ATM	14.17 ± 3.75b	1290.76 ± 5.98b	0.0118 ± 0.0002b	37.50 ± 4.60c	67.50 ± 1.77a	4.40 ± 0.28b	2.15 ± 0.07ab	20.64 ± 1.81bc
ATB	12.63 ± 4.22b	1118.22 ± 51.82b	0.0126 ± 0.0008b	60.50 ± 0.35b	89.50 ± 6.01a	6.35 ± 0.32a	1.49 ± 0.31bc	25.53 ± 0.62a
F values	8.80**	24.71**	33.58**	50.92**	11.90**	15.75**	8.37**	6.05**
CV (%)	46.03	9.88	18.34	9.86	17.89	14.82	17.69	16.94**

Mean values followed by the same letter do not differ significantly based on Tukey's test at a 5% probability

**Significant at 1% probability ($p < 0.01$), *significant at 5% probability ($p < 0.05$), and ns denotes not significant ($p \geq 0.05$). Values are mean ± SE ($n = 3$)

Additionally, rootstocks may affect the scion in different ways, and these alterations can also be observed in the production of secondary metabolites and in ionic accumulation (Huang et al. 2015; Penella et al. 2015, 2017).

We observed that ungrafted (CTR) and autografted (ATA) plants presented little variation in gas exchange rates throughout the day. Therefore, it suggests that the rootstock may increase gas exchange rates.

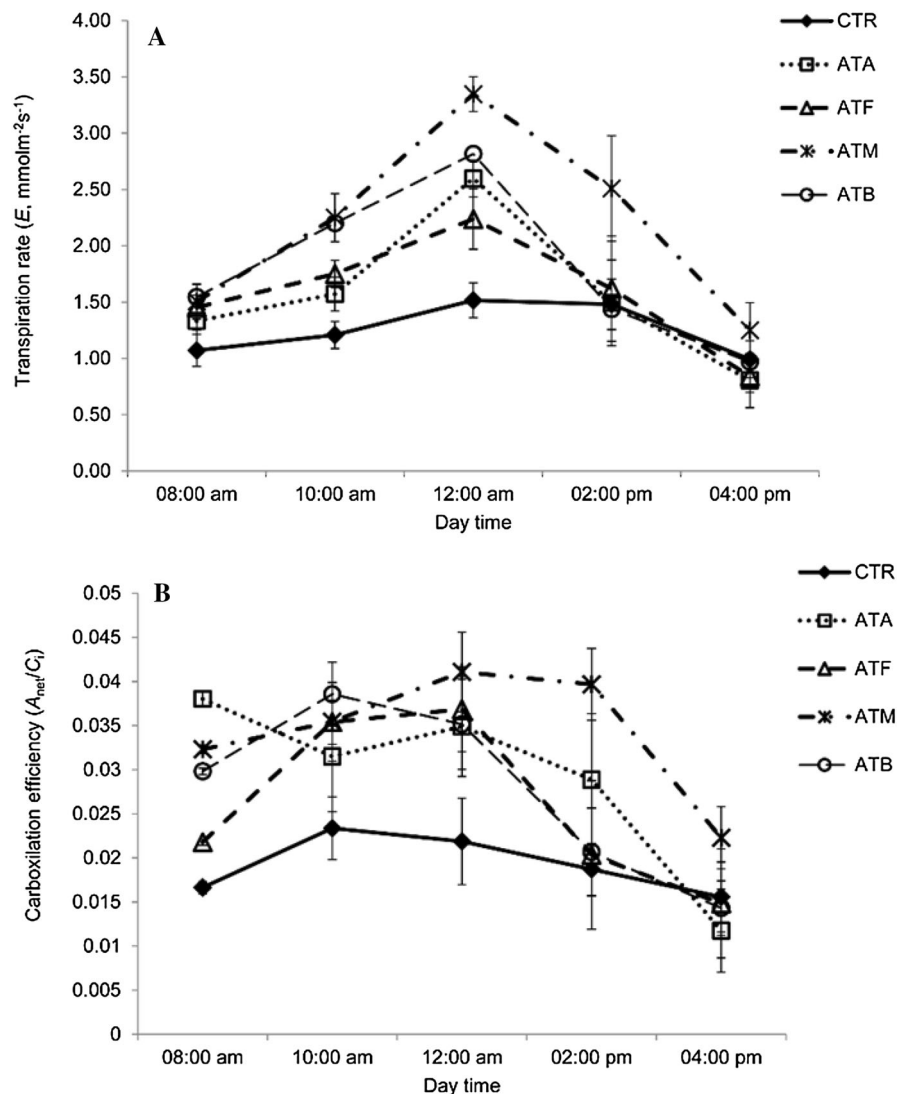
In this study, ATM plants exhibited greater efficiency in keeping the stomata open, presenting higher g_s ($p < 0.05$) even during the hottest hours of the day (12:00 a.m. and 02:00 p.m.). This provided higher A_{net} than other combinations used ($p < 0.05$), and it also exhibited higher carboxylation efficiency at 02:00 p.m. ($p < 0.05$) and the lowest $V_{\text{cmáx}}$ ($p < 0.01$). Improved A_{net} can result in improved growth, dry matter accumulation, yields, and fruit quality.

The literature reports the evolutionary origin and domestication of both *A. emarginata* var. *mirim* (sin. *Rollinia emarginata* Schltdl.) and *A. emarginata* var. *terra-fria* predominantly occurred in subtropical and tropical regions of the globe, experiencing higher environmental temperatures (Paul et al. 1992). However, the *terra-fria* variety has exhibited the greatest adaptation to milder temperature conditions (Baron et al. 2014; Tokunaga 2005), which may explain the greater stomatal opening of ATM plants during the hottest hours of the day.

The type of rootstock used influences the absorption, synthesis, and the translocation of water, minerals, and plant hormones (Martínez-Ballesta et al. 2010). This increases the availability of water in the plant, causing an increase of the water flow that keeps the stomata open, even during the hottest hours of the day, providing high rates of CO_2 assimilation (Amaro et al. 2014).

According to Baron et al. (2017), *araticuns* and *biribá* do not restrict the ionic flow to atemoya-grafted plants. These authors observed that combinations between atemoya grafted onto ATF and ATM show great accumulation of K^+ in their leaves. Furthermore, Maathuis (2009) suggests that K^+ is responsible for several changes in the turgor of guard cells during stomatal movement, which results in greater stomatal opening. Additionally, this mineral element is a cofactor of enzymes involved in respiration and

Fig. 2 **a** Transpiration rate (E , $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) and **b** carboxylation efficiency (A_{net}/C_i), in atemoya scions grafted onto atemoya (ATA), *araticum-de-terra-fria* (ATF), *araticum-mirim* (ATM), and *biribá* (ATB) rootstocks, and ungrafted atemoya (CTR), from 8:00 a.m. to 4:00 p.m. Values are mean \pm SE ($n = 12$)



photosynthesis (Jin et al. 2011), which explains the results obtained in the present study.

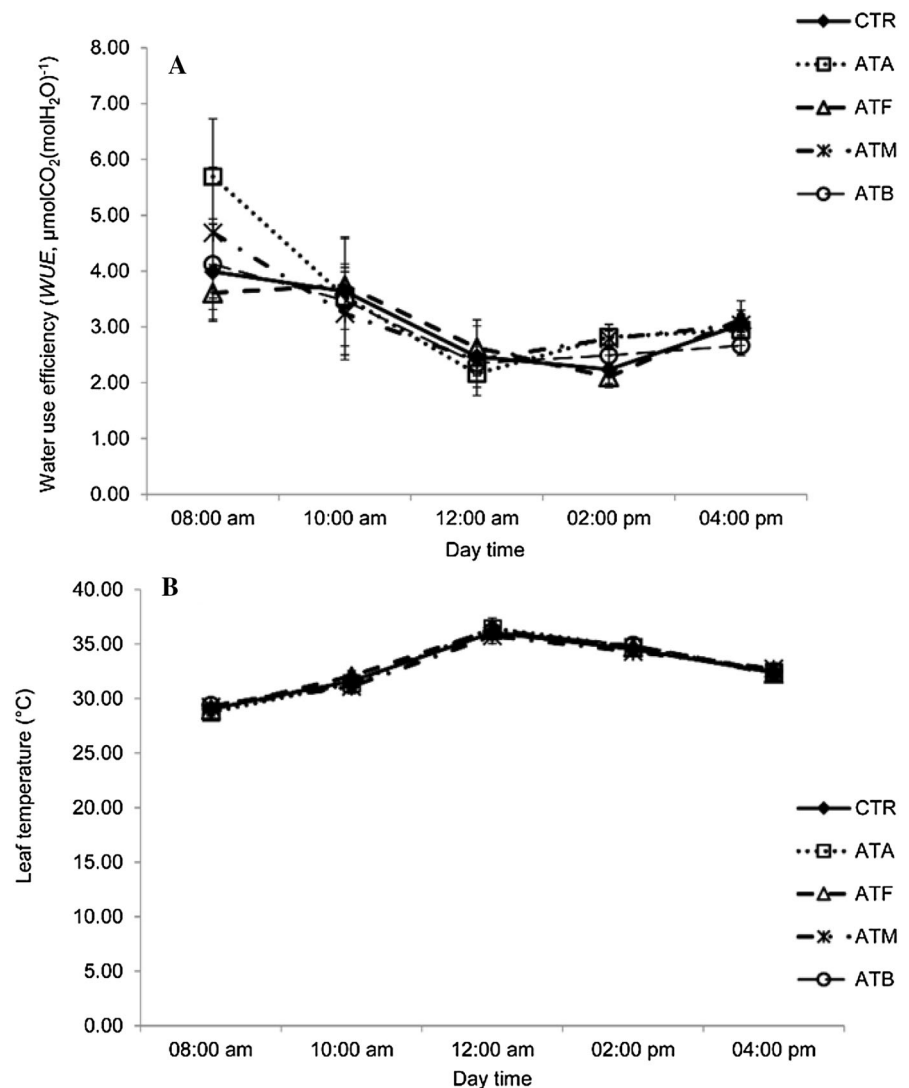
Gas exchange in grafted plants seems to be influenced by the rootstock because the rootstock can modify the gene expression, vigor, and productivity of the scion (Colla et al. 2012; Merli et al. 2016), as evidenced by the compatibility between scion/rootstock, the chlorophyll content (Etehadnia et al. 2008; Liu et al. 2007; Rouphael et al. 2008) and photosystem II efficiency (PS II) (Ahn et al. 1999; He et al. 2009; Zheng et al. 2009).

Over the past decade, the use of grafting in “model species” such as *Arabidopsis thaliana* L. and tobacco (*Nicotiana tabacum* L.) in plant physiology studies has

contributed to advances in genomic/proteomic studies. With the important contribution of knowledge regarding the transmission of floral stimuli, proteins, and long-distance RNAs in plants, it has been shown that possible signaling from rootstock to scion (Corbesier et al. 2007; Harada 2010; Kasai et al. 2011; Notaguchi et al. 2008, 2009) may interfere with the metabolism of the whole plant (Kanehira et al. 2010; Le Hir et al. 2008).

The highest light compensation point (τ) presented by ATA plants was not evidenced in the other rootstock combinations. This result may be due to the fact that ATA showed the highest respiratory rate (R_d^*), indicating that these plants require more

Fig. 3 a Water use efficiency [WUE , $\mu\text{mol CO}_2$ ($\text{mol H}_2\text{O})^{-1}$] and **b** leaf temperature ($^{\circ}\text{C}$), in atemoya scions grafted onto atemoya (ATA), *araticum-de-terra-fria* (ATF), *araticum-mirim* (ATM), and *biribá* (ATB) rootstocks, and ungrafted atemoya (CTR), from 8:00 a.m. to 4:00 p.m. Values are mean \pm SE ($n = 12$)



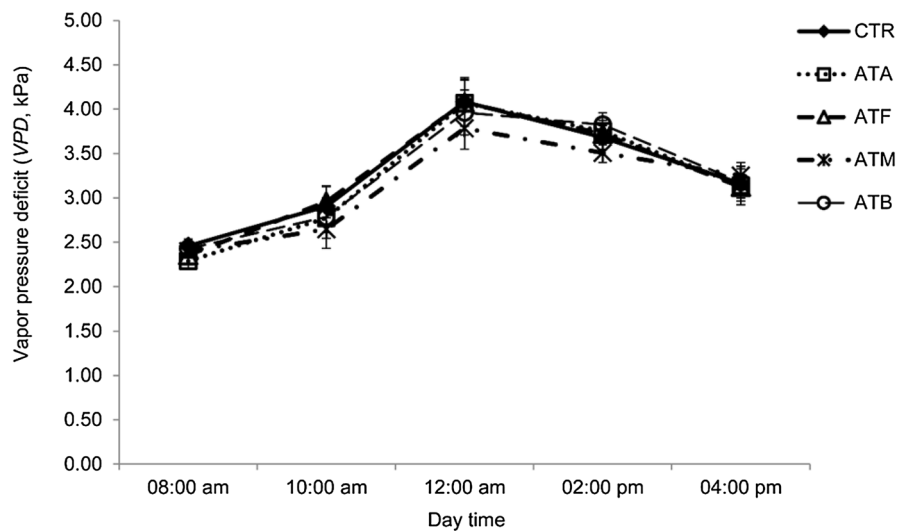
photosynthetic photons to assimilate the same amount of CO_2 compared to other rootstocks combinations. Although apparent CO_2 exchange does not occur, CO_2 uptake will follow the linear increase of radiation until reaching the light saturation point if the stomata are open and other environmental factors do not limit the gas exchange after the light compensation point.

The light saturation point of atemoya was affected by the rootstocks, regardless of the combination used, although this result did not change J , with the exception of ATF plants, which besides presenting the lowest light saturation, also showed the lowest J . Notably, this rate is used to estimate the maximum rate of electron transport under saturating light, and

it is based on the number of electrons required to reduce NADP to $\text{NADPH} + \text{H}^+$, as used by triose phosphate in the regeneration of RuBP (Sharkey et al. 2007). This indicates that ATF is the most efficient scion for utilizing irradiance.

Photosynthesis may be limited by three biochemical processes: (1) the maximum carboxylation rate of the RuBisCO enzyme, (2) the regeneration rate of RuBP, or (3) *TPU* limitation (Ding et al. 2017; Sharkey et al. 2007). ATF plants showed the highest maximum quantum yield of photosynthesis (Φ), suggesting that this rootstock species increased the efficiency of the Calvin cycle in terms of the use of ATP and $\text{NADPH} + \text{H}^+$, as also observed in tomato

Fig. 4 Vapor pressure deficit (VPD, kPa), in atemoya scions grafted onto atemoya (ATA), *araticum-de-terra-fria* (ATF), *araticum-mirim* (ATM), and *biribá* (ATB) rootstocks, and ungrafted atemoya (CTR), from 8:00 a.m. to 4:00 p.m. Values are mean \pm SE ($n = 12$)



plants (*Solanum lycopersicum* L.) (Ding et al. 2017). However, for ATF plants, photosynthesis was limited by the *TPU*, which was the lowest among all combinations. In combinations of watermelon [*Citrullus lanatus* (Thunb.) Mansf.] grafted onto calabash (*Lagenaria siceraria* Standl.), it has been reported that the rootstock contributes significantly to the expression of key enzymes involved in the Calvin cycle and the tricarboxylic acid cycle (Yang et al. 2012). Finally, ATB plants presented the highest *TPU*, indicating an increased export rate of photoassimilates, which could be directed to growth and yield.

We accepted our hypothesis, suggesting that grafting may affect the photosynthetic metabolism of the atemoya hybrid, regardless of the combination used. In addition, we demonstrate that grafting seems to promote increased A_{net} and lower maximum carboxylation rates of RuBP and light saturation points.

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