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ORIGINAL ARTICLE

UGP gene expression and UDP-glucose pyrophosphorylase enzymatic activity in grafting annonaceous plants

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Abstract Grafting is commonly used to propagate commercial fruit species to ensure that the genetic characteristics of selected clones are maintained. However, the biochemical and molecular mechanisms involved in the graft incompatibility of woody trees are not well understood. We investigated the effect of grafting in vegetative growth, UDP-glucose pyrophosphorylase expression and activity of Annonaceous grafted plants: atemoya (*Annona cherimola* Mill. x *Annona squamosa* L.) 'Thompson' grafted onto different rootstocks, *araticum-de-terra-fria* (*Annona emarginata* Schltdl. H. Rainer "var. terra-fria"), *araticum-mirim (Annona emarginata* Schltdl. H. Rainer "var. mirim") and *biribá* (*Annona mucosa* Schltdl. H. Rainer) at different post-grafting times. The growth of atemoya grafted onto *araticum-mirim* was lower than that of the rootstocks *araticum-de terra-fria* and *biribá*. The results also indicated that grafting alters UGPase gene expression; showing the combination atemoya grafted onto *araticum-de-terra-fria* (a compatible union) the higher levels of gene expression during the early stages of grafting development. However, no significant differences were detected in UGPase enzyme activity between the graft combinations. In addition, SDS-PAGE and MALDI-TOF analyses detected similar UGPase amino acid sequences in ungrafted atemoya samples to cherimoya (*Annona cherimola* Mill.), a female parent of the atemoya hybrid. These findings suggest that expression of the UGPase protein is related to graft compatibility in grafted *Annona* plants.

Keywords Annonaceae · Enzymatic activity · Graft incompatibility · Plant propagation · *UGP*

Abbreviations

CTAB	Hexadecyl trimethyl ammonium bromide
DAG	Days after grafting
MALDI-TOF	Matrix-assisted laser desorption/ ionisation—time of flight
UGPase	UDP-glucose pyrophosphorylase
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis

Introduction

The production areas for Annonaceae, such as atemoya (*Annona cherimola* Mill. x *Annona squamosa* L.) and soursop (*Annona muricata* L.) have dramatically increased and many issues of incompatibility have been observed in the field. In orchards, Annonaceae species of economic importance are grafted to ensure that the genetic characteristics of productive scions are maintained (Almeida et al. 2010; Encina et al. 2014), however, graft incompatibility is frequently observed. Graft incompatibility is generally

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defined as the interruption in cambial and vascular continuity between rootstock and scion (Hartmann et al. 2011; Kostopoulou and Therios 2014; Li et al. 2012). Further understanding of the mechanisms related to graft incompatibility in Annonaceae is valuable, especially for commercial species, such as sweetsop and atemoya.

The rootstocks most often used to graft atemoya, a hybrid fruit with high organoleptic quality, are *araticum-de-terra-fria* (*Annona emarginata* Schltdl. H. Rainer "var. terra-fria") and *araticum-mirim* (*Annona emarginata* H. Rainer "var. mirim"). Atemoya grafted onto *araticum-de-terra-fria* rootstock results in further development of the scion and tolerance to cave nematodes [*Radopholus similis* (Cobb) Thorne], stem borers (*Cratosomus bombina* F.) and water stress (Tokunaga 2005). On the other hand, *araticum-mirim* rootstock causes dwarfism, which is considered beneficial because it facilitates the management of commercial orchards (Prassinos et al. 2009). Despite research indicating graft incompatibility between atemoya and *biribá* (Almeida et al. 2010), this authors suggested that wild Annonaceae species, such as '*biribás*' and '*araticuns*', are potential rootstocks in breeding and in selection of new fruits.

It is widely known that the more closely related rootstock-scion are, the better the chances for the graft to be successful facilitating new tissue formation and re-establishment of vascular connection (Hartmann et al. 2011; Pina and Errea 2005). Several factors are attributed to graft incompatibility including physiological intolerance at the cellular level between rootstock and scion (Andrew and Marquez 1993; Nocito et al. 2010; Pina and Errea 2005; Pina et al. 2012). Currently, molecular and proteomics approaches are contributing in the study of graft union formation and incompatibility, highlighting that it involves a network of different metabolic pathways (Cookson et al. 2013; 2014; Irisarri et al. 2015; Liu et al. 2013; Pina and Errea 2008; Prinsi et al. 2015; Zhang et al. 2015).

The enzyme UDP-glucose pyrophosporylase (UGPase) has been suggested as a candidate marker for re-establishment during plant post-grafting in fruit trees (Pina and Errea 2008). This enzyme (UGPase; EC 2.7.7.9), catalyses the interconversion of starch and sucrose being responsible for the synthesis of UDP-glucose, which is an important precursor in the biosynthesis and regulation of plant cell wall components, including cellulose, hemicellulose and pectin (Ciereszko et al. 2001; Kleczkowski 1994, 2004; Kleczkowski and Decker 2015).

A decrease in the accumulation and activity of the UDP-glucose pyrophosphorylase was associated to graft incompatibility in apricot/plum combinations (Pina and Errea 2008). Likewise, Prinsi et al. (2015) suggested that the pear/quince incompatibility is somehow associated with an alteration of the carbohydrate metabolism in pear tissues. However, UGPase expression and activity have not been studied during graft union formation in the genus *Annona*. Therefore, considering the need to understand the underlying mechanisms involved in *Annona* graft incompatibility, the aim of this study was to determine the effect of grafting in *UGP* gene expression and enzymatic activity in various graft combinations of atemoya scion grafted onto three different rootstocks: *araticum-de-terra-fria*, *araticum-mirim* and *biribá* plants.

Materials and methods

Plant material and treatments

The experiment was conducted in a greenhouse at the Instituto de Biociências (IB), Universidade Estadual Paulista (UNESP), Botany Department, Botucatu, São Paulo, Brazil (altitude 850 m). Seeds of three rootstocks *araticum-de-terra-fria*, *araticum-mirim*, *biribá* 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 fibre. The whip and tongue graft technique was performed according to Tokunaga (2005). The rootstocks 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 'Thompson' was prepared using stem segments (12 cm in length, 8–10 mm in diameter) from the same plant. Experiments were performed independently for each graft combination (atemoya scions grafted onto *araticum-de-terra-fria*, *araticum-mirim* and *biribá* rootstocks). Ungrafted genotypes were used as controls. The vegetative growth of grafted and ungrafted plants was evaluated using different parameters: stem diameter, plant height, number of fully expanded leaves and number of shoots 60 and 90 days after grafting (DAG).

Real time qRT-PCR expression analyses

Total RNA was isolated from stem bark tissue containing 5 cm of the different graft interfaces and ungrafted plants 30 and 60 DAG using the CTAB protocol described by Korimbocus et al. (2002). RNA integrity was confirmed using denaturing agarose gel electrophoresis and concentration was measured using a NanoDrop ND1000 Spectrophotometer (NanoDrop® Technologies).

Total RNA (2 μg) was treated with RNase-free DNase I (Fermentas®) and then used for cDNA synthesis using the High Capacity RNA-to-cDNA Master Mix kit (Invitrogen®) and the SuperScript III Reverse Transcriptase Kit (Invitrogen®) according to the manufacturer's instructions.

Gene-specific primer pair (Forward) 5′-AGAACTA ATCCATCAAACCCT-3′ and (Reverse) 5′-CACCAAAC CATACATCACCA-3′ were used for real time amplification. Each reaction was performed in a total volume of 10 µl containing 60 ng of cDNA and 0.2 µM of each primer, using the following cycle conditions: 5 min at 95 °C, followed by 45 cycles of 15 s at 95 °C and 60 s at 60 °C and a final dissociation curve in a StepOnePlus Real Time PCR System (Applied Biosystems®). Relative expression levels were normalised using the 18S rRNA gene from cherimoya plants using the forward primer (F) 5′-CGCAAATTACCCAATCTTGA-3′ and reverse 5′-ACTCATTCCAATTACCAGACTC-3′ (R) (González-Agüero et al. 2011). Relative quantification was determined using the $2^{-\Delta\Delta Ct}$ method as described previously by Livak and Schmittgen (2001). Amplification efficiencies were calculated from the amplification plots using the program LinRegPCR (Ramakers et al. 2003). The quantitative data were analysed using the Relative Expression Software Tool (Rest2009®Qiagen), and differences were considered significant compared to the ungrafted and homograft (atemoya grafted onto atemoya) control at $p \leq 0.05$.

Protein extraction, UGPase activity assays and MALDI-TOF analysis

Total protein was extracted from each graft combination in four replicates. The extraction was performed in 100 mM Tris–HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM EDTA, 2 mM DTT, 0.05 % (v/v) Triton-X-100 and 1 % (w/v) PVPP. The samples were centrifuged at 9000*g* for 20 min at 4 °C, and the supernatants were collected as described previously (Ciereszko et al. 2001). To ensure equal loading of the samples, aliquots of the crude protein extracts were used to determine the protein concentrations using the Bio-Rad protein assay using bovine serum album (BSA) as the standard. UGPase enzymatic activity was examined following the methodology described by Ciereszko et al. (2001) with minor modifications. Protein extracts (2 µl) were mixed in extraction buffer containing 100 mM of Tris–HCl, 5.0 mM of $MgCl_2$, 0.8 mM of NAD, 0.8 mM of UDP-glucose as substrate, glucose 1,6-bisphosphate, 4 phosphoglucomutase units and dehydrogenaseglucose-6-phosphate (Sigma®). Finally, 2 mM of pyrophosphate (PPi) was added to initiate the reaction. UGPase activity was measured based on the absorbance at 340 nm, and reported in units of µmol min^{-1} (g FW)$^{-1}$. Activity levels were indicated for the media ($n = 4$) and ± standard error (SE).

Proteins from ungrafted stem tissues of atemoya, *araticum-de-terra-fria*, *araticum-mirim* and *biribá* were separated by SDS-PAGE and putative UGPase bands (of approximately 55 kDa) were excised from the gel, washed several times with sterilised water and subjected to MALDI-TOF analysis essentially as described in Pina and Errea (2008).

Experimental design and statistical analysis

The experiment was conducted using a randomised complete block design with four blocks of scion/rootstock unions for vegetative growth, gene expression and enzyme activity. There were at least three replicates per group for grafted plants at 30 and 60 DAG, and four replicates were evaluated for ungrafted plants. The data were analysed using the software SAS 9.2. Variance homogeneity of the treatment groups was analysed using Levene's Test. The data were then analysed using ANOVA, and the group means were compared using Tukey's Test at 5 % probability ($p \leq 0.05$).

Results

Plant growth

At the time of grafting, the ungrafted plants were of uniform diameter and height, which facilitated grafting. However, ungrafted atemoya and *araticum-mirim* plants differed in leaf number (Table 1). Atemoya had fewer leaves than *araticum-mirim* but did not differ from *araticum-de terra- fria* or *biribá*, even though all species were grown under the same environmental conditions.

At 60 DAG, the stem diameter, number of leaves and shoots did not differ between the grafted plant combinations. There were significant differences ($p \leq 0.05$) in plant height between the graft combinations atemoya onto *araticum-mirim* and atemoya onto *biribá* (Table 1). At 90 DAG, there were significant differences ($p \leq 0.05$) between atemoya onto *araticum-de-terra-fria* and atemoya onto *biribá* with respect to plant height with other graft combinations atemoya onto atemoya and atemoya onto *araticum-mirim*. A significant increase in plant height of grafted atemoya onto the rootstocks *araticum-de-terra-fria* and *biribá* was obtained with longer graft length for these grafted plants. Regarding number of leaves, the combination atemoya onto *biribá* had the higher number of leaves than the other graft combinations, but the number of shoots did not differ between them (Table 1).

UGP gene expression in grafted and ungrafted Annonaceae plants

To examine the effects of grafting on *UGP* expression, total RNA from grafted plants (at 30 and 60 DAG) and ungrafted control plants was subjected to RT-qPCR. The

Table 1 Growth differences, as measured by stem diameter, plant height, number of leaves and number of shoots, before grafting and at 60 and 90 days after grafting

Moment before grafting	Stem diameter (cm)	Plant height (cm)	Number of leaves (unit)
Atemoya ungrafted	0.9 ± 0.3a	159.3 ± 15.7a	45.3 ± 19.5b
araticum-de-terra-fria ungrafted	1.2 ± 0.2a	121.0 ± 11.3a	107.3 ± 18.7ab
araticum-mirim ungrafted	1.4 ± 0.3a	148.3 ± 5.8a	136.5 ± 18.6a
biribá ungrafted	1.1 ± 0.2a	121.3 ± 12.6a	74.3 ± 17.8ab
F value	1.06^{ns}	2.65^{ns}	4.51*
LSD	0.9567	49.966	78.302

60 days after grafting	Stem diameter (cm)	Plant height (cm)	Number of leaves (unit)	Number of shoots (unit)
Atemoya ungrafted	1.4 ± 0.2a	24.8 ± 1.4b	14.4 ± 3.62a	2.2 ± 0.42a
araticum-de-terra-fria ungrafted	1.5 ± 0.2a	29.4 ± 1.4ab	15.8 ± 2.7a	2.2 ± 0.22a
araticum-mirim ungrafted	1.5 ± 0.1a	25.6 ± 4.9b	13.8 ± 1.1a	2.6 ± 0.27a
biribá ungrafted	1.4 ± 0.1a	37.2 ± 2.1a	17.8 ± 2.6a	2.8 ± 0.22a
F value	0.02^{ns}	4.96*	0.6^{ns}	1.29^{ns}
LSD	0.5754	10.296	9.2176	1.0705

90 days after grafting	Stem diameter (cm)	Plant height (cm)	Number of leaves (unit)	Number of shoots (unit)
Atemoya ungrafted	1.5 ± 0.14a	29.6 ± 4.72b	17.2 ± 3.3b	2.0 ± 0.0a
araticum-de-terra-fria ungrafted	1.6 ± 0.12a	66 ± 5.65a	21 ± 2.32ab	2 ± 0.0a
araticum-mirim ungrafted	1.5 ± 0.11a	33.6 ± 7.29b	14.2 ± 2.68b	1.2 ± 0.22a
biribá ungrafted	1.7 ± 0.08a	70.6 ± 6.65a	27.6 ± 3.47a	2.0 ± 0.0a
F value	0.91^{ns}	15.02*	5.38*	16^{ns}
LSD	0.4211	22.27	11.11	0.4046

Means (±standard error, SE) followed by the same letter in the column do not differ according to Tukey's test at 5 % probability, $n = 5$

LSD least significant difference

results indicated that the expression of the gene coding for the UGPase was detected in all ungrafted genotypes (Fig. 1a). The constitutive UGPase transcription level depends on the genotype, ranging from high levels in the rootstock *biribá* to lower levels in atemoya, *araticum-de-terra-fria* and *araticum-mirim* (Fig. 1a). As shown in Fig. 1, significant differences in relative *UGP* expression were observed between ungrafted and grafted plants and the mRNA expression levels followed the same trend between graft combinations at 30 and 60 DAG (Fig. 1b, c). *UGP* expression levels were significantly different between the tested homograft atemoya onto atemoya and the heterografts atemoya onto *araticum-de-terra-fria* and atemoya onto *araticum-mirim.* The graft combination atemoya onto *araticum-de-terra-fria* exhibited higher UGP expression levels than atemoya onto *araticum-mirim* which showed lower UGP expression than the homograft and ungrafted atemoya throughout graft union development (Fig. 1b, c). *UGP* expression levels in ungrafted *biribá* were higher than those determined for atemoya onto *biribá* and the atemoya reference plant, respectively. Overall, these results indicated that *UGP* expression differs between the investigated graft combinations at the transcriptional level.

UGPase activity and protein identification

To determine whether UGPase activity is affected by grafting, we measured enzyme activity in ungrafted and grafted plants immediately after grafting and at 30 and 60 DAG (Table 2). Among the ungrafted plants, atemoya exhibited the greatest UGPase activity compared to the other genotypes. In contrast, the homograft atemoya onto atemoya exhibited lower UGPase activity compared to ungrafted atemoya at 60 DAG, but not 30 DAG (Table 2). The graft combination atemoya onto *araticum-de-terra-fria* exhibited greater UGPase activity than the ungrafted rootstock *araticum-de-terra-fria*; this difference was of equal magnitude to the difference in UGPase activity observed between the ungrafted rootstocks *araticum-de-terra-fria* and atemoya. The graft combination atemoya onto *araticum-mirim* showed the same trend as atemoya

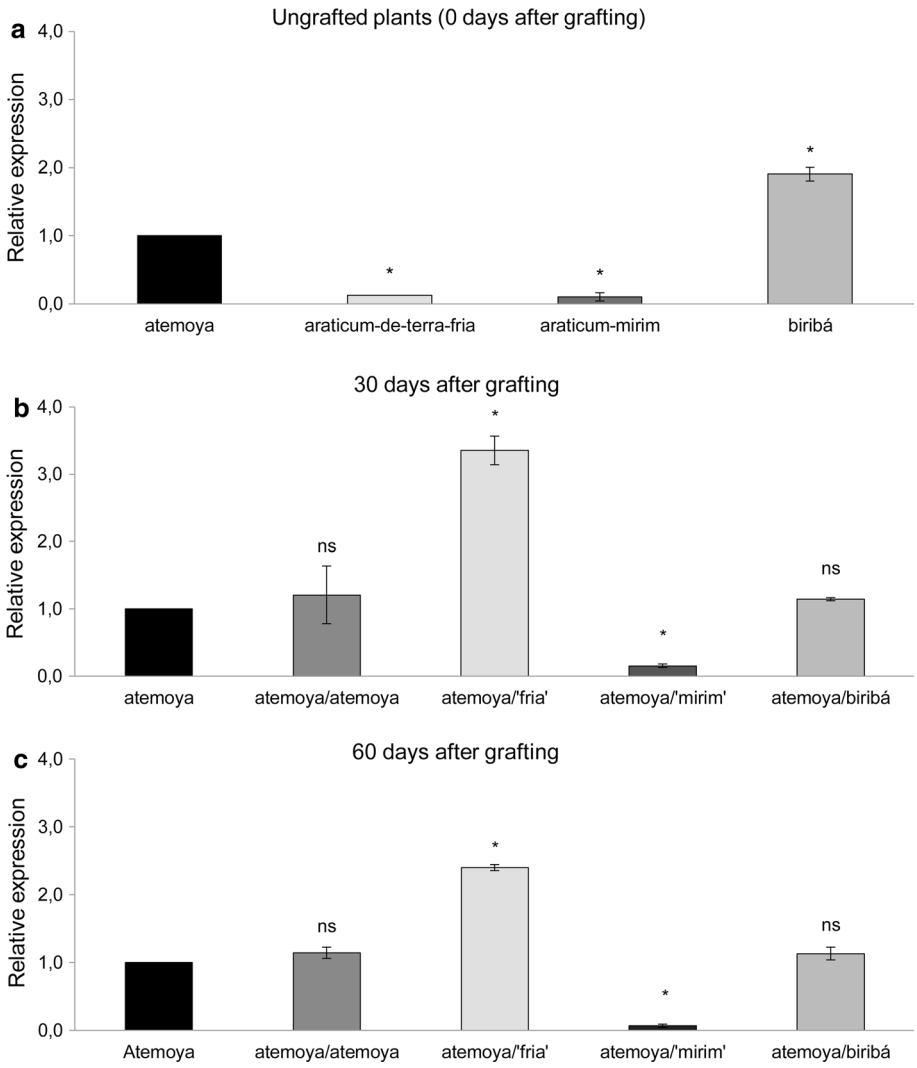

Fig. 1 Relative *UGP* gene expression in *Annona* plants. **a** Ungrafted plants (0 DAG); **b** Grafted plants at 30 DAG; **c** Grafted plants at 60 DAG. The data represent averages and standard errors obtained from three biological replicates (*$p \leq 0.05$). Ungrafted atemoya was used as the reference (expression arbitrarily set to 1)

Table 2 UGPase activity assessed in ungrafted and grafted Annonaceous plants

UGPase activity [μmol min^{-1} $(g\ FW)^{-1}$]				
DAG (days after grafting)	Atemoya onto atemoya	Atemoya onto *araticum-de-terra-fria*	Atemoya onto *araticum-mirim*	Atemoya onto *biribá*
0	17.90 ± 0.16Aa	14.80 ± 0.19Bb	8.1 ± 0.80Dc	10.5 ± 0.18Cc
30	15.63 ± 1.35BCab	18.80 ± 0.43Ba	12.7 ± 0.50Cb	22.1 ± 0.42Aa
60	13.17 ± 0.28Bb	19.90 ± 1.44Aa	18.58 ± 0.47Aa	13.95 ± 0.74Bb

Means followed by the same capital letter (lines) and lower case letter (columns) do not differ according to Tukey's Test at 5 % probability

UGPase activity levels are reported for ungrafted atemoya, *araticum-de-terra-fria*, *araticum-mirim* and *biribá* immediately before grafting (0 DAG), and for atemoya onto atemoya (atemoya onto *araticum-de-terra-fria*, atemoya onto *araticum-mirim* and atemoya grafted onto *biribá* at 30 and 60 DAG

a = <0.0001* (treatments); b = < 0.0001* (time); a × b = <0.0001* (treatments × time)

onto *araticum-de-terra-fria* during graft union development, reaching values similar to ungrafted atemoya at 60 DAG. These data showed that grafting plants with atemoya increases UGPase activity (Table 2). The ungrafted rootstock *biribá* exhibited lower UGPase activity than ungrafted atemoya and *araticum-de-terra-fria*. However, when grafted atemoya onto *biribá*, this union exhibited greater UGPase activity at 30 DAG compared to ungrafted *biribá*, which turned to be similar to that of ungrafted atemoya. At 60 DAG, UGPase activity in this graft

01 **MAAVDTEKLSKLQSAAAGLNQISENEKA**GFVSLVSHYLSGEAQQIEWSKI
51 QTPTDDVVVPYDSMEAAPEDPAQTKSLLDKLVVLKLNGGLGTTMGCTGPK
101 SVIEVRNGLTFLDLIVKQIESLNSKYGCDVPLLLMNSFNTHDDTLKIVE**K**
151 **YANSNIQIHTFNQSQYPRL**VVEDFLPLPSKGQTGK**DGWYPPGHGDVFPSL**
201 **RN**SGKLDALLSQGKEYVFVANSDNLGAVVDLKILHHLIKHKNEYCMEVTP
251 KTLADVKGGTLISYEGKVQLLEIAQVPDEHVNEFKSIEKFKIFNTNNLWV
301 NLSAIKRLVEADALKMEIIPNPKEVAGV**KVLQLETAAGAAIR**FFNHAIG
351 **VNVPRS**RFLPV**KATSDLLLVQSDLYTINDGFVLRN**SA**RTNPSNPSIELGP**
401 **EFKKV**SDFLSRFKSIPSILALDSLKIVGDVWFGAGVVLKG**KVSITADPGV**

Fig. 2 The UGPase amino acid sequence obtained using peptide maps generated from in-gel trypsin digests. UGPase of the atemoya rootstock is approximately 55 kDa in mass. The protein was excised from a 15 % polyacrylamide gel, eluted, and analysed using mass spectrometry. Trypsinised peptides matching UGPase from cherimoya are shown in bold. Peptide mass fingerprints were searched using the Mascot search engine

combination decreased to similar levels to those observed in atemoya onto atemoya at 60 DAG (Table 2).

In addition, we evaluated the presence of UGPase protein in ungrafted atemoya, *araticum-de-terra-fria*, *araticum-mirim* and *biribá* immediately before grafting. UGPase identification was performed using SDS-PAGE in combination with MALDI-TOF. Protein bands around 55 kDa were subjected to trypsin digestion and MALDI-TOF analysis to rapidly calculate the peptide masses. The MALDI-TOF spectrum identified 470 peptides comprising the trypsin fragments from atemoya genotype, and these fragments were used as queries against the Mascot database (Fig. 2). Of these peptides, 31 % (145) matched cherimoya, UDP-glucose pyrophosphorylase (accession number NCBI: ACN50183.1). However, UGPase derived peptides were not identified in the ungrafted rootstocks *araticum-de-terra-fria*, *araticum-mirim* and *biribá*.

Discussion

UDP-glucose pyrophosphorylase (UGPase) plays an important role in the production/metabolism of UDP-glucose, a key metabolite for sucrose and cell wall biosynthesis (Lerouxel et al. 2006). Graft incompatibility has also strong effects on regulation of UGPase gene in apricot/plum combinations (Pina and Errea 2008). In this context, early increased expression of this gene is indicative of a rapid union of plant tissues after grafting. The *UGP* expression results described here corroborate the existence of a significant interaction between scion and rootstock after grafting. According to our results, *UGP* expression levels were higher in grafts of atemoya onto *araticum-de-terra-fria* than in ungrafted atemoya species, which exhibited naturally lower *UGP* expression. These findings are consistent with the literature indicating that atemoya grafts with *araticum-de-terra-fria* are suitable for seedling formation in commercial orchards (Kavati 1992, 2013; Tokunaga 2005).

In contrast, no increase in *UGP* expression was detected when atemoya was grafted as scion onto *araticum-mirim* rootstock, suggesting that no significant interaction occurred between these species, as observed with the *araticum-de-terra-fria* plants. Intriguing, the observed *UGPase* expression pattern clearly did not impair the post-graft restoration of the plants, especially because the corresponding graft combination survived the entire experimental observation period (60 DAG). Nevertheless, a previous report suggests that *araticum-mirim* rootstock induces plant dwarfing, and as consequence, the resulting plants do not survive longer than 4 years in orchards due to growth restrictions in the grafted stem tissue (Tokunaga 2005). This response is supposed to reflect incompatibility in the induction or repression of genes between plants (Prassinos et al. 2009).

In addition, previous research describes *biribá* species as being immediately incompatible after grafting. It has been reported that they lack a stem tissue union during the nursery phase after grafting, and consequently cannot survive (Almeida et al. 2010; Santos et al. 2005). However, *biribá* plants can be restored during the initial period post-grafting (until 60 DAG). Here, we show that UGPase gene expression in the graft combination atemoya onto *biribá* was similar to that in the homograft atemoya onto atemoya and ungrafted atemoya but lower than that in ungrafted *biribá*. Several studies have found incompatibility symptoms when atemoya was grafted on the rootstock *biribá* 1 year of orchard growth that include: shoot death, drying out of scion branches (Kavati 2013) and blackening at the graft interface due to the presence of phenolic compounds (Almeida et al. 2010).

It is evident from the present study that the atemoya scion affected UGPase activity depending on the rootstock/scion combination. In this context, enzymatic activity in ungrafted plants was lower than in ungrafted atemoya. All tested graft combinations exhibited higher UGPase activity than the rootstock and ungrafted atemoya; however, none of the grafts exhibited symptoms of incompatibility. Surprisingly, only the atemoya onto *araticum-de-terra-fria* showed a positive correlation between *UGP* gene expression and UGPase activity, while this correlation was moderated in atemoya grafts with both *araticum-mirim* and *biribá* at 30 and 60 DAG, respectively. A previous study found little or no correlation between *UGP* gene expression and activity between ungrafted and grafted combinations of plum and nectarine 2 weeks after grafting (Pina and Errea 2008). In this case, the suggestion that UGPase is involved in the success of the graft is supported by the observation that UGPase transcripts were diminished in the rootstock from incompatible combinations belonging to the genus

Prunus (plum and apricot) compared with compatible ones (Pina and Errea 2008). The same study found that there was no correlation between the expression and activity of UGPase in the apricot cultivar under different graft partners (ungrafted, homo- and heterografts), suggesting a posttranscriptional/translational regulation of the enzyme.

Regarding the MALDI-TOF results, we found that only ungrafted atemoya presented UGPase-derived amino acid sequences that were similar to that of the UGPase described in cherimoya. One possible explanation is that atemoya is an interspecies cross between sweetsop and cherimoya (Sanewski 1991). On the other hand, the UGPase derived amino acid sequences from ungrafted *araticum-de-terra-fria*, *araticum-mirim* and *biribá* could not be aligned to known plant UGPases sequences. Although the entire amino acid sequences could not be found within the generated UGPase profiles, *UGP* expression and enzyme activity were both detected and quantified in our study.

The possible existence of other UGPase isoforms playing a role in the regeneration of the grafted plants is also plausible. In effect, previous studies have shown that the expression of homologous *UGP* genes is associated with the formation of multiple UGPase isoforms (Chen et al. 2007; Meng et al. 2007, 2009). Two mechanisms, alternative splicing and post-translational modifications (such as phosphorylation, glycosylation and acetylations), have been proposed as important for the regulation of UGPase activity and the formation of UGPase variants (Chen et al. 2007; Meng et al. 2007, 2009). Recently, a UGPase from sugarcane has been found to be phosphorylated in vivo (Soares et al. 2014). Moreover, post-translational modifications are known to facilitate the formation of multiple UGPase isoforms in various plant species, such as *Arabidopsis*, rice and potato (Abe et al. 2002; Chen et al. 2007; Kleczkowski et al. 2004; Meng et al. 2009).

Conclusion

Our study expands upon the current understanding of the molecular mechanisms involved in Annonaceae grafting compatibility. We found that increases in *UGP* expression and UGPase activity are related to the manner that these species react during the attachment of rootstock and scion and the resumption of plant growth.

Author contribution statement Daniel Baron, Gisela Ferreira and Ivan G. Maia contributed to the idea of the study. Daniel Baron, Juliana P. Bravo and Ana Pina collected data of the qRT-PCR, UGPase activity and UGPase amino acid profile. Daniel Baron, Gisela Ferreira, Ivan G. Maia and Ana Pina contributed to assistance with the literature review and statistical analysis. All the authors contributed with manuscript preparation and approved the manuscript in its final form.

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