

Abscisic acid and compatibility of atemoya (*Annona x atemoya* Mabb.) grafted onto native species

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Abstract - Grafting is an effective technique used in the cultivation of commercial fruit species given the necessity to guarantee the genetic characteristics of productive species using selected clones. Although grafting is a common and widespread technique and phytohormones play a key role in the formation of tissues, the relationship between phytohormones, such as abscisic acid, and mechanisms of incompatibility is not yet well elucidated. Thus, the objective of this study was to establish whether a correlation exists between variations in abscisic acid and the compatibility of the atemoya (*Annona x atemoya* Mabb.) cultivar ‘Thompson’ grafted onto biribá [*Annona mucosa* (Bail.) H. Rainer], araticum-mirim [*Annona emarginata* (Schltdl.) H. Rainer ‘var. mirim’] and araticum-de-terra-fria [*Annona emarginata* (Schltdl.) H. Rainer ‘var. terra-fria’]. Plant cultivation was carried out at the Botany Department of Instituto de Biociências (IB), Unesp, Botucatu, São Paulo, Brazil. The plant material of grafted plants (stem above the grafted area, stem containing the grafted region, and stem below the grafted region) and ungrafted plants (stem 20 cm above ground) was collected 500 days after grafting (DAG) for the extraction and quantification of abscisic acid. The results of this study show that ungrafted *Annona* plants exhibit variations in the concentration of abscisic acid among the native rootstock species. When grafted, the most commonly used grafting combinations, araticum-de-terra-fria and araticum-mirim, present the same concentrations of abscisic acid in the graft region as self-grafted atemoya. It was concluded that the observed variations in the concentrations of abscisic acid in the graft region did not cause incompatibility in the combinations of atemoya grafted onto different native species.

Index terms: phytohormones, rootstock, post-grafting plant restoration.

Ácido abscísico e compatibilidade de atemoia (*Annona x atemoya* Mabb.) enxertada em espécies nativas

Resumo - A enxertia é utilizada de maneira eficaz para o cultivo de espécies frutíferas comerciais, uma vez que é necessário garantir as características genéticas de espécies produtivas com o emprego de clones selecionados. Apesar de a enxertia ser técnica comum e amplamente difundida, e os hormônios terem papel-chave na formação de tecidos, a relação de fitormônios como o ácido abscísico com mecanismos de incompatibilidade ainda não está bem elucidada. Desta forma, o objetivo deste estudo foi avaliar se existe correlação entre as variações do ácido abscísico com a compatibilidade de atemoia (*Annona x atemoya* Mabb.) cultivar ‘Thompson’, enxertada em biribazeiro [*Annona mucosa* (Bail.) H. Rainer], e plantas de araticum-mirim [*Annona emarginata* (Schltdl.) H. Rainer variedade mirim] e araticum-de-terra-fria [*Annona emarginata* (Schltdl.) H. Rainer variedade terra-fria]. O cultivo das plantas foi realizado no Departamento de Botânica do Instituto de Biociências de Botucatu (IB), Unesp, município de Botucatu-SP. O material vegetal de plantas enxertadas (caule acima da região enxertada, caule contendo a região enxertada e caule abaixo da região enxertada) e não enxertadas (caule situado a 20 cm acima do solo) foi coletado aos 500 dias após a enxertia (D.A.E.) para a extração e quantificação do ácido abscísico. Os resultados deste estudo evidenciam que as anonáceas (pé-francos) apresentam variações na concentração de ácido abscísico entre as espécies de porta-enxertos nativas, e quando enxertadas; além do mais, as combinações mais utilizadas atualmente, araticum-de-terra-fria e araticum-mirim, apresentam as mesmas concentrações de ácido abscísico na região enxertada que da atemoia enxertada nela mesma. Conclui-se que as variações observadas nas concentrações de ácido abscísico na região enxertada não provocaram incompatibilidade nas combinações de atemoia enxertada em diferentes espécies nativas.

Termos de indexação: fitormônios, porta-enxerto, restabelecimento vegetal pós-enxertia.

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Brazil occupies a prominent place in the global commercial production of *Annonas*, mainly due to its climatic diversity, which allows for production of the fruit from the north to the south of the country (SÃO JOSÉ et al., 2014). The emphasis is on atemoya (*Annona* x *atemoya* Mabb.), which is a hybrid between the sweetsop (*Annona squamosa* L.) and cherimoya (*Annona cherimola* Mill.) (RÂBELO et al., 2015). In Brazil, atemoya seedlings are produced by grafting using native rootstock species, such as *araticum-de-terra-fria* [*Annona emarginata* (Schltdl.) H. Rainer 'var. terra-fria'], that, when compared to the native species *araticum-mirim* [*Annona emarginata* (Schltdl.) H. Rainer 'var. mirim'], provide greater longevity to the scion, do not develop grafted tissue hypertrophy ("elephant's foot") and do not cause dwarfism in the grafted plant (TOKUNAGA, 2005). *Biribá* has been considered by several authors to be incompatible as a rootstock for atemoya (SANTOS et al., 2005; KAVATI, 2013). However, plants evaluated three months after grafting exhibited a high survival rate (85%) (BARON et al., 2016).

Compatibility between scion and rootstock includes a number of physiological mechanisms with immediate responses to injury such as callus formation and the establishment of new functional vascular tissue between the partners. The ability to form a compatible scion and rootstock combination is also due to hormonal and biochemical characteristics (MELNYK; MEYEROWITZ, 2015). Abscisic acid (ABA) regulates tolerance responses to a large number of abiotic stresses, such as salinity and lack of water (LIU et al., 2016; KUMAR et al., 2017). In addition, studies with grafted tomatoes have shown that the ABA level in the scion plays a fundamental role in reducing the size of grafted plants, independently of the genotype (ALBACETE et al., 2015).

According to Tworkoski and Fazio (2015), ABA is one of the main factors responsible for triggering the dwarfing process in otherwise tall plants. Dwarfing apple tree rootstocks (*Malus* sp.) contain large amounts of ABA, in addition to having a high ratio of ABA to IAA (auxin), compared to vigorous rootstocks of the same species (LORDAN et al., 2017); however, there is no full understanding of how ABA affects the survival rates of grafted plants. Most attempts by studies to explain incompatibility refer to the initial stages after grafting in herbaceous systems (KÜMPERS et al., 2015; MELNYK et al., 2015), and few studies have been carried out on woody plants (PINA et al., 2012) due to the difficulties inherent in investigating species that require a longer period of time for evaluation. In light of the fact that tissue formation between scion and rootstock requires hormonal action, research on ABA action may help further our understanding of the physiological mechanisms of incompatibility.

The experiment was implemented and conducted in a greenhouse at the Botany Department of Instituto de Biociências (IB), Universidade Estadual Paulista Júlio de Mesquita Filho (Unesp), Botucatu campus, São Paulo, Brazil, located at 22°52'S, 48°26'E at an altitude of 850 m.

For the production of the rootstocks and ungrafted plants, seeds were extracted from fruits of *araticum-de-terra-fria* (*Annona emarginata* Schltdl. H. Rainer 'var. terra-fria'), *araticum-mirim* (*A. emarginata* 'var. Mirim'), *biribá* (*A. mucosa*) and atemoya (*Annona* x *atemoya* Mabb. 'Thompson'). After seed extraction, sowing was carried out in polystyrene trays (72 cells) filled with vermiculite of medium granulometry, using one seed per cell. The seedlings, when presenting the first leaf completely expanded above the third node of the epicotyl, were called young plants (seedlings). Seedlings of ± 10 cm in length were transplanted into plastic bags with a volumetric capacity of 17 dm³ containing approximately 5 dm³ of *pinus* bark mixture, fertile eutroferic Latosol soil of areno-clayey texture, vermiculite, and coconut fiber of average granulometry (1:2:1:1 v/v). When these reached a stem diameter of 10 mm at 20 cm of soil height (about 500 days after sowing [DAS]), they were grafted.

The whip and tongue graft technique was utilized (TOKUNAGA, 2005). Scions obtained from a single adult atemoya plant were grafted onto *biribá*, *araticum-de-terra-fria*, *araticum-mirim* and atemoya rootstocks grown from seed. In addition to the grafting combinations, ungrafted plants (atemoya, *biribá*, *araticum-de-terra-fria* and *araticum-mirim*) were used. The plant material of grafted plants (stem tissue at the interface of the grafting region, collected 15–20 cm from the neck of the plant, stem above the grafted region, stem containing the grafted region and stem below the grafted region) and of ungrafted plants (stem at 20 cm above the collar, where the grafting would be carried out) were collected at the phenological stage that represents the establishment period, simulating a possible transplant of seedlings to the field, at 500 DAG. The samples were conditioned in liquid nitrogen and stored in an ultra-low freezer until analysis.

The plant samples were pulverized for extraction and quantification of the ABA hormone (MA et al., 2013). Plant material (100 mg) was homogenized in 500 μ l of methanol-MeCN extraction solution: methanol, acetonitrile, Mil-Q water and acetic acid (40/40/20/1, v/v/v/v). Subsequently, this mixture was blended using a vortex mixer for 2 minutes before being submerged in an ultrasonic bath for 30 minutes and in an ice bath for 1 minute. The homogenate was centrifuged at 12000 rpm (4 °C). The resulting supernatant was transferred to a separate tube and the pellet was mixed into the extraction solution, according to the methodology described above, which is known as "double extraction". The chromatographic separation was performed using the Shimadzu Prominence high-performance liquid chromatograph (HPLC), which

is composed of a mobile phase degasser DGU-20A, quaternary pumping system consisting of an LC-20AD pump, a SIL-20AHT self-sampler, a CBM-20A controller, and CTO-20AC column oven. The Sinergi 2.5 Hydro RP-100A 50 x 4.6 mm column was used, which was maintained at 40 °C during the determinations. The column effluent was introduced into an AB Sciex 4500 triple quadrupole mass spectrometer equipped with an ESI-type ionization source (electrospray) in the interface. The results were expressed in nmol per gram of fresh mass (nmol g⁻¹ FM) (MA et al., 2013).

The experiment was conducted in a randomized block design with eight treatments (atemoya scion grafted onto atemoya; *araticum-de-terra-fria*; *araticum-mirim* and a *biribá* rootstock other than atemoya; *araticum-de-terra-fria*; ungrafted *araticum-mirim* and *biribá*) with nine replicates of each plant per treatment. The data were submitted to a variance of homogeneity test (“Levene’s test”) and variance analysis (ANOVA), and the means were compared using the Tukey test ($p \leq 0.05$). During the course of the experiment, crop handling procedures, such as mineral nutrition and thinning, were carried out. These comprised removing any branch or leaves that had grown from the stems of the seedlings between the level of the soil to 25 cm in height), with the aim of ensuring the sanity and uniformity of the plants.

The results of this study show that *Annonas* present variations in the concentration of ABA among ungrafted species (Table 1) and grafted species (Table 2). The most commonly used combinations, *araticum-de-terra-fria* and *araticum-mirim* (TOKUNAGA, 2005), presented the same concentrations of ABA in the grafted region as self-grafted atemoya (Table 2).

In all grafting combinations, the concentration of ABA in the scion (the region above the graft) was greater than in the region below the graft, which may be a reflection of the stress caused by the grafting and is not necessarily related to incompatibility, since this fact is also observed in self-grafted atemoya, in which there would be no genetic reason for incompatibility.

Regarding gene expression in tissue formation, it was evident that, in atemoya grafted onto *araticum-de-terra-fria*, the expression of UGPase occurs earlier than in other grafting combinations, which evidences faster tissue formation in the graft region (BARON et al., 2016). In addition, promoter hormones such as gibberellins (GA) and auxins (AX) act on tissue formation, as proposed by Hartmann et al. (2011), which explains the lower concentration of ABA found in the graft region. Atemoya grafted onto *biribá* presented the highest concentrations of ABA in the graft region, yet this fact cannot be considered to reflect incompatibility, since the graft survival rate was 80–85% (data not shown).

In studies of UGP gene expression and the UDP-glucose pyrophosphorylase enzymatic activity responsible for cell wall formation, *biribá* rootstocks have been shown to form tissues later than is the case with *araticum-de-terra-fria* and *araticum-mirim*. This may occur due to the action of ABA, which reduces or prevents the action of hormones such as gibberellins, which is involved in the formation of tissues. Thus, it appears that the concentrations of ABA observed in *biribá* are responsible for delaying the formation of tissues; however, this does not change the survival rate of grafted plants following their complete cicatrization in nursery conditions. Nevertheless, *biribá* is not considered a good option as a rootstock for atemoya and sweetsop (*A. squamosa* L.) (ALMEIDA et al., 2010; KAVATI, 2013).

Santos et al. (2005) reported a survival rate of 4% at 45 days after grafting sugar apple onto *biribá*; however, the physiological explanations that support the existence of negative reactions in the formation of callus or vascular systems are not presented. Scientific evidence indicates that atemoya grafted onto *biribá* has a proliferation of parenchyma and differentiation of the vascular system, which establishes connections between the scion and rootstock tissues (BARON et al., 2014). According to Kavati (2013), producers report incompatibility after years of commercial orchard formation. Although there are reports that the concentration of ABA can be used as an efficient marker in the early selection of dwarfing rootstocks (HARTMANN et al., 2011), in this study, it was not possible to indicate such condition, since *araticum-mirim*, which is reported as a dwarfing rootstock to atemoya (TOKUNAGA, 2005; KAVATI, 2013), presented similar ABA concentrations to that exhibited by the other grafting combinations that are not considered to be dwarfing.

Incompatibility may be expressed by “unsuccessful” grafts, deficient or abnormal growth of the grafted plant, hypertrophy of the graft attachment point, or poor mechanical strength of the grafting union, which, in extreme cases, may result in tissue rupture at the site of the graft. This incompatibility may manifest immediately or emerge only after many years (KAVATI, 2013). A distinction must be made between incompatibility provoked by the viral action of the “negative” reaction between the grafting partners (ROWHANI et al., 2017), reactions that result in tissue hypertrophy in the grafted region (“elephant’s foot”) (TOKUNAGA, 2005), or reactions caused by environments that are unsuitable for certain species.

In this context, this study allowed us to verify, in nursery conditions over an approximately 18-month period, that the observed variations in the concentrations of ABA in the grafted region do not provoke incompatibility in the combinations of atemoya grafted onto *biribá*: *araticum-de-terra-fria* and *araticum-mirim*.

Table 1. Concentration of abscisic acid (ABA) expressed in nmol per gram of fresh mass (nmol g⁻¹ FM) in the stem region at 20 cm above ground, in ungrafted atemoya, *biribá*, *araticum-de-terra-fria*, and *araticum-mirim* at 540 days after sowing (DAS).

| Ungrafted | Stem region at 20 cm above ground |
|-------------------------------|-----------------------------------|
| <i>araticum-de-terra-fria</i> | 94,32 ± 28,4 c |
| atemoya | 176,41 ± 30,3 ab |
| <i>araticum-mirim</i> | 250,17 ± 36,8 a |
| <i>biribá</i> | 145,20 ± 19,0 bc |
| C.V. (%) | 17,6 |

Means followed by the same lowercase letters do not differ in Tukey's test at 5% probability (\pm standard deviation, $n = 9$).

Table 2. Concentration of abscisic acid (ABA) expressed in nmol per gram of fresh mass (nmol g⁻¹ FM) in the stem above grafting region, stem below grafting region and stem in the grafting region of self-grafted atemoya (ATE x ATE), atemoya grafted onto *araticum-de-terra-fria* (ATE x FRIA), atemoya grafted onto *araticum-mirim* (ATE x MIRIM), and atemoya grafted onto *biribá* (ATE x BIR) 500 days after grafting (DAG).

| Scion x rootstock | ABA (nmol g ⁻¹ FM) | | |
|-------------------|-------------------------------|-----------------------------|----------------------------|
| | Stem above grafting region | Stem in the grafting region | Stem below grafting region |
| ATE x ATE | 237,1 ± 37,0 Aa | 93,27 ± 5,2 Bb | 102,30 ± 4,91 Ba |
| ATE x FRIA | 91,03 ± 12,5 Ac | 71,50 ± 3,7 ABb | 58,30 ± 13,8 Bb |
| ATE x MIRIM | 130,10 ± 0,8 Ab | 85,13 ± 4,6 Bb | 64,00 ± 3,0 Bb |
| ATE x BIR | 134,63 ± 3,9 Ab | 149,40 ± 14,4 Aa | 99,30 ± 16,2 Ba |
| C.V. Total (%) | 12,61 | | |

Means followed by the same lowercase letters do not differ in Tukey's test at 5% probability (\pm standard deviation, $n = 9$).

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