

Full Length Research Paper

Can early peroxidase quantification detect graft-compatible in anonaceous rootstocks?

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Received 22 March, 2018; Accepted 26 April, 2018

This study aimed to quantify the class III peroxidase activity in young plants of different species belonging to Annonaceae botany family, with high potential as a rootstock, for early detection of graft-compatible atemoya (*Annona x atemoya* Mabb.). The experimental design was randomized block, that evaluated the species *araticum-de-terra-fria* (*Annona emarginata* (Schltdl.) H. Rainer 'variety terra-fria'), *araticum-mirim* (*Annona emarginata* (Schltdl.) H. Rainer 'variety mirim) and *biribá* (*Annona mucosa* (Schltdl.) H. Rainer), with rootstock potential, and atemoya (scion), which were divided into four blocks, each with four plants. The peroxidase was quantified on the stem before grafting. Statistical analysis showed that only the *biribá* presented different peroxidase activity compared to atemoya plants; and *araticum-mirim* and *araticum-de-terra-fria* were similar to atemoya plants. Thus, the peroxidase activity could not be possible or be used as a tool in the early diagnosis of the graft-compatible in atemoya combinations.

Key words: *Annona emarginata*, *Annona mucosa*, *Annona x atemoya*, compatibility, grafting.

INTRODUCTION

The hybrid atemoya is a fruit used worldwide in the food industry and it is propagated by vegetative methods such as grafting, to ensure commercial characteristics (Heenkenda et al., 2009). The rootstocks most often used

to graft the atemoya are *araticum-de-terra-fria*, *araticum-mirim*, *biribá* and atemoya itself (Baron et al., 2016).

Grafting is a technique of vegetative propagation, which involves the union of two parts of plants through the

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tissue regeneration, so that the assembly constitutes a new plant (Melnik and Meyerowitz, 2015; Xu et al., 2016). The mechanism of reestablishment post-grafting is not yet elucidated and several hypotheses have been raised in an attempt to explain them (Melnik and Meyerowitz, 2015; Xu et al., 2016), besides, predicting the incompatibility of the graft is very important to select graft-compatible and graft-incompatible. The reasons for reestablishing post-grafting are complex and involve many physiological and biochemical processes, such as the peroxidase. Peroxidase is intrinsically linked to the beginning of grafting process, because during the lignification there are specific functions in the lignin biosynthesis (Fernández-Pérez et al., 2015; Mo et al., 2017).

The peroxidase act as a scavenging excess reactive oxygen species induced by wounding (Rogers and Munné-Bosch, 2016), which might play an important role in the graft process. It uses hydrogen peroxide as its electron receptor to oxidize the cinnamic acid and convert the ferulic acid in diferulic, which acts in the bridge of hemicelulose binding the cinnamic acid to the proteins and the carbohydrates of the cellular wall favoring the consolidation (Liu, 2012).

The presence of peroxidase may be used as a marker to predict the compatibility of pecan (*Carya illinoensis*) (Mo et al., 2017); likewise, are found similarly in peroxidase activity between scion and rootstock in interstock of the *Prunus* genus (Telles et al., 2009). Besides, studies claim that the presence of peroxidase isozymes in grafting is an experimental approach to predict the incompatibility reaction (Irisarri et al., 2015).

Therefore, the aim of this study was to quantify the class III peroxidase activity, in young plants of different Annonaceae species, with high potential as rootstock, to investigate the possibility of using this analysis for early prediction of graft-compatible atemoya.

MATERIALS AND METHODS

The experiment was conducted in greenhouse located in the experimental area belonging to Botany Department of *Instituto de Biociências, Universidade Estadual Paulista* (Unesp), Botucatu municipality, São Paulo State, Brazil, which has the following geographical coordinates: 48° 24' 35"W, 22° 49'10"S and 850 m of average altitude above sea level.

The seeds of *araticum-de-terra-fria* (*Annona emarginata* (Schltdl.) H. Rainer "variedade terra-fria"), *araticum-mirim* (*Annona emarginata* (Schltdl.) H. Rainer "variedade mirim"), *biribá* (*Annona mucosa* (Bail.) H. Rainer) and atemoya (*Annona x atemoya* Mabb.) were disinfected with sodium hypochlorite (10% a.i.) and N-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide (CAPTANA) (CAS 133-06-2) fungicide (1.2 g a.i. kg⁻¹ of seeds).

Thereafter, seeds were sown in polystyrene trays filled with vermiculite, until their emergence. The seedlings presented ± 10 cm in length (neck to the stem apex), and were transplanted to plastic bags with a capacity of 5 L, containing a mixture of fertile soil, vermiculite medium texture, coconut fiber and decomposed pine

bark (1:1:1:1, v/v). The experimental design was a randomized block with four blocks, each one with four plants. The cultivation was done in a greenhouse and Hoagland and Arnon n°2 was applied, diluted to 50% of its ionic strength, electrical conductivity (EC) of 1.0 miliSiemens cm⁻¹ (300 ml per pot) and pH 5.5-6.5, according to Baron et al. (2013).

For biochemical analysis, when the plants were one year old, approximately 5 cm of stem was collected, which was a band cut (bark + wood), 20 cm above the cervical region, in which grafting is usually performed by commercial nurseries. For enzyme extract, 300 mg of the samples 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 polyvinylpyrrolidone (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 class III peroxidase activity.

The determination of class III peroxidase activity (PRX, EC 1.11.1.7) was performed according to Teisseire and Guy (2000). The reaction system was composed of 30 µL of the enzyme extract, 500 µL of potassium phosphate buffer (50 mM, pH 6.5), 250 µL of pyrogallol (1,2,3-benzenetriol, 20 mM) and 220 µL of hydrogen peroxide (H₂O₂, 5 mM), in a final volume of 1000 µL. The reaction was conducted at room temperature conditions for 5 min. The purpurogallin formation was measured on 430 nm using spectrophotometer (SP-220, Biospectro, Brazil) and its molar extinction coefficient (2.5 mmol L⁻¹ cm⁻¹) was used to calculate the specific activity of enzyme (µmol of purpurogallin min⁻¹mg⁻¹protein).

The soluble protein content was obtained using the Bradford method (Bradford, 1976). The reaction system was composed of 100 µL of the enzymatic extract and 5000 µL of Bradford-reactive. The reaction was conducted at room temperature for 15 min, and the absorbance reading was measured on 595 nm using spectrophotometer (SP-220, Biospectro, Brazil). Whereas its absorbance readings was performed in 595 nm using spectrophotometer (SP-220, Biospectro, Brazil), utilizing casein as a reference protein.

The statistical package used for the data analysis was SAS 9.2 (SAS Institute Inc., Cary, NC). The Levene test was used to verify the homogeneity of variances of the treatments. Comparisons were made with the hybrid atemoya with each species with potential use for rootstock (*araticum-mirim*, *araticum-de-terra-fria* and *biribá*). Thus, the results were submitted to the Student t test at 5% probability for independent samples (unpaired), comparing their averages two to two.

RESULTS AND DISCUSSION

The Levene test showed that the variances were homogeneous among groups. Thus, it is observed in Figure 1, that only the *biribá* showed different peroxidase activity from atemoya, presenting lower values. On the other hand, *araticum-mirim* and *araticum-de-terra-fria* presented similar peroxide activity to atemoya plants (Figure 1). The *biribá* plants had lower peroxidase activity than atemoya plants. The literature reports that atemoya scion graft onto *biribá* rootstock presents a survival rate lower than the combination of atemoya graft onto *araticum-de-terra-fria* (Santos et al., 2005).

However, Baron et al. (2016) cultivated atemoya scion graft onto *biribá* rootstock at 60 and 90 days after

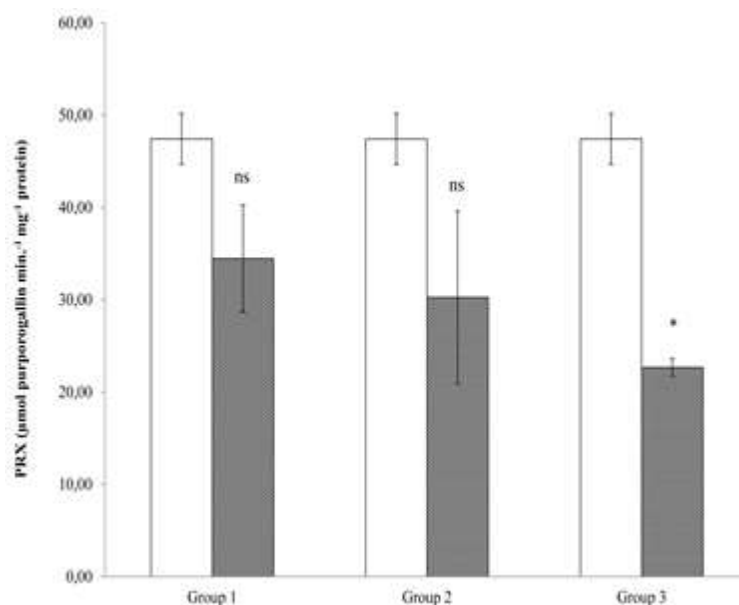


Figure 1. Class III peroxidase activity mean values (PRX, $\mu\text{mol de purpurogallin min}^{-1} \text{mg}^{-1} \text{protein}$) of stem in different Annonaceae species. Group 1 [atemoya (not hatch) and *araticum-de-terra-fria* (hatch)]; group 2 [atemoya (not hatch) and *araticum-mirim* (hatch)]; group 3 [atemoya (not hatch) and *biribá* (hatch)]. Columns represent the mean and the error bars represent standard error ($n = 4$). *Significant at 5% probability ($P \leq 0.05$); and ns denotes not significant ($P > 0.05$).

grafting, that UGP gene expression was similar to that graft-compatible, for example, atemoya graft onto araticum-de-terra-fria and araticum-mirim rootstock. This gene encoded UDP-glucose pyrophosphorylase (UGPase) protein plays an important role in many physiological processes, including carbohydrate metabolism, sucrose and cellulose formation in cell walls (Lerouxel et al., 2006; Janse Van Rensburg et al., 2018). Despite its important regulatory role, little is known about the expression of this gene associated with graft-compatible (Pina and Errea, 2008). Thus, early increased expression of this gene indicates a rapid union of plant tissues after grafting.

Nevertheless, Rodrigues et al. (2002) reports that rootstocks of peach and plum, for example 'mirabolano', presented higher peroxidase activity, indicating that this species, when grafted onto cultivars with lower activity, present graft-incompatibilities. Moreover, in cherry, cultivars with lower peroxidase activity can predict graft-incompatible (Güçlü and Koyuncu, 2012).

Several studies assert that peroxidase and phenolic compounds are involved in tissue lignification (Hiraga et al., 2001; Liu, 2012), which are important in the early stages of the connection between the graft and rootstock (Irisarri et al., 2015), 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).

When the peroxidase activities of *araticum-mirim* and *araticum-de-terra-fria* were evaluated it was found that both did not differ, suggesting graft-compatible species. These results corroborate those previously found in the union of *araticum-de-terra-fria* and atemoya, and are consistent with those obtained by Baron et al. (2016, 2017), who reports that atemoya graft onto *araticum-de-terra-fria* are suitable for seedling formation in commercial orchards.

Araticum-de-terra-fria rootstock compared to *araticum-mirim* rootstock is more agronomical advantageous, because its field duration is larger and do not show signs of "elephant foot" and dwarfism. However, dwarfism in fruit trees is an advantageous feature because it reduces manufacturing costs by increasing the density of the plants in the cultivated area (López-Marín et al., 2017). The atemoya scion grafted onto *araticum-de-terra-fria* is stronger than the one grafted on *araticum-mirim*, while the development is identical between both, however there is need to wait for an additional year for the atemoya produce fruits when grafted on *araticum-de-terra-fria*, compared to *araticum-miri m* (Scaloppi and Martins, 2013).

Rainer (2007) previously reports taxonomic rearrangement, which the sinonimized and ranked both, araticum-de-terra-fria and araticum-mirim, as *Annona*

emarginata (Schltdl.) H. Rainer and, perhaps, justifying the similarity between the activities of peroxidase found in this study for both rootstocks, however, there are morphological and origin center differences between the *araticum-mirim* and *araticum-de-terra-fria* species, such as lanceolate leaves of *araticum-de-terra-fria*, seeds and fruits morphologies (Couvreur et al., 2012).

CONCLUSION

The peroxidase activity could not be used as a tool in the early diagnosis of the graft-compatible in atemoya combinations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors wish to thank the Foundation for Research Support of the State of São Paulo (FAPESP). Daniel Baron was the recipient of FAPESP Grant n° 2011/00853-8 and FAPESP B.E.P.E. Grant n° 2013/22036-7.

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