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Histological analysis and performance of sour passion fruit populations under different rootstocks resistant to *Fusarium* spp

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Abstract - The present study aimed to describe histological traits of the graft welding process and evaluate the effect of rootstocks on the physical and chemical traits and the productivity of sour passion fruit populations. The experiment was arranged in a randomized block design, in a 4x2 + 4 factorial scheme (cultivars / canopy x rootstocks), three replicates and nine plants per plot. Commercial cultivars and populations and *Passiflora alata* and *P. nitida* rootstocks, in addition to ungrafted plants were used as canopy. Cleft grafting was the grafting type adopted. Traits analyzed such as productivity and physical and chemical quality of fruits were submitted to analysis of variance and compared by the Tukey test. The canopy morphology was evaluated according to descriptors of the Ministry of Agriculture, Livestock and Supply. Grafting was carried out for each graft/rootstock combination for histological analysis. Sections were obtained by freehand cuts, stained and mounted on semi-permanent slides, examined under optical microscope and photomicrographed. Rootstocks affected the early cultivation and reduced productivity and number of fruits of passion fruit populations. Grafting did not affect the quality of fruits or the canopy morphology. Considering the complete culture cycle, *P. alata* species is able to be used as rootstock. The anatomical study demonstrates the occurrence of better compatibility in the connection of *P. edulis* tissues on *P. alata,* compared to grafting on *P. nitida*.
Index Terms: Grafting, plant tissues, Productivity, *Passiflora edulis.*

Análise histológica e desempenho de populações de maracujazeiro-azedo sob diferentes porta-enxertos resistentes ao *fusarium* spp

Resumo - O objetivo do presente trabalho foi descrever as características histológicas do processo de soldadura da enxertia e avaliar a influencia dos porta enxertos sobre as características físicas e químicas do fruto e na produtividade de populações de maracujazeiro azedo. O delineamento utilizado foi o de blocos ao acaso, num esquema fatorial 4x2+4 (cultivares/copas x porta enxertos), com três repetições e nove plantas por parcela. Foram utilizadas como copa cultivares comerciais e populações e os porta enxertos *Passiflora alata* e *P. nitida,* além do pé-franco. O tipo de enxertia foi o convencional por garfagem no topo em fenda cheia. As características analisadas como produtividade e qualidade físico química dos frutos foram submetidas à análise de variância e comparadas pelo teste de Tukey. E a morfologia da copa foi avaliada de acordo com descritores do MAPA. Foi realizada a técnica de enxertia de cada combinação enxerto/porta enxerto para a analise histológica, onde as secções foram obtidas à cortes a mão livre, coradas e montadas em lâminas semipermanente e analisadas sob microscópio óptico e fotomicrografadas. Os portas enxertos influenciaram no período inicial de cultivo reduzindo a produtividade e o número de frutos das populações de maracujazeiro, e a enxertia não influenciou na qualidade dos frutos e na morfologia da copa. Considerando o ciclo completo da cultura, a espécie *P. alata* possui condições de ser utilizada como porta enxerto. O estudo anatômico demonstra a ocorrência de uma melhor compatibilidade na união dos tecidos de *P. edulis* sobre *P. alata* quando comparados a enxertia sobre *P. nitida.*
Termos para Indexação: Enxertia, tecidos vegetais, produtividade, *Passiflora edulis.*

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Introduction

Brazil is the world's largest producer and consumer of passion fruit (*Passiflora edulis* Sims). However, its productivity is only 14,488 kg ha^{-1} $year^{-1}$(IBGE, 2014), far below the productive potential of the culture. Low productivity is related to inappropriate farming techniques, insignificant use of improved cultivars and phytosanitary problems (LIMA, 2004; GONÇALVES et al., 2007), which have reduced the useful life of orchards (LIMA et al., 2004; CAVICHIOLI et al., 2011b). Several diseases affect passion fruit cultivation, including soil diseases called fusarium wilt and collar rot (RUGGIERO et al., 1996).

Fusarium wilt is caused by fungus *F. oxysporum f. sp. Passiflora*e, which colonizes pots of plants, blocks the xylem and prevents the flow of water, leading to wilt and plant death (INDEX FUNGORUM, 2015). The causative agent of collar rot is fungus *Haematonectria haematococca*, which in its imperfect form is called *Fusarium solani* Martius. Its early symptoms include swelling and formation of lesions in the plant collar (INDEX FUNGORUM, 2015).

The occurrence of these diseases discourages many sour passion fruit producers, since plants die at the beginning of the production cycle. They reduce crop longevity and productivity and cause production losses, considering that costs involved in the implementation of the orchard are high. Therefore, the search for technologies for the management of diseases caused by *Fusarium solani* and *F. oxysporum f. sp. passiflorae* is a significant research demand (FALEIRO et al., 2006).

An alternative for mitigating losses caused by soil diseases is the use of genetic resistance observed in some wild species, including *P. quadrangularis, P. nitida, P. alata and P. Foetida*, resistant to fungus *F. solani* (FISCHER et al., 2005; PREISIGKE et al., 2015) and *P. alata, P. laurifolia, P. suberosa, P. coccinea, P. gibertii* and *P. setace*, resistant to *F. oxysporum f. sp. Passiflorae* (MENEZES et al., 1994; MELETTI and BRUCKNER, 2001; RONCATTO et al., 2004). Thus, these species can be used in breeding programs, mainly as rootstocks. Grafting is a viable technique for passion fruit cultivation (CHAVES et al., 2004; SILVA et al., 2005; CORRÊA et al., 2010), aiming to solve problems in this culture related to soil diseases (JUNQUEIRA et al., 2006). The conventional cleft grafting technique has been used in the formation of sour passion fruit seedlings, and reached 76.3% of fixation of *P. edulis* grafted on *P. alata*; 98.8% on *P. gibertii*; and 100 % on *P. edulis* (CORRÊA et al., 2010).

However, in addition to the fixation index, the effect of rootstock on sour passion fruit productivity and physical and chemical traits is another important factor that deserves attention. It is important to point out that the compatibility between rootstock and graft is fundamental for successful grafting, and may be related to physiological and anatomical factors (NAVARRO 1988; PIO et. al., 2001; ANSELMINI and ZANETTE, 2008). Therefore, histological analysis can provide the characterization of structures involved in the union of tissues and greater understanding of grafting results (ESTRADA - LUNA et al., 2002).

Thus, the present study aimed to describe the histological traits of graft welding and evaluate the effect of rootstocks on productivity and physical and chemical traits of sour passion fruit populations.

Material and Methods

The experiment was installed in the experimental area of the State University of Mato Grosso (UNEMAT), municipality of Tangará da Serra, state of Mato Grosso, Brazil (14º39' S and 57º25' W and 321 m a.s.l.). Soil is classified as clayey Dystroferric Red Latosol, with flat to slightly wavy relief (EMBRAPA, 2006). The climate is tropical, with well defined dry and rainy seasons, average annual rainfall ranging from 1300 to 2000 mm $year^{-1}$, and annual temperature ranging from 16 to 36°C (MARTINS et al., 2010).

Seedlings were produced in a protected environment in plastic bags (10x20 cm) containing substrate (LIMA et al., 2004), through propagation by cleft grafting. For such, seeds of *P. alata* and *P. nitida* rootstocks were sown in September 2013, 60 days prior to grafting due to the time needed for germination and growth. *P. edulis* genotypes used as grafts were sown in November 2013. Grafting was carried out 60 days after the emergence of plants, when plants used as rootstocks and graft showed stem diameter around 3 mm. For the grafting construction, plants used as rootstocks were cut at the height of 10-12 cm from the collar, where a longitudinal slit of 1 to 2 cm was made, in which a fork with two internodes and the wedged base were introduced. This region of the stem was wrapped with plastic film to maintain the graft in tight contact with the rootstock and to protect the set (CAVICHIOLI et al., 2011b).

The experiment was installed in the field and arranged in a randomized block design, in a 4x2 + 4 factorial scheme (canopies x rootstocks), with three replicates and nine plants per plot. Two populations from the sour passion fruit breeding program conducted by UNEMAT, called UNEMAT S10 and UNEMAT S30, and commercial cultivars 'FB 200 Yellow Master' and 'FB 300 Araguari' were used as canopies. Wild *P. alata* and *P. nitida* species resistant to *F. oxysporum f. sp. passiflorae and F. solani* (FISCHER et al., 2005; PREISIGKE et al., 2015) were used as rootstocks. 'FB 200 Yellow Master' and 'FB 300 Araguari' cultivars, UNEMAT S10 populations and ungrafted UNEMAT S30 were the four controls used in the experiment.

The experiment was planted in February 2014, with spacing of 3.0 m between plants and 3.5 m between planting rows to allow the use of machinery within the experiment. Plants were conducted in vertical support structure with 2.5 m fence posts, with spacing of 6.0 m and flat wire number 12, at height of 2.0 m from the soil. Liming and fertilization on plants and cover were carried out according to soil analysis, according to recommendations of Borges et al. (2006). The other cultural practices used, such as pruning, pest and disease control were those recommended for passion fruit culture (BRUCKNER and PICANÇO, 2001). Irrigation was performed using the micro-sprinkler system.

The following traits were evaluated (KRAUSE et al., 2012): Productivity (Prod) in kg ha^{-1}, as evidenced by the sum of total fruit harvests carried out during the experiment; number of fruits (NF) per plant, number of fruits collected per plot throughout the experiment and divided by the number of plants per plot. Prod and NF traits were measured based on the first year of cultivation (from 02/2014 to 01/2015), second year of cultivation (from 02/2015 to 10/2015) and the complete cycle (from 02/2014 to 10/2015) of the sour passion fruit culture.

The other traits were measured based on the analysis of 20 fruits per plot in January and February 2015: fruit mass (MF) in g, calculated by the weight of 20 fruits per plot divided by the total number of fruits harvested; average length (CF) of fruits in mm, obtained using the arithmetic mean of the longitudinal dimensions of fruits; average diameter (DF) of fruits in mm, obtained with the arithmetic mean of the transversal dimensions of fruits; fruit shape obtained by dividing CF by DF; average shell thickness in mm (EC), determined by the arithmetic mean of measures of four external points of the peel in the middle portion of fruits (cross cut towards the largest diameter).

Pulp percentage (PP) was obtained by weighing the pulp (seeds with arils), dividing this value by the total weight of fruits and multiplying the result by 100; total soluble solids (TSS) was obtained by refractometry, using portable digital refractometer, with reading ranging from 0 to 32º Brix. Readings were performed with aliquots of juice obtained from fruit pulp, using at least two fruits; pulp color (CP) was obtained by visual assessment of the fruit pulp color, using scale ranging from one to six for color rating, so that 1 (whitish yellow), 2 (light yellow), 3 (yellow), 4 (gold), 5 (light orange), 6 (orange) (LINHALES, 2007).

Total titratable acidity (TTA) was determined according to methodology recommended by AOAC - Association of Official Agriculture Chemists (1990) and modified by Araujo (2001), by diluting 5 ml of juice composed of at least two fruits, in distilled water at a ratio of 5: 1, using 5 drops of phenolphthalein g L^{-1} as indicator. Then, with the help of Digital Burette (Digitrate Pro 50 mL – Jencons), sample were titrated with NaOH 0.1 mol L^{-1}, under stirring. Results were expressed in grams of citric acid per 100 ml of juice, after applying the following formula:

$$G = \frac{V \,.\, f \,.\, N \,.\, PE}{P}$$

Where L = equivalent of citric acid per 100 ml of juice, V (L) = volume of NaOH 0.1 mol L 1 used in titration, f = correction factor due to the standardization of 0.96, N = normality of NaOH (L^{-1} eq) is 0.1, EW = equivalent weight of citric acid (g q^{-1}) 64 and P = volume of 5 mL of juice. The hydrogenionic potential (pH) measured through digital pH meter (glass electrode potentiometer) (MA-PA200). The total soluble solids/ total titratable acidity (SST/ATT) ratio was conducted using the simple division of SST value obtained by the ATT value obtained.

Analysis of variance was carried out for all assessed traits. Means were compared by the Tukey test at 5% probability. Statistical analyses were performed using the R software system (R DEVELOPMENT CORE TEAM, 2011).

In addition to productivity, physical and chemical traits of fruits, 17 morphological traits were assessed, in compliance with instructions of the national bureau of cultivar protection of the Ministry of Agriculture, Livestock and Supply.

Ten seedlings of each graft x rootstock combination described above were used for histological analysis by the cleft graft propagation method. Sixty days after grafting, samples of the connection region between graft and rootstock were collected for histological observation of the wound healing and vascular recovery of grafted plants. The median stem regions of ungrafted *P. Edulis, P. alata and P. nitida* seedlings were assessed for comparison with grafted seedlings.

Segments with 3 cm in length from the graft location were removed, whose size corresponds to that of the cut made for grafting. The plant material collected was fixed in FAA (formaldehyde: acetic acid: 50% ethanol, 5: 5: 90) for 24 hours and stored at 70% ethanol (JOHANSEN, 1940), for further anatomical analysis. Segments were positioned so that the longitudinal and cross cuts were parallel to the grafting direction, which allowed full view of the welding process. Sections were obtained freehand, with the help of a razor, stained with astra blue and basic fuchsin (ROESER, 1962) and mounted on semi-permanent histological slides with glycerinated gelatin (KAISER, 1880).

Samples were analyzed by Leica ICC50 photo microscope coupled to a computer and analyzed using the LAZ EZ software system version 1.7.0. Boards were assembled to show anatomical general standards for the studied species, seeking to highlight the diagnostic traits, which will be used for comparative analysis.

Results and Discussions

There was no significant interaction between factors rootstock and canopy. However, factor rootstock significantly affected ($p \leq 0.01$) the number of traits of fruits and productivity in the first year of cultivation and in the complete productivity cycle ($p \leq 0.05$), as shown in Table 1. The results show no significant difference among canopies of populations UNEMAT S10 and UNEMAT S30 when compared to commercial cultivars 'FB 200 Yellow Master' and 'FB 300 Araguari' for variables number of fruits and productivity. Thus, it is important to point out that populations from the UNEMAT sour passion fruit genetic breeding program, UNEMAT S10 and UNEMAT S30, can be used as canopies, since their productive potential is the same as that of cultivars already launched in the market.

In the first year of cultivation, ungrafted plants showed higher number of fruits (24,356 un ha^{-1}) and higher productivity (5,010 kg ha^{-1}) than when *P. alata* (12,768 ha^{-1}, 2,213 kg ha^{-1}) and *P. nitida* (11,375 un ha^{-1}, 2,030 kg ha^{-1}) were used as rootstocks (Table 1). In the assessment of the complete crop cycle, ungrafted plants (11,262kg ha^{-1}) also showed productivity higher than those grafted on *P. alata* (7,597 kg ha^{-1}) and *P. nitida* (6,579 kg ha^{-1}) (Table 1). In the early development, which comprises planting, formation of the curtain and early production, rootstocks did not reduce the number of fruits or productivity. This is an important trait, since the higher the productivity in the first year of cultivation, the faster the producers will obtain the return on their investment for implementing the culture. Rootstocks did not affect these traits, if we consider only the second year of cultivation, when plants are already formed.

In the assessment of the complete crop cycle, no difference was found between rootstocks and ungrafted plants for trait number of fruits. Plants grafted on *P. alata* rootstock presented the same productivity as ungrafted plants, which demonstrates its potential for use as rootstock, since it is resistant to fungus *F. solani*. *P. nitida* rootstock showed lower productivity, due its poor productivity in the first year.

The effect of rootstock on canopies has been reported by Hartmann et al. (2011), who mention that rootstock is very important for plant formation, since it affects the canopy development and strength, production quantity and quality, precocity, advance or delay in fruit ripening, resistance to diseases and pests, and ability to adapt to unfavorable climate and soil conditions.

There was a significant effect ($p \leq 0.05$) for the physical and chemical traits of fruits only for the rootstock factor for variable fruit diameter (Table 2). Fruits with larger diameters were obtained from plants without grafting with 79.7 mm (Table 2). However, according to the Brazilian program for the improvement of horticultural trade patterns and packaging, classes were determined using a numerical scale (1-5), by measuring the fruit diameter. Rootstocks of *P. nitida* and *P. alata* and ungrafted plants were rated in class 4 (≥75 to <85 mm), which is considered an optimal rating standard. Thus, it was observed that rootstocks did not affect the physical and chemical traits of fruits, which demonstrates their potential for use.

Regarding the 17 morphological traits of assessed canopies, it was observed that the canopy traits of grafted plants remained the same as control (ungrafted plants). This shows that the rootstocks used did not affect the development of these traits in sour passion fruits.

Thus, the most vigorous rootstocks have greater capacity to perform the absorption and translocation of water and nutrients and present higher growth levels, stimulating substances, which favors the canopy development. Rootstocks play an important role in the adaptation to environmental factors, as they connect the soil and the canopy, mainly affecting the absorption of nutrients and adapting to the soil physical and chemical characteristics (HARTMANN and KESTER, 1990). In addition, vegetative studies through morphological traits should be carried out to assess plant growth and development (BASTOS et al., 2002). They can also add agronomic and economic values to the culture and provide guidance for breeding programs (VANDERPLANK, 2000).

Cavichioli et al. (2011a) evaluated cleft grafting with pliers in sour passion fruits on three rootstock (*P. edulis*, *P. alata* and *P. gibertii*), plus control without grafting (free foot). The authors verified that the three rootstock presented different compatibility levels. In addition, *P. edulis* plants grafted on rootstocks of the same species and free standing plants were more vigorous than plants grafted on different species.

The results of the anatomical assessment of ungrafted *P. edulis*, *P. alata* and *P. nitida* seedlings showed uniseriate epidermis, cortical parenchyma, phloematic fibers, phloem, xylem and similar cord (Fig. 1-c). *P. alata* and *P. nitida* showed similar uniseriate epidermis, cortical parenchyma, phloematic fibers, phloem, xylem and marrow (Fig. 1a-c). The structure of the cortical region is important for successful grafting, since the apex is inserted in this region (NAVARRO et al., 1975; RIBEIRO et al., 2008). According to Estrada-Luna et al. (2002), cortical parenchyma cells are involved in the callus and establishment of vascular connection between apex and rootstock. Drusen (Fig. 1a-c) were also observed in the three species studied. These ergastic substances are the most common type of calcium oxalate crystal in dicotyledons (NAVARRO, 1975) and are related to different functions, such as the mechanical defense against herbivorous o que??, providing structural support, calcium reserves and maintenance of ionic balance (RIBEIRO, 2006).

The analysis of the cross and longitudinal cuts of *P. edulis* grafted on *P. edulis* showed that the parts had been welded and completely filled with parenchymal tissue. In the cortical parenchyma of *P. edulis* graft, cells differed and joined the cortical parenchyma region of the *P. edulis* rootstock. It was evident that these tissues were regenerated in regions of vascular tissues (xylem), and the connection between graft tissues and rootstock tissues had been established, which allowed the restoration of the sap flow (Fig. 1e-d). It was also observed that cells of the medullary region of the *P. edulis* graft differed, and the region was connected to the vascular tissues of the *P. edulis* rootstock (Fig. 1d). Structures considered as healing or callus (ESAU, 1974) were observed in the contact regions between graft and rootstock. Anatomically, grafting comprises three main processes: adhesion between graft and rootstock, proliferation of callus cells in the graft / rootstock interface and vascular differentiation in the graft interface (MOORE, 1984). These events were observed and confirmed that the process had been successfully completed. The cortical, medullary, and vascular regions between canopy and rootstock showed intense differentiation activity, which demonstrates excellent union among species, with the formation of healing regions (callus) and absence of rejection tissue. These results were expected, since grafting was conducted using the same species.

Through cross and longitudinal section cuts of *P. edulis* grafted on *P. nitida*, it was possible to see the connection between tissues. The area of the cortical parenchyma of *P. edulis* graft and that of *P. nitida* rootstock presented totipotent cells, which have the ability to differentiate. Thus, one region was connected to the other. It was observed that the medullary region of *P. edulis* went through a process similar to that observed in the cortical parenchyma and joined the medullary region of *P. nitida*. The healing processes were easily observed, with the formation of callus (cell differentiation), in areas where tissues are connected (Fig. 1h). The longitudinal section cuts of *P. edulis* on *P. nitida* show that, at the top end of the rootstock slot, there is plenty of meristem tissue under differentiation, cortex tissues and vascular tissues. It is important to highlight that vascular tissues of the graft connect to rootstock tissues in a disorganized way. Tissues were not exactly juxtaposed, which could delay the canopy development, but it did not prevent the formation of the grafting connection. The reordering of the vascular tissues direction was verified, so that they touched each other and connected (Fig. 1i). This may affect the normal plant development, since the delayed recovery of vascular tissues may affect other factors such as the translocation of water and nutrients from the rootstock to the graft and from the canopy to the rootstock (DIAS et al., 2009).

In the cross and longitudinal sections in the area of *P. edulis* on *P. alata* graft, it was possible to identify regions where tissues are connected, with cells under cell differentiation. It was observed that both *P. edulis* and *P. alata* vascular tissue cells were connected and with abundant number of cells under modification (Fig. 1f-g). In the welding process between *P. edulis* and *P. alata* (Fig. 1f), a large amount of parenchyma cells from the graft and rootstock, which were divided and filled the space between them was observed, giving rise to the so-called callus, parenchymal tissue resulting from the proliferation of various parenchymal cells close to the graft surface, which is also responsible for restoring the cambial continuity, when the vascular cambium is cut due to injury (ESAU, 1974). Thus, it is evident that the grafting of *P. edulis* on *P. alata* shows compatible tissue formation, since the sequences of structural events occurring during graft fixation are generally similar to those described by Hartmann et al. (2011).

Table 1. Average number of fruits and productivity in the first and second years of cultivation and in the complete cycle in two cultivars and two sour passion fruit populations grafted on *P. nitida*, *P. alata* and control (ungrafted cultivars and populations), Tangará da Serra-MT, 2015.

Rootstock	1st year	2nd year	Complete cycle
	Number of fruits (um ha^{-1})		
P. nítida	11.375b	26.975a	38.350a
P. alata	12.768b	31.816a	44.585a
Ungrafted	24.356a	34.717a	59.074a
	Productivity (kg ha^{-1})		
P. nítida	2.030b	4.549a	6.579b
P. alata	2.213b	5.384a	7.597ab
Ungrafted	5.010a	6.251a	11.262a

Averages followed by the same letters do not differ by the Tukey test at 5% probability.

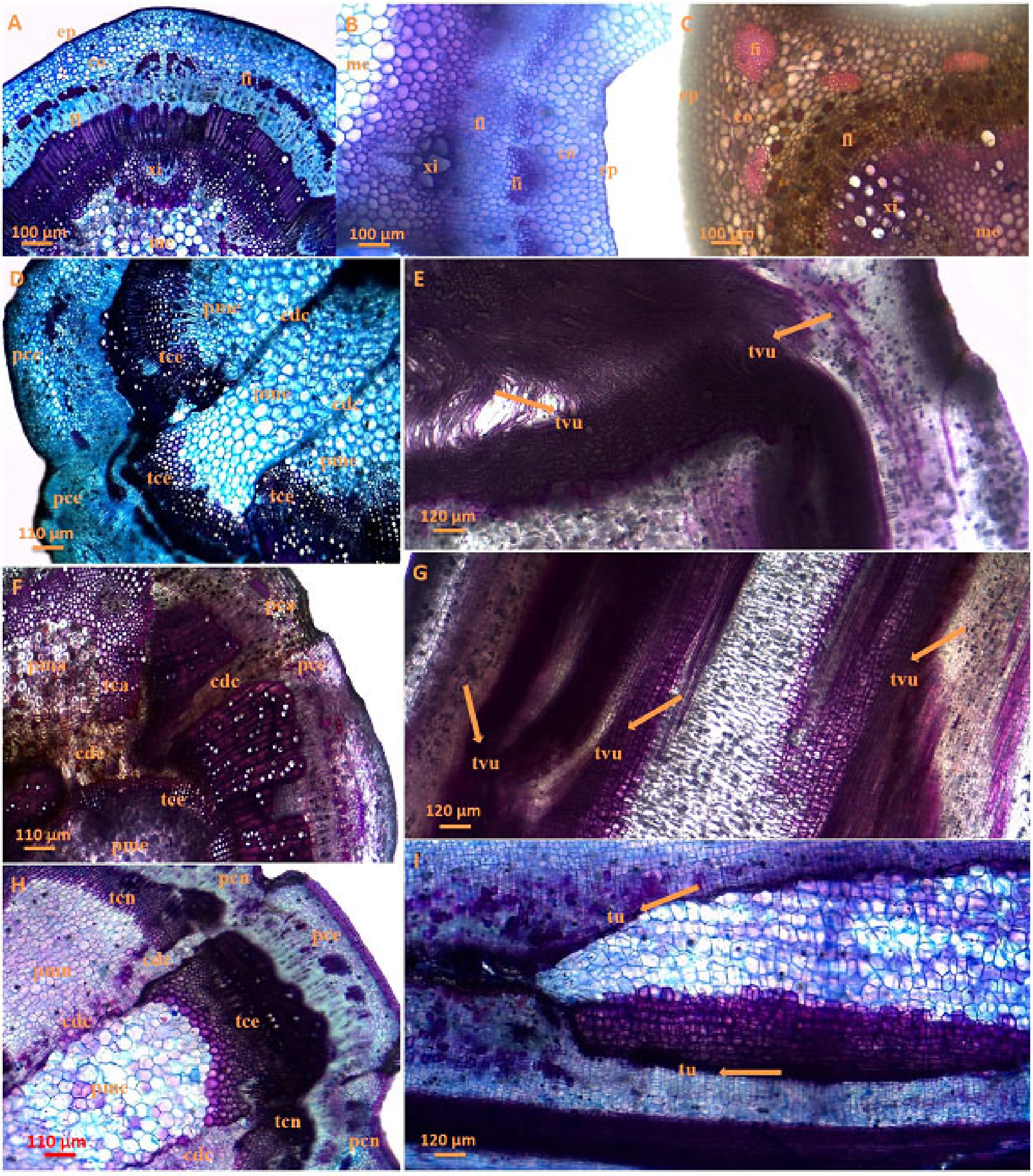

Figure 1. Cross and longitudinal sections of the grafting area. a. Cross section of *Passiflora edulis* stem region; b. Cross section of *Passiflora nitida* stem region; c. Cross section of *Passiflora alata* stem region; d, e. General view, showing connected regions of *P. edulis*/*P. edulis*. f, g General view showing joint regions of *P. edulis*/*P. alata*. h, i General view showing united regions of *P. edulis/P. nitida*. (Ep: epidermis; Co: cortex; fi: phloematic fibers; fl: phloem; xi: xylem; me: marrow; pce: cortical parenchyma; tce: conductive tissue; pme: marrow parenchyma; cdc: cells in cell differentiation (calluses); tvu: united vascular tissues; tu: united tissues).

Table 2. Average fruit mass (MF), fruit length (CF), fruit diameter (DF), shell thickness (EC), pulp percentage (PP), fruit shape (FF), pulp color (CP), total soluble solids (TSS), hydrogenionic potential (PH), total titratable acidity (TTA), SST / ATT ratio in two cultivars and two sour passion fruit populations grafted on *P. nitida*, *P. alata* and control (cultivars and ungrafted populations), Tangará da Serra – MT, 2015.

Traits	Rootstock		
	P. nítida	*P. alata*	Testemunha
MF (mm)	188.9a	189.6a	209.9a
CF (mm)	87.1a	86.9a	89.1a
DF (mm)	76.2b	76.1b	79.7a
EC (mm)	8.1a	7.5a	8.0a
PP (%)	40.5a	40.4a	40.5a
FF	1.12a	1.14a	1.12a
CP	3.9a	3.9a	3.7a
TSS (°Brix)	12.99a	12.91a	12.96a
PH	3.2a	3.31a	3.45a
TTA (g 100 mL^{1})	3.14a	3.02a	3.17a
Ratio	4.21a	4.36a	4.10a

Averages followed by the same letters in the lines do not differ by the Tukey test, at 5% probability.

Conclusion

The results show that rootstocks affected the early cultivation and reduced productivity and the number of fruits in sour passion fruit populations. In general, grafting did not affect the physical and chemical traits of fruits or the morphology of canopies. Considering the complete crop cycle, *P. alata* species has conditions to be used as rootstock for the grafting of sour passion fruit. The results of the histological analysis revealed compatibility in the grafting of *P. edulis* on *P. alata* rootstock, since better results were obtained for the process that connects graft and rootstock, compared to grafting on *P. nitida*.

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