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Grafting relations in atemoya (*Annona* x *atemoya* Mabb.) plants: peroxidase and phenolic compounds

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Abstract

The aim of this study was to evaluate if variations in the peroxidase activity (PRX) and the phenolic compounds (PC) concentration in atemoya scion (*Annona* x *atemoya* Mabb.) grafted onto different rootstocks are related to incompatibility. The experiment was conducted in a greenhouse, with atemoya scion grafted onto *Annona* species rootstocks [*A. emarginata* 'var. terra-fria'; *A. emarginata* 'var. mirim; *A. mucosa* and atemoya (homograft)] and these species ungrafted. The evaluations were performed at 30 and 60 days after grafting (DAG) in the hypocotyl region, where the grafting was performed, in each grafting combination and the same region corresponding to the stem tissue in ungrafted plants. The atemoya scion grafted onto *A. mucosa* showed higher PRX than *A. mucosa* ungrafted, although smaller than *A. emarginata* 'var. mirim' rootstock. In atemoya grafted onto *A. emarginata* 'var. fria' rootstock, no significant change in PRX compared to *A. emarginata* 'var. fria', atemoya ungrafted and homograft. The PC concentration at 30 DAG showed no significant differences in the grafted plants, although higher than in their respective ungrafted. At 60 DAG, no differences between grafted plants were observed, however, the PC concentrations in atemoya grafted onto *A. emarginata* 'var. terra-fria' and 'var. mirim' rootstocks were higher than those observed in *A. emarginata* 'var. terra-fria' and 'var. mirim' ungrafted, respectively. We concluded that variations in PRX and the PC are not related or triggered to incompatibility between atemoya scion grafted onto *A. emarginata* 'var. terra-fria', 'var. mirim' and *A. mucosa*.

Keywords: Annonaceae; incompatibility; plant biochemistry; plant propagation; plant physiology.
Abbreviation: PRX_peroxidase activity,
PC_phenolic compounds

Introduction

The mechanisms of incompatibility in grafting are not fully elucidated yet, although some studies, such as those reported with the participation of class III peroxidases in lignin synthesis, have been studied in many plant species and tissue culture systems. Santamour (1988) reports that differences in peroxidase (PRX) concentration between the rootstock and the scion may lead to abnormal lignification and lack of vascular connections in the graft area. The author also reports that in plants that show the existence of similarity of peroxidase activity between canopy and rootstock, problems of incompatibility are rarely encountered. This suggests that peroxidases have a specific role in lignification and that generalizations about their role in incompatibility should be proposed with caution.

According to Errea et al. (2001), Rodrigues et al. (2002) and Telles et al. (2009) peroxidase activity (EC 1.11.1.7) and phenolic compounds concentrations in the xylem tissue of scion and rootstock would be a prognosis to verify incompatible grafting combinations. Several studies assert that peroxidase and phenolic compounds are involved in tissue lignification (Hiraga et al., 2001; Vanholme et al., 2008; Liu, 2012), which are important in the early stages of the connection between the graft and rootstock (Errea, 1998; Pina and Errea, 2005; Hartmann et al., 2011), because the cell walls of xylem tissues are dynamic structures composed of polysaccharides, proteins, minerals and phenolic compounds such as lignin (Herrero et al., 2014).

The presence of phenolic compounds is considered an important parameter in incompatibility evaluation (Errea et al., 2001; Rodrigues et al., 2001; Rodrigues et al., 2002; Telles et al., 2009). Phenolic compounds are described as implied in cell division, vegetative development processes and internal differentiation of new tissues, in grafting region, by regulating AIA-oxidase synthesis, particularly *p*-coumaric

acid, the lignin precursor, showing primary and secondary plant functions (Errea, 1998; Mng'omba et al., 2008). However, in incompatible grafting combinations an accumulation of *p*-coumaric acid occurs in the grafted tissue, possibly leading to non-differentiation and degradation on tissue interface graft/rootstock.

The plants that are the object of this study belong to the Annonaceae family, which presents approximately 110 genera and 2400 species (Chatrou et al., 2012), however, it has only four species with relevant economic interest, the *Annona squamosa* L. (sweet sop), *A. muricata* L. (soursop), *A. cherimola* Mill. (cherimoya) and the hybrid *Annona* x *atemoya* Mabb. (Cheong et al., 2011; Gupta-Elera et al., 2011; Pareek et al., 2011). The atemoya is used in the production of sorbets, jams, juices, liqueurs, in the *in natura* consumption. It is propagated by vegetative methods such as cutting, layering, grafting and micropropagation, while the seeds are used for the production of rootstocks in areas without soil pathogens (Heenkenda et al., 2009; Kavati, 2013).

Tokunaga (2005) reports that it is difficult to find a rootstock species for atemoya, that is resistant to pathogens (*Phytophthora nicotianeae* var. *parasitica*, *Pythium* sp. e *Rhizoctonia solani*) and not involving incompatibility with the scion. Despite the reports showed that wild Annonaceae species, such as *biribás* and *araticuns* (*Annona mucosa*, *A. reticulata*, *A. sylvatica* e *A. emarginata*) are potential for the use as rootstock (Santos et al., 2005; Almeida et al., 2010), there is no information about incompatibility when these species are used as rootstock to atemoya.

In order to test our scientific hypothesis, that different grafting combinations have different reestablishment post-grafting responses, the aim of this study was to evaluate if variations in the peroxidase activity and the phenolic compounds concentration in atemoya scion (*Annona* x *atemoya* Mabb.) cv. "Thompson" grafted onto different rootstocks are related to incompatibility.

Results

Peroxidase in grafted and ungrafted plants

There was 98% survival of grafted plants of all grafting combinations evaluated. The data of total peroxidase activity (PRX) and total phenolic compounds concentration, measured at 30 and 60 days after grafting (DAG) are presented in Table 1.

In the evaluations carried out at the 30 DAG, it was verified that when *A. emarginata* 'var. mirim' is used as rootstock for atemoya, there is an increase in the peroxidase enzymatic activity in relation to the other ungrafted species and grafting combinations (Table 1). Over time, at 60 DAG, it was found that the *A. emarginata* 'var. mirim' grafted onto atemoya, which had provided the highest peroxidase activity at 30 DAG, showed the lowest activity between scion/rootstock combinations at 60 DAG, resembling the atemoya grafted onto *A. emarginata* 'var. terra-fria' (Table 1.).

On the other hand, in atemoya grafted onto atemoya and atemoya grafted onto *A. mucosa*, no differences were detected in relation to their ungrafted plants. It is also observed that atemoya grafted onto *A. mucosa* presented the highest peroxidase activity, differing from all other treatments at 60 DAG, either in grafted plants or ungrafted plants. When the *A. mucosa* is used as a rootstock, the union region presents higher peroxidase activity than in the ungrafted *A. mucosa*, indicating that this union also increases peroxidase activity, although less than the effect of the atemoya grafted onto *A. emarginata* 'var. mirim'. It is worth mentioning that, although the atemoya grafted onto *A. mucosa* showed higher peroxidase activity than its ungrafted, the values resemble both ungrafted atemoya and atemoya homografted (Table 1.).

However, peroxidase activity in the atemoya grafted onto *A. emarginata* 'var. mirim' is higher than the peroxidase activity observed in the ungrafted plants. In the atemoya grafted onto *A. emarginata* 'var. terra-fria' there is no significant change in the peroxidase activity compared the *A. emarginata* 'var. terra-fria' ungrafted, or to the atemoya ungrafted or homografted. Thus, the increase in peroxidase activity in the grafted region is dependent on which rootstock was used.

Phenolic compounds in grafted and ungrafted plants

The concentration of phenolic compounds at 30 DAG was not altered in the grafted region of the scion/rootstock combinations, these values were higher than those observed in their respective ungrafted plant. At 60 DAG no differences were observed between the grafting combinations, however, the concentrations of phenolic compounds in atemoya grafted onto *A. emarginata* 'var. terra-fria'and atemoya grafted onto *A. emarginata* 'var. mirim' were higher than those *A. emarginata* 'var. terra-fria' and *A. emarginata* 'var. mirim' ungrafted plants, respectively.

The results evidenced variations in the peroxidase activity (PRX) and phenolic compound concentrations between scion/rootstock combinations. However, the interface of the grafting was continuous in all grafting combinations, with the radial parenchyma proliferation of the xylem and differentiation of the conductive elements in the graft region (Fig 1. and 2.). It should be noticed that in this study 98% survival of the grafted plants was obtained throughout the evaluated period.

Discussion

The elevated reestablishment post grafting obtained in this study differs from that described in the literature, especially in relation to the *A. mucosa*, which is considered incompatible (Kavati, 2013). Almeida et al. (2010) also evaluated the survival rate of grafts on different days after the grafting and observed that 105 DAG differences occurred between atemoya grafted onto *A. emarginata* 'var. terra-fria' (70% survival) and *A. mucosa* (0% survival). Nevertheless, in this experiment there was no observed grafted incompatibility reactions on the tissues, demonstrating that biological conditions, as well as environmental, were satisfactory. Santos et al. (2005) observed that sweetsop (*Annona squamosa* L.) grafted onto *A. mucosa* using different methods of grafting presented 19.2% until 4.0% survival post-grafting at 45 DAG. These authors reported that the period immediately after grafting is the most critical for the graft survival, in this case, the small number of surviving grafts remained alive until this time due to their reserves.

Table 1. Total peroxidase activity (PRX, µmol min^{-1} mg^{-1} of protein) and phenolic compounds concentration (mg g^{-1} of fresh weight) of ungrafted plants and grafted plants at 30 and 60 days after grafting (DAG).

	Total peroxidase activity (PRX, µmol min^{-1} mg^{-1} of protein)		Phenolic compounds concentration (mg g^{-1} of fresh weight)	
	30 DAG	60 DAG	30 DAG	60 DAG
Atemoya ungrafted	46.49 ± 1.3 bc	35.35 ± 3.0 de	0.309 ± 0.016 c	0.357 ± 0.031 a
'Fria' ungrafted	52.00 ± 4.3 bc	42.21 ± 2.6 d	0.307 ± 0.006 c	0.270 ± 0.005 c
'Mirim' ungrafted	48.31 ± 2.5 bc	38.88 ± 2.6 de	0.314 ± 0.008 bc	0.258 ± 0.012 c
'Mucosa' ungrafted	41.43 ± 4.1 c	33.54 ± 0.5 e	0.252 ± 0.012 d	0.250 ± 0.009 c
Atemoya x Atemoya	49.64 ± 1.3 bc	76.46 ± 1.3 b	0.367 ± 0.014 a	0.327 ± 0.009 ab
Atemoya x 'Fria'	56.30 ± 2.2 b	60.17 ± 1.3 c	0.359 ± 0.012 ab	0.324 ± 0.002 ab
Atemoya x 'Mirim'	76.40 ± 2.2 a	56.96 ± 1.1 c	0.367 ± 0.013 a	0.337 ± 0.005 ab
Atemoya x 'Mucosa'	53.90 ± 1.1 b	163.94 ± 1.4a	0.335 ± 0.008 abc	0.287 ± 0.007 bc
	Homogeneity Test		Homogeneity Test	
Value F	2.11	1.90	17.95	3.44
Pr > F	0.1027	0.1358	0.0456	0.0193
	ANOVA		ANOVA	
Value F	23.70*	67.97*	17.95*	13.84*
DMS	10.55	7.63	0.046	0.052

Means followed by the same letter in the column do not differ by Tukey's Test at 5% probability. LSD (least significant difference). 'Atemoya ungrafted (*Annona atemoya* Mabb.); 'Fria' ungrafted [*Annona emarginata* (Schltdl.) 'var. terra-fria' ungrafted]; 'Mirim' ungrafted [*Annona emarginata* (Schltdl.) 'var. mirim' ungrafted]; 'Mucosa' ungrafted [*Annona mucosa* (Bail.) H. Rainer]; Atemoya x Atemoya (atemoya scion grafted onto atemoya rootstock); Atemoya x 'Fria' [atemoya scion grafted onto *Annona emarginata* (Schltdl.) H. Rainer 'var. terra-fria' rootstock]; Atemoya x 'Mirim' (atemoya scion grafted onto *Annona emarginata* (Schltdl.) H. Rainer 'var. mirim' rootstock); Atemoya x Mucosa (atemoya scion grafted onto *Annona mucosa* (Bail.) H. Rainer rootstock). (*n* = 9, ± standard error).

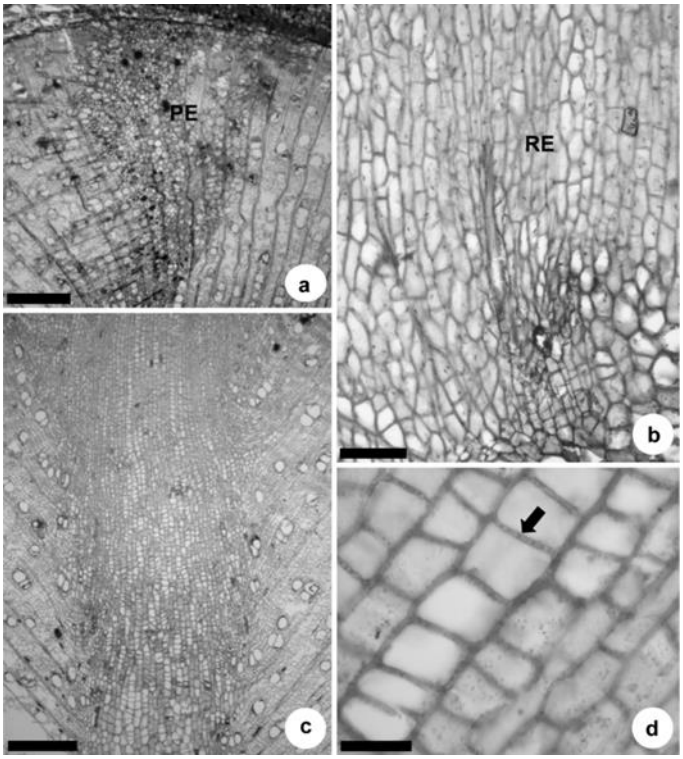

Fig 1. Transverse sections of hypocotyl region, where the grafting was performed, at 30 days after grafting. a - Proliferation of radial xylem wood parenchyma of atemoya scion grafted onto *Annona emarginata* (Schltdl.) H. Rainer 'var. terra-fria' rootstock. b - Proliferation of radial wood xylem parenchyma of atemoya scion grafted onto atemoya rootstock. c - Conductive differentiation of elements xylem (vessels) of atemoya scion grafted onto atemoya rootstock. d - Parenchyma cells with conspicuous pits (arrow) of atemoya scion grafted onto atemoya rootstock. (PE: parenchyma of hypocotyl region, where the grafting was performed; RE: grafted region). Scale bars: 500µm (a, b), 100µm (c), 50µm (d).

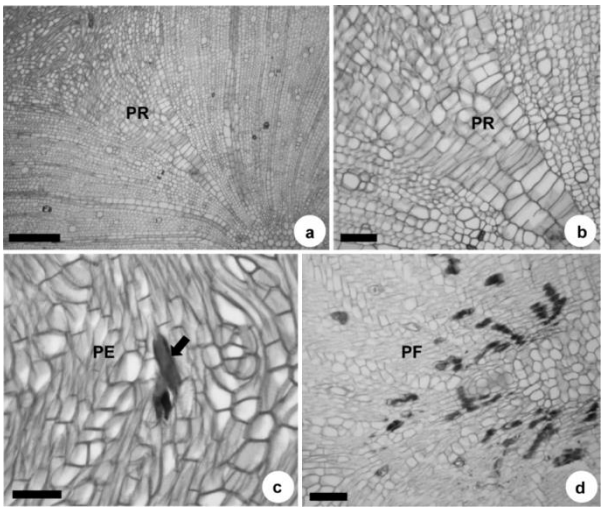

Fig 2. Transverse sections of hypocotyl region, where the grafting was performed, at 60 days after grafting of atemoya scion grafted onto *Annona mucosa* (Bail.) H. Rainer' rootstock. a - Proliferation of radial wood xylem parenchyma. b – Detail of 'a' photo showing the proliferation of radial wood xylem parenchyma). c - Conductive differentiation of elements xylem (arrow) from parenchyma cells. d – Proliferation of phloem parenchyma with differentiating conductiong cells. (PR: radial parenchyma of xylem; PE: parenchyma of grafted region; PF: phloematic parenchyma). Scale bars: 500µm (a), 150µm (b, c), 100µm (c).

It should be noted that, although studies report *A. mucosa* as rootstock incompatibility to atemoya or sweetsop (Santos et al., 2005; Almeida et al., 2010; Kavati, 2013) these experiment shows no physiological incompatibility explanations. In this way, it is believed that the reported incompatibility is not related to anatomical features, because in this experiment both *A. mucosa* (Fig 2b, c and d) as the other species studied (Fig 1.) showed parenchymal proliferation and differentiation of the vascular system, establishing connection between scion/rootstock grafted.

In the present study, at 30 DAG there was an increase peroxidase activity in atemoya grafted onto *A. emarginata* 'var. mirim' and at 60 DAG there was an increase in all grafted plants in relation to their ungrafted, besides the increase of peroxidase activity of atemoya homografted. It must be emphasized that from the moment in which atemoya homografted increases peroxidase activity, this could not be considered an adverse effect of graft, because scion/rootstock utilizing same species is reported with higher compatibility (Kavati, 2013; Lemos, 2013).

Perhaps the grafting, which is considered a 'stress activity' for plants may have caused an increase in peroxidase activity of atemoya homografted, which resulted in increased tissues lignification, as proposed by Passardi et al. (2005). Likewise, Fernández-García et al. (2004) also observed an increased peroxidase activity in compatible grafting combinations of *Solanum lycopersicum* L. (tomato) 'fanny' (scion) and 'AR9704' (rootstock) throughout time (8-15 DAG). In contrast, when there is an increase in peroxidase activity in grafted plants previously known as incompatible, such as between scion *Prunus persica* cv. 'Summergrand' (nectarine) grafted onto *Prunus cerasifera* cv. 'Myrobalan GF 3–1' (apricot) and *Prunus domestica* cv. 'Damas GF 1869' (apricot), Zarrouk et al. (2010) claim that there is a higher disorganization of cambium cells, minor differentiation of vascular tissue, degeneration of phloem and xylem and accumulation of phenols in the grafted region with plants evaluated at 5 months after grafting, indicating that peroxidase activity could be regarded as an indicator of early incompatibility. However in none of the grafting combinations of the present study were verified tissue damage or death cell, on the contrary, it was observed proliferation of radial xylem parenchyma in the graft region (Fig 1A, 1B, 2A, 2B), differentiations of the xylem conducting elements (Fig 1C, 2C), besides parenchyma cells with conspicuous pits in the graft union tissue, which represented the reestablishment of tissue and vascular system formation which will be responsible for both water transport and translocation of assimilates (Fig 2D).

In this way, although it is of great importance the early diagnosis of incompatibility evaluating peroxidase activity of grafted union tissue, as proposed by Fernández-García et al. (2004) and Zarrouk et al. (2010), in this study it was found that neither higher peroxidase activity in grafted combinations (relative to their own ungrafted) nor the increase in activity found over time (30-60 days) may be considered evidence of incompatibility early diagnosis in anonaceous plants, since the peroxidase activity appears to be related to the cell wall lignification in plants and not with tissue degradation.

In addition to the peroxidase activity, there is an interaction of this with the phenolic compounds, because peroxidases use the phenolic compounds, mainly those of low molecular weight, as substrate for peroxidase synthesis, presenting inversely proportional balance, which suggests the interrelation between these two compounds (Hiraga et al., 2001; Passardi et al., 2005; Liu, 2012). In the grafted plants combinations evaluated, despite of the phenolic compounds concentration be greater than *A. emarginata* 'var. mirim', *A. emarginata* 'var. terra-fria' and *A. mucosa* ungrafted, grafted plants did not show an increase in relation to atemoya ungrafted, neither atemoya homografted at 60 DAG, indicating that these combinations can be considered compatible.

On the other hand, Pina et al. (2012) reports that there is high phenolic compounds concentration in incompatible

grafted callus tissue. Furthermore, the accumulation of phenols (anthocyanins, flavonones, *p*-coumaric acid and hydroxybenzoic acid) has been associated with reduced compatibility in early stages and late on restoring after grafting of apricot (Errea et al., 2001; Pina and Errea, 2008), *Uapaka kirkiana* (Mng'omba et al., 2008) and peach (Zarrouk et al., 2010). These accumulations reduce auxin content, which directly affects the differentiation of the xylem and phloem vessel elements, as well as lignification (Errea, 1998; Liu, 2012) or even in their own degradation, which disrupts cell function or chemical reactions (Hartmann, 2011; Mng'omba et al., 2008). However, more studies are needed to elucidate if accumulation of phenols in compatible combinations is the cause or consequence in the cellular level incompatibility reactions.

From the present study is evident that the grafted combinations did not present signs of an early or immediate incompatibility in atemoya grafted onto *A. mucosa*, disagreeing with statements reported by Almeida et al. (2010), which claim that this combination showed incompatibility immediately after grafting. However, this does not mean that subsequently the incompatibility does not appear in the orchard over useful life of the grafted plant, it should be in mind what would be the incompatibility caused by other factors, for example, virus infection, which would not exactly be a reaction between partners in grafting (Creasap et al., 2005). Or even, incompatibility caused by tissue hypertrophy in the region below, above or in the grafted region ('elephant's foot') (Tokunaga, 2005) or caused due to unsuitable environments.

Another question that must be observed is that few authors have reported the existence of specimens of the study deposited in herbarium, what is very important, since the species can be known by different common names, which can lead to confusion in obtaining seeds. The *A. mucosa*, for example, is commonly used in different regions of Brazil to refer to *Annona muricata* (soursop), *Annona glabra* (pond-apple), *Annona montana* (mountain soursop) e *Annona reticulata* (custard apple), which are reported as incompatible rootstock with scion atemoya (George and Nissen, 1987; Kavati, 2013).

Moreover, we should also consider the influence of abiotic factors, such as environmental temperature appropriate after grafting (24 to 27ºC), air relative humidity equal or above the saturation point and the ability of grafting experts, in order to distinguish if physiological incompatibility is caused by biochemistry and/or anatomical, for example, inability of grafting experts (Hartmann et al., 2011; Lemos, 2013).

Materials and methods

Plant material

The fruits of atemoya (*Annona* x *atemoya* Mabb.) cv 'Thompson' and *Annona emarginata* (Schltdl.) H. Rainer 'variety terra-fria' were collected in the São Bento do Sapucaí Municipality, São Paulo State, Brazil (*Latitude* S 22°41'20'' and *Longitude* W 45°43'51'). The fruits of *Annona emarginata* (Schltdl.) H. Rainer 'variety mirim' were collected in the Tietê Municipality, São Paulo State, Brazil (*Latitude* 23º06'07' S e *Longitude* 47º42'53' W). The fruits of *Annona mucosa* (Schltdl.) H. Rainer were collected in the Piraju Municipality, São Paulo State, Brazil (*Latitude* S 23º06'15' and *Longitude* W 49º20'02').

The exsiccates vouchers were deposited in the 'Irina Delanova de Gemtchujnicov' (BOTU) Herbarium, Department of Botany, Institute of Biosciences of Botucatu, IBB, University of São Paulo State, Unesp, Botucatu *campus*. The vouchers numbers 28233, 28279, 27600 and 27596 were deposited to *A. emarginata* 'var. terra-fria', *A. emarginata* 'var. mirim', *A. atemoya* and *A. mucosa*, respectively.

Pre-grafting experimental implementation

The experiment was conducted in a greenhouse at the Instituto de Biociências (IB), Universidade Estadual Paulista "Júlio de Mesquita Filho" (Unesp), Botany Department, Botucatu *campus*, Brazil. The study site is located at 48° 24' 35" W and 22° 49' 10" S, and an altitude of 850 m. Seeds of three rootstocks *A. emarginata* 'var. terra-fria', *A. emarginata* 'var. mirim', *A. mucosa* and atemoya were sown in polystyrene trays containing vermiculite, according to Baron et al. (2011). When the seedlings developed fully expanded leaves, they were transplanted to plastic pots (approximately 17 dm^3) containing a mixture substrate with fertile soil, textured vermiculite and coconut fiber.

Grafting and treatments

The whip and tongue graft technique was performed according to Tokunaga (2005). The rootstocks atemoya, *A. emarginata* 'var. terra-fria', *A. emarginata* 'var. mirim' and *A. mucosa* were prepared 18 months after sowing, when the plants had stem diameters ranging from 8 to 10 mm and 15 cm in height. The atemoya cv. 'Thompson' was used as scion, employing stem segments (12 cm in length, 8–10 mm in diameter) from the same plant.

The evaluations were performed in the combinations between the scion atemoya grafted onto rootstock atemoya, *A. emarginata* 'var. terra-fria', *A. emarginata* 'var. mirim' and *A. mucosa*, and, also, in ungrafted plants of atemoya, *A. emarginata* 'var. terra-fria', *A. emarginata* 'var. mirim' and *A. mucosa*.

Experimental design

The experiment was conducted using a randomized block design consisting of eight treatments (four grafted combinations and four ungrafted species) with nine replicates per treatment at 30 and 60 days after grafting (DAG).

Collection and preparation of samples

Approximately 5 cm of the hypocotyl region, where the grafting was performed, in each combination and the same region corresponding to the stem tissue in ungrafted plants were sampled with stainless steel pruning shears at different times (time before grafting, 30 and 60 DAG).

Samples were collected between 09:00am–10:00am and immediately stored in falcon tubes protected from light, frozen in liquid nitrogen and stored in were stored in a -80 °C freezer for further analysis.

Peroxidase assay (PRX, EC 1.11.1.7)

For enzyme extract, the samples (300 mg) were pulverized in liquid nitrogen and homogenized in 4 mL of pre-cooled potassium phosphate buffer (0.1 M, pH 6.8) and 200 mg PVP. The homogenates were centrifuged at 10,000 x g for 10 min at 4 °C, and the resulting supernatants were used for enzyme assay (Kar and Mishra, 1976).
The supernatant from the extraction was used to determine the total peroxidase activity (Teisseire and Guy, 2000). The soluble protein content determined using casein as standard (Bradford, 1976).

Quantification of total phenolic compounds

The quantification of total phenolic compounds was performed according to Herrig et al. (2002), with minor modifications. Samples (250 mg) were homogenized in 5 mL HCl 2N and were poured for 30 min. After boiling, samples were cooled for 3 min and were centrifuged at 5,000 x g for 10 min at 4 °C. 100 µL of this supernatant were diluted in 4.5 mL H_2O Mili-Q, 750 µL sodium carbonate (Na_2CO_3) 1.9M and 250 µL Folin-Ciocalteau (RFC) reagent. These mixtures were kept in the dark during 60 min. After the elapsed time, a read was performed in a spectrophotometer (SP-220, Biospectro, Brazil) at a wavelength of 750 nm. Ferulic acid was used as standard, according to Blum et al. (1991). The results were expressed as total phenolic compounds mg g^{-1} of fresh weight (FW).

Histological sectioning

Stem tissue from the interface of the graft region were obtained in different times, 30 and 60 DAG, fixed on FAA 70 (formaldehyde, acetic acid and ethanol) (Johansen, 1940) then put in the vacuum pump for 5 min, preserved by ethanol 70% and included in polyethylene glycol-1500 (*Merck-millipore*®) (Mari, 2007). After the step of including, transverse sections were performed in the graft tissue. A glue solution 'PVC' (Tigre®) + Butyl Acetate utilizing 'hair dryer' was adopted on the sample that was sectioned (graft stem tissue) promoting the drying of the plant material. After forming a very thin film on the material, the cross sectioning of the sample with a cutting thickness of 17 µm in a slide microtome was performed. As the cuts were performed, they were kept on glass blades and visualized with the aid of a micrometric eyepiece coupled under a light microscope. After their visualization, they were wrapped with aluminum foil, labeled and stored in a refrigerator (4 ºC) for further processing.
The second step consisted of removing the cuts from the refrigerator and placing them in a petri dish with water at 40 °C for 5 min until the polyethylene glycol-1500 dissolved completely. After this process, the sections were washed twice with deionized water and added with a dropper 50% sodium hypochlorite, leaving for 1 to 2 min until the cuts were discolored. After this process, the cuts were washed twice with deionized water and then added with a dropper water-acetic, leaving for 1 to 2 min, to neutralize the sodium hypochlorite and then rinsed twice with deionized water. The sections were stained with Astra Blue and Safranin (Mari, 2007).
Serial alcoholic dehydration was performed with 10%, 30%, 50%, 70%, 90%, 96% e 99,8% ethyl alcohol. After this process, a drop of albumin adhesive was added drop-wise onto a glass blade, spread and rubbed with the finger until the glass blade was sticky on the finger. Subsequently, the glass blades with the slices were placed in glass vats with butyl acetate + ethanol 100% in a ratio of 1:1 for 5 min. After this time, the slides were withdrawn from this vat by transferring them to another vat containing pure butyl acetate for another 5 min. Finally, the blades were assembled with Entelan® and waited seven days for it to completely harden.

Statistical analysis

The statistical package used for the peroxidase activity and the phenolic compounds concentration analysis was SAS 9.2 (SAS Institute Inc., Cary, NC). The Levene test was used to verify the homogeneity of variances of the treatments. The data were examined using analysis of variance (ANOVA). The means were compared using Tukey test ($P \leq 0.05$).

Conclusion

We concluded that variations in peroxidase and the phenolic compounds are not related or triggered to incompatibility between atemoya scion grafted onto *A. emarginata* 'var. terra-fria', 'var. mirim' and *A. mucosa*.

Acknowledgments

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