Why study the UGP gene in grafted woody plants?

Por que estudar o gene UGP em plantas lenhosas enxertadas?

¿Por qué estudiar el gen UGP en plantas leñosas injertadas?

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ABSTRACT

Grafting is a technique for cultivating commercial fruit species that guarantees genetic characteristics using selected clones. However, candidate genes involved in the re-establishment of grafted woody plants remain poorly understood. In this way, our manuscript aimed to present current knowledge on UDP-glucose pyrophosphorylase (UGP) gene expression and its enzymatic activity (UGPase) by incorporating Brazilian wild to elucidate the role of “players” in re-establishing post-grafting in woody species. Considerable benefits were observed in grafted plants, explained by the correlation between gene expression, such as that of UGP, and UGPase biosynthesis and enzymatic activity. This enzyme is directly responsible for cell wall formation, which explains faster tissue formation in some grafting combinations than others. Besides, UGP promotes the re-establishment of the vascular connection in plant grafting, thus regulating short- and long-term grafting compatibility in woody plants. These crucial experiments are also highly relevant to many nurseries. In this study, we report that the Annona genus is a valuable plant model for studying plant grafting in wood species.

Keywords: atemoya, gene expression, plant grafting, UGPase.

RESUMO

A enxertia é uma técnica de cultivo de espécies frutíferas comerciais que garante as características genéticas a partir de clones selecionados. No entanto, os genes candidatos envolvidos no restabelecimento de plantas lenhosas enxertadas permanecem pouco compreendidos. Este manuscrito apresenta o conhecimento atual sobre a expressão do gene UDP-glicose pirofosforilase
(UGP) y su actividad enzimática (UGPase) a través de la incorporación de una especie silvestre brasileña como planta modelo. Estas evaluaciones genéticas y bioquímicas fueron implementadas para elucidar el papel de los “atores” en el restablecimiento de post-enxertia en especies lenhosas. Beneficios considerables fueron observados en plantas enxertadas, explicados por la correlación entre la expresión genética, como a la UGP, y la biosíntesis y actividad enzimática de la UGPase. Esta enzima es directamente responsable de la formación de la pared celular, lo que explica la formación de tejido más rápida en algunas combinaciones de enxertos que en otras. Además, la UGP promueve el restablecimiento de la conexión vascular en plantas enxertadas, regulando así la compatibilidad del enxerto a corto y largo plazo en plantas lenhosas. Estos experimentos relevantes también son altamente oportunos para muchos viveiros. En este estudio, relatomos que el género Annona es un valioso sistema modelo para el estudio de enxertia de plantas en especies lenhosas.

Palavras-chave: atemoia, expresión génica, enxertia de plantas, UGPase.

RESUMEN
El injerto es una técnica de cultivo de especies frutales comerciales que garantiza características genéticas a partir de clones seleccionados. Sin embargo, los genes candidatos implicados en el restablecimiento de plantas leñosas enxertadas siguen siendo poco conocidos. Este manuscrito presenta el conocimiento actual sobre la expresión del gen UDP-glucosa pirofosforilasa (UGP) y su actividad enzimática (UGPasa) mediante la incorporación de una especie silvestre brasileña como planta modelo. Estas evaluaciones genéticas y bioquímicas se implementaron para dilucidar el papel de los “actores” en la restauración del postinjerto en especies leñosas. Se observaron beneficios considerables en plantas enxertadas, explicados por la correlación entre la expresión de genes, como la UGP, y la biosíntesis y actividad enzimática de la UGPasa. Esta enzima es directamente responsable de la formación de la pared celular, lo que explica la formación de tejido más rápida en algunas combinaciones de injertos que en otras. Además, la UGP promueve el restablecimiento de la conexión vascular en plantas enxertadas, regulando así la compatibilidad del injerto a corto y largo plazo en plantas leñosas. Estos experimentos relevantes también son muy oportunos para muchos viveiros. En este estudio, informamos que el género Annona es un sistema modelo valioso para estudiar el injerto de plantas en especies leñosas.

Palabras clave: atemoia, expresión génica, injerto de plantas, UGPasa.
1 INTRODUCTION

The orchard areas of plant species belong to the Annonnaceae family, such as sugar apple or sweetsop (*Annona squamosa* L.), soursop (*A. muricata* L.), cherimoya (*A. cherimola* Mill.), and atemoya hybrid (*A. x atemoya* Mabb.) have been increased around the world. For example, the atemoya fruits are used in the production of ice cream, jams, juices, and liqueurs, in *natura* consumption and it is propagated by an asexual technique (e.g. grafting). In contrast, the sexual technique (seeds) is used for rootstock production in areas without soil pathogens (Heenkenda *et al.*, 2009; Kavati, 2013; Ferreira *et al.*, 2019). Thus, becomes necessary to ensure the genetic characteristics, however, graft incompatibility is frequently observed (Baron, Daniel *et al.*, 2016).

The plant rootstocks most often used to graft atemoya are araticum-de-terra-fria (*A. emarginata* Schltdl. H. Rainer “var. terra-fria”) and araticum-mirim (*A. emarginata* H. Rainer “var. mirim”). Atemoya grafted onto ‘araticum-de-terra-fria’ rootstock causes further development and tolerance of the scion to cave nematodes, stem borers, 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).

Although research indicates graft incompatibility between atemoya and other wild *Annona* species, there are potential rootstocks in the breeding of new fruits. Graft incompatibility is generally defined as the interruption in cambial and vascular continuity between rootstock and scion (Melnyk *et al.*, 2015; Hartmann *et al.*, 2018; Baron *et al.*, 2019) and urges for further understanding of the mechanisms related to graft incompatibility in Annonaceae. Despite the long history of grafting as a scientific research tool in plant propagation, the molecular mechanisms involved in post-grafting remain poorly understood (Melnyk, 2017; Phanu *et al.*, 2023).

Moreover, investigating the *Annona* grafting re-establishment (woody plants) is challenging as these individuals need a longer period than plant
vegetables to assess the success of the grafting. Many “candidates” responsible for compatibility are directly or indirectly related to reestablishing the vascular connection, but so far, a very small diversity of species has been used to study this subject (Pina and Errea, 2008). If the researcher or producer of grafted seedlings pays attention to overcoming possible obstacles in obtaining grafted plants and still faces difficulties getting compatible plants, biomolecular events may be the reason for the failed grafting.

In this way, our manuscript aimed to present current knowledge on UDP-glucose pyrophosphorylase (UGP) gene expression and its enzymatic activity (UGPase) by incorporating Brazilian wild to elucidate the role of “players” in re-establishing post-grafting in woody species.

2 THEORETICAL FRAMEWORKS

In general, plant grafting combines two parts of living plants, such as rootstock (root system) and scion (shoot system) to create a new whole plant (Hartmann et al., 2018; Loupit et al., 2023). Several biotic and abiotic factors are involved in graft incompatibility. One of the most relevant involves the professional skills required improving the conditions post-grafting is crucial for a successful re-establishment whether certain scion-rootstock combinations are “truly incompatible” (e.g., anatomical mechanisms or unexperienced grafting practice). Nursery producers, responsible for producing commercial rootstocks and scions, generally apply slight physical pressure, using a wrapping tape around a graft tissue joint to improve the successful formation and complete realignment of the cambial tissues of both two parts of living plants (Baron et al., 2019).

One concern is that incompatibility may manifest years later through hypertrophy of tissues in the scion/rootstock joining region, forming an “elephant’s foot.” This damage is mainly caused by poorly choosing the plant species involved in the grafting combination, primarily due to incorrect taxonomical identification of the seeds or seedlings (Hartmann et al., 2018).
phylogenetics proximity of scion and rootstock species is relevant; however, few authors report taxonomic information about the species used in their grafting studies. Besides, we speculate that the number of exsiccates of *Annona* grafted deposited in herbariums is low, which may result in identification mistakes and difficulties obtaining the right seeds since the species may be known by different common names (Baron *et al*., 2019). For example, *Annona mucosa* Jacq. is popularly referred to as biribá in different regions of Brazil. It is a compatible rootstock used to create the atemoya hybrid and, at this present moment, there are no physiological incompatibility responses immediately after grafting (0–15 days after grafting [DAG]) and until 500 DAG (Baron, Daniel *et al*., 2016). However, the same popular name is used to refer to other Brazilian native species, such as *A. muricata* L., *A. glabra* L., *A. montana* Macfad., and *A. reticulata* L., which are reportedly incompatible as rootstocks with atemoya scions (Ferreira *et al*., 2019).

If, given the above information and *sine qua non* considerations, the researcher or producer of grafted seedlings pays attention to overcoming possible obstacles in obtaining grafted plants and still faces difficulties getting compatible plants, biomolecular events may be the reason for the failed grafting. *UGP* codes for UGPase (EC 2.7.7.9), a key enzyme in the production of sugar nucleotides and interconversion of UDP-glucose sugar, a relevant precursor in the biosynthesis of cellulose, hemicellulose, and pectin components (cell wall) (Kleczkowski *et al*., 2004; Amos and Mohnen, 2019; Zhang *et al*., 2021). To evaluate compatibility relationships in grafted plants, a pioneering study investigated the role of *UGP* in plants (Pina and Errea, 2008). The authors observed that *UGP* transcripts were poorly detected in the graft of incompatible combinations compared with compatible ones, suggesting a potential role for this gene to re-establishment in woody plants. Likewise, the lower gene expression in previously observed incompatible combinations led to decreased UGPase activity at 10 DAG in the Prunus genus. Possible UGPase activity in the graft is described for several other species, including *Populus* sp. and arabdopsis plants (Meng *et al*., 2007; Liu *et al*., 2021). *UGP* expression during the initial period after
grafting (15 DAG) in *Annona emarginata* (Schltdl.) ‘terra-fria’ and ‘mirim’ rootstocks was higher than in the ungrafted atemoya hybrid (Baron *et al.*, 2016). Consequently, the same authors reported that UGPase activity was also higher in *A. mucosa* and the two *A. emarginata* varieties compared to the atemoya hybrid, indicating tissue production and rapid union of plant tissues after grafting.

Preliminary results suggested that a group of candidate genes may be directly responsible for producing cellulose microfibrils, one major cell wall constituent (González-Agüero *et al.*, 2011). *UGP* expression is extremely responsive to subtropical climatic conditions (low and/or chilling temperature) (Ciereszko *et al.*, 2001; Meng *et al.*, 2009) that may contribute to successful re-establishment of atemoya hybrid grafting. According to nursery growers’ recommendations, in subtropical regions of Brazil, grafting of fruit trees should be performed preferably during winter (vegetative rest). Although the gene expression and cold combination may indicate graft compatibility in *Annona* plants, no data is available in the literature. In general, it is widely known that the more closely related rootstock-scion species are the better the chances for the graft to be successful facilitating new tissue formation and re-establishment of vascular connection (Baron *et al.*, 2019). In the last decade, 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.*, 2014; Irisarri *et al.*, 2015).

Ungrafted Annonaceae species displayed different profiles and similarities with other plants based on the alignment of the UGPase amino acid sequences (Baron, D. *et al.*, 2016). The atemoya hybrid had a similar UGPase amino acid sequence only with cherimoya species, explained by cherimoya being one of the atemoya hybrid’s parents. In *A. mucosa*, *A. emarginata* var. “terra-fria”, and *A. emarginata* var. “mirim”, no UGPase amino acid sequence was detected. These observations suggest and lead to speculate that the UGPase have a multiple UGPase isoforms. However, more studies are needed to confirm these hypotheses in other woody plants. Advances in molecular biology studies in grafted plants led to understanding the correlation between gene expressions—
such as UGP—and compatibility. In addition, the UGPase biosynthesis and enzymatic activity are directly responsible for cell wall formation, explaining faster tissue formation in some grafting combinations than others, such as Annona x atemoya Mabb. grafted on A. emarginata ‘terra-fria’ (Baron et al., 2016; Baron et al., 2019). Undoubtedly, these authors expand upon the current understanding of the molecular mechanisms involved in Annonaceae grafting compatibility (Table 1).

3 CONCLUSION

We speculate that the Annona genus is an excellent candidate for studying grafting in woody plants and the role of UGP in facilitating the re-establishment of vascular connections and regulating short- and long-term grafting compatibility. Nevertheless, additional experiments are necessary to further understand woody species.

Table 1. Gene expression patterns of UGPase (mRNA UGP) in published transcriptomic datasets on ungrafted and/or graft compatibility.

<table>
<thead>
<tr>
<th>Specie(s)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apricot (Prunus armeniaca L. cv. ‘Moniqui’)</td>
<td>(Pina and Errea, 2008)</td>
</tr>
<tr>
<td>Plum (P. cerasifera × P. munsoniana cv. ‘Marianna 2624’)</td>
<td></td>
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<tr>
<td>Atemoya (Annona x atemoya Mabb. cv. ‘Thompson’)</td>
<td></td>
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<tr>
<td>Araticum-de-terra-fria (A. emarginata var. “Araticum-de-terra-fria”)</td>
<td>(Baron et al., 2016; Baron et al., 2019)</td>
</tr>
<tr>
<td>Araticum-mirim (A. emarginata var. “Araticum-mirim”)</td>
<td></td>
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<tr>
<td>Biribá (A. mucosa Jacq.)</td>
<td></td>
</tr>
<tr>
<td>Populus (Populus sp.)</td>
<td>(Meng et al., 2007)</td>
</tr>
<tr>
<td>Rice (Oryza sativa L.)</td>
<td>(Chen et al., 2007; Huang et al., 2011)</td>
</tr>
<tr>
<td>Arabdopsis (Arabidopsis thaliana L.)</td>
<td>(Meng et al., 2009; Liu et al., 2021)</td>
</tr>
<tr>
<td>Tomato (Solanum lycopersicum L.)</td>
<td>(Sowokinos, 2001)</td>
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</tbody>
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Source: Authors
REFERENCES


