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Grafting height does not affect *Fusarium* wilt control or horticultural performance of *Passiflora gibertii* N.E.Br. rootstock

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ABSTRACT

The influence of the grafting height (5, 10, 20 and 30 cm above the root collar) of *P. edulis* on *P. gibertii* was evaluated on the incidence of *Fusarium* wilt and horticultural performance. Plants of *P. gibertii* grafted on *P. edulis* and non-grafted plants of both species were also studied. In addition, histopathological studies were also performed on the roots of non-grafted *P. edulis* collected at three severity stages of *Fusarium* wilt. In greenhouse, the graft take was inversely related to the grafting height in general. In the field conditions, the plant growth of *P. gibertii* grafted on *P. edulis* was superior to its reciprocal grafting, even though the former combination was susceptible to *Fop*. Plants of *P. edulis* grafted on *P. gibertii* at all grafting heights did not present symptoms of *Fop,* and the number of fruit yield and quality were equivalent, but plant growth was decreased in relation to the non-grafted plants. Starch depletion in the root system of *P. edulis* was directly related to the severity of the *Fusarium* wilt. *P. gibertii* was confirmed as a *Fusarium* wilt resistant rootstock of *P. edulis*, with minimal influence of the grafting height for the control of the disease.

Key words: *Passiflora edulis*, *Fusarium oxysporum* f. sp. *passiflorae*, anatomy, propagation, resistance.

INTRODUCTION

Soil-borne diseases are the main causes of economic losses in the yellow passion fruit (*Passiflora edulis* Sim) cultivation (Ortiz and Hoyos-Carvajal 2016, Morgado et al. 2017). *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. *passiflorae* (*Fop*), is considered the most important because it attacks the vascular system causing sudden wilting and early death of the plant (McGovern 2015). *Fop* has been decreasing the productivity and lifespan of *P. edulis* crop worldwide (Fischer and Rezende 2008).

The grafting onto resistant rootstocks is used for the control of soil-borne diseases in several crops (Prunier et al. 1999, Pavlou et al. 2002, Sayler et al. 2002) either anticipates the production (Castle 1995, Karlidag et al. 2016). The grafting of the yellow passion fruit on several resistant wild species was studied as an alternative for the production in

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areas with a history of *Fop* (Cavichioli et al. 2011a, Lima et al. 2017). *Passiflora gibertii* N.E. Br. was highlighted with additional desirable traits, such as rapid plant growth, high seed germination and good anatomical compatibility for grafting with *P. edulis* (Cavichioli et al. 2011a, Yockteng et al. 2011, Lima et al. 2017).

Studies with passion fruit, in general, do not make inferences of the effect of grafting height on the horticultural performance and incidence of *Fop,* even though this influence is reported for other grafted crops and diseases (Prunier et al. 1999, Sayler et al. 2002, Chalise et al. 2013, Yazdani et al. 2016, Karlidag et al. 2016). Nakasone and Paull (1998) report that higher grafting height of the purple passion fruit on the yellow one is associated to a decreased incidence of *Fusarium* wilt. Cleft grafting of *P. edulis* on *P. gibertii* at 6 to 8 cm above the soil level resulted in high plant survival to *Fusarium* wilt in São Paulo State, Brazil (Cavichioli et al. 2011a). However, infected plants of this combination were observed in Bahia State, Brazil, which was attributed to the low height in hypocotyledonar grafting (Santos et al. 2016).

In this work, we evaluated the influence of the grafting height of *P. edulis* on *P. gibertii* on the incidence of *Fusarium* wilt and horticultural performance. In addition, the grafting of *P. gibertii* on *P. edulis* and histopathological aspects of the latter's root system were investigated to understand this pathogen-host relationship.

MATERIALS AND METHODS

LOCATION OF EXPERIMENT AND PLANT MATERIAL

Experimental works were carried out in the municipality of Cruz das Almas, Bahia State, Brazil (12° 39' 25'' S, 39° 07' 27'' W, 222 m. a. s. l.). Plantlets were grown in greenhouse from January to April 2016, at 28±2 °C and relative humidity of 60%. Later, the plantlets were transplanted into a field with a high incidence of *Fusarium* wilt (Santos et al. 2016) and cultivated from April 2016 to August 2017. During the field evaluation period, the average air temperature was 25.4 °C, with rainfall of 1047 mm and relative humidity of 77%.

The scion variety was BRS Rubi do Cerrado, a commercial yellow passion fruit hybrid (*P. edulis*), and the wild species *P. gibertii* (BGP008 accession) was used as the rootstock. Non-grafted seedlings of both species were controls. Reciprocal grafting was also performed to evaluate if the *P. gibertii* scion would induce some resistance to *Fop* in the rootstock of *P. edulis* (Figure 1).

EVALUATION IN GREENHOUSE

The experimental design in the greenhouse was completely randomized blocks in a factorial scheme (2 x 4 + 2), with two scion-rootstock combinations (*P. edulis*/*P. gibertii* and *P. gibertii*/*P. edulis*) and four grafting heights (5, 10, 20 and 30 cm above the root collar, Figure 1), plus two additional treatments (non-grafted seedlings of each species), distributed in four replications of 10 plants per plot.

The rootstock seedlings grafted at 20 and 30 cm height were sown 30 days before those grafted at 5 and 10 cm height. This procedure was performed so that the grafts were performed at the same time at all grafting heights studied. The seeds were planted in trays using vermiculite potting mix. Fifteen days after emergence, the rootstock seedlings were transplanted into polyethylene bags (22 x 12 cm) containing a mixture of humus, vermiculite, coconut fiber and commercial potting mix Vivato® (2:1:1:1, v:v).

At 45 days after transplanting, the cleft grafting method was performed and the rootstocks were cut with scissors at heights of 5, 10, 20 and 30 cm. At the cutting site, a longitudinal cut of 1.0 cm was opened with a scalpel blade. In the open cut, the scion with about 4.0 cm long, obtained from the meristematic apex of seedlings from passion fruit seeds 'BRS Rubi do Cerrado', was inserted. The base of the scion was cut into wedge-shaped double bevel and its leaves were cut in 1/3 of its area. Subsequently, the graft was inserted in the rootstock carefully, in order to coincide with the cambium tissues; the grafting region was protected by clip grafted (Figure 1).

The variables studied were graft length (GL, in cm) measured from the graft region to the tip of the plant, diameter of the scion stem (DC, in mm) measured with a pachymeter at 2 cm above the grafting region, and graft take (%) at 60 days after grafting (DAG).

EVALUATION IN FIELD CONDITIONS

At 60 DAG, all the grafted plantlets and seedlings were transplanted into the field to evaluate the incidence of *Fusarium* wilt and the horticultural performance. The experimental design was the same of the greenhouse, except that six plants were used in the plot. The soil of the experimental area is classified as allic distrocohesive yellow latosol, with the following chemical characteristics (0-20 cm): pH (water) 6.5, phosphorus (P) 60.85 mg dm^{-3}, potassium (K) 0.20, calcium (Ca) 2.26, magnesium (Mg) 0.99, aluminum (Al) 0.0, sodium (Na) 0.09, hydrogen + aluminum (H + Al) 1.25, base saturation (SB) 3.24, cation exchange capacity (CTC) 4.79 $cmol_c$ dm^{-3}, base saturation percentage (V%) 73.65%, and organic matter (OM) 13.2 g kg^{-1}.

The plants were trained by single-wire trellises with 2.0 m height and spacing between plants was 1.8 m x 2.0 m. Drip irrigation was carried out three times a week with a duration of 30 min each, with an average water volume of 10 L per plant per irrigation. The plants were fertilized every 20 days for the first six months with 30 g of ammonium sulphate per plant (afterward increased to 80 g)

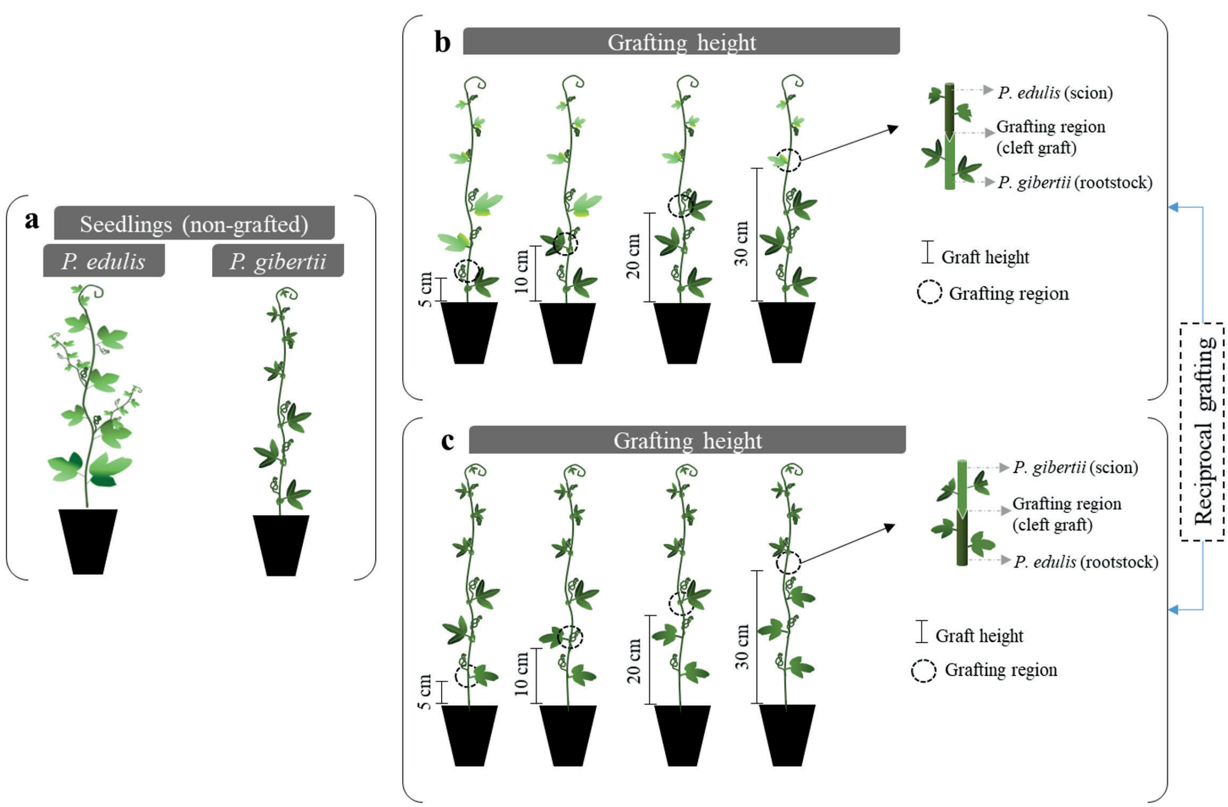

Figure 1 - (**a**) Non-grafted *P. edulis* and *P. gibertii* plants used as controls (**b**) grafting of *P. edulis/P. gibertii* at four grafting heights and (**c**) their reciprocal grafting of *P. gibertii/P. edulis*.

plus 30 g of potassium chloride and 20 g of simple superphosphate. The other cultural practices were carried out following the recommendations for cultivation of passion fruit of Lima et al. (2011).

The plant growth index (GRI) was estimated up to 220 days after transplanting as established by Jesus et al. (2016). The number of fruits per plant and crop yield (kg ha^{-1}) were also measured in the first production cycle. To evaluate fruit quality, five ripe fruits were randomly collected per plot at peak plant production during the months of November and December 2016. Fruit quality was studied only for the plants of the *P. edulis*/*P. gibertii* combination and the non-grafted seedlings of *P. edulis.* The physical and chemical characteristics measured were: fruit mass (g), length and diameter (cm), skin thickness (mm) and mass (g), pulp mass (g), soluble solids content (SS, in °Brix), titratable acidity (TA) expressed in mg citric acid per 100 mL juice, as determined through titration with NaOH at 0.1 mol L^{-1}, and the maturity index (SS/TA) (Jesus et al. 2017).

HISTOPATHOLOGICAL ANALYSIS OF ROOTS

To support the understanding of the interaction *P. edulis* x *Fop*, histopathological analysis was performed on non-grafted seedlings of BRS Rubi do Cerrado (n = 3) at different *Fusarium* wilt severity stages: (i) visually healthy plants or controls (Figures 2a, c), (ii) plants with slight leaf wilt (onset of symptoms) (Figures 2b, d, stage 1), (iii) generalized presence of wilted leaves adhered to the plant (Figures 2e, g, stage 2), and (iv) necrosis of the roots with most of the leaves already fallen (Figures 2f, h, stage 3). Secondary root fragments (n = 6, with a mean diameter of 1.0 mm and 2-4 cm length) were collected throughout the evaluation period from plants at all wilt stages, and were analyzed to confirm the presence of the pathogen as the causal agent (Figures 2d, g, h).

Root samples were fixed, embedded, sliced in sections and visualized according to the method described by Lima et al. (2017). Three slides containing 10 sections were prepared for each treatment, totaling 30 units. To determine the presence of starch, sections containing 5 µm thick root segments were stained with Lugol's iodine for 5 min for observation of cell structure with dark blue or brown color (Berlyn and Miksche 1976).

For fluorescence microscopy, the root segments were stained with Lugol's iodine for 3 min, then washed with tap water and stained with 1% aniline for 8 min and finally in Lugol again for 30 s and assembled with tap water (Kraus and Arduin 1997). The blue aniline dye produces a blue color in callose tissue and Lugol acts on the cell walls, giving gray and yellowish coloration to lignified ones. To verify the presence of callose in the *Fop*-infected yellow passion fruit root system, a fluorescence microscope with ultraviolet filter was used (Axioskop2, Carl Zeiss, Jena, Germany). The slides were also analyzed under a light microscope.

Hyphae of the pathogen were visualized using fresh root fragments 2-4 cm long to evaluate the colonization of host tissues by *Fop*, using the clearing and staining technique (Phillips and Haymann 1970).

STATISTICAL ANALYSES

Data were submitted to analysis of variance and the means were compared by the Tukey test at 5% probability. When necessary, data were transformed by to satisfy the premises of normal distribution. Survival analysis was performed using the nonparametric Kaplan-Meier (KM) curve (Kaplan and Maier 1958). In this study, the event of interest was the death of the plant caused by the *Fusarium* wilt, which was recorded every two days based on the typical visual symptoms (Figure 2). The differences between survival curves in the different combinations of scion-rootstock and grafting heights were tested using the

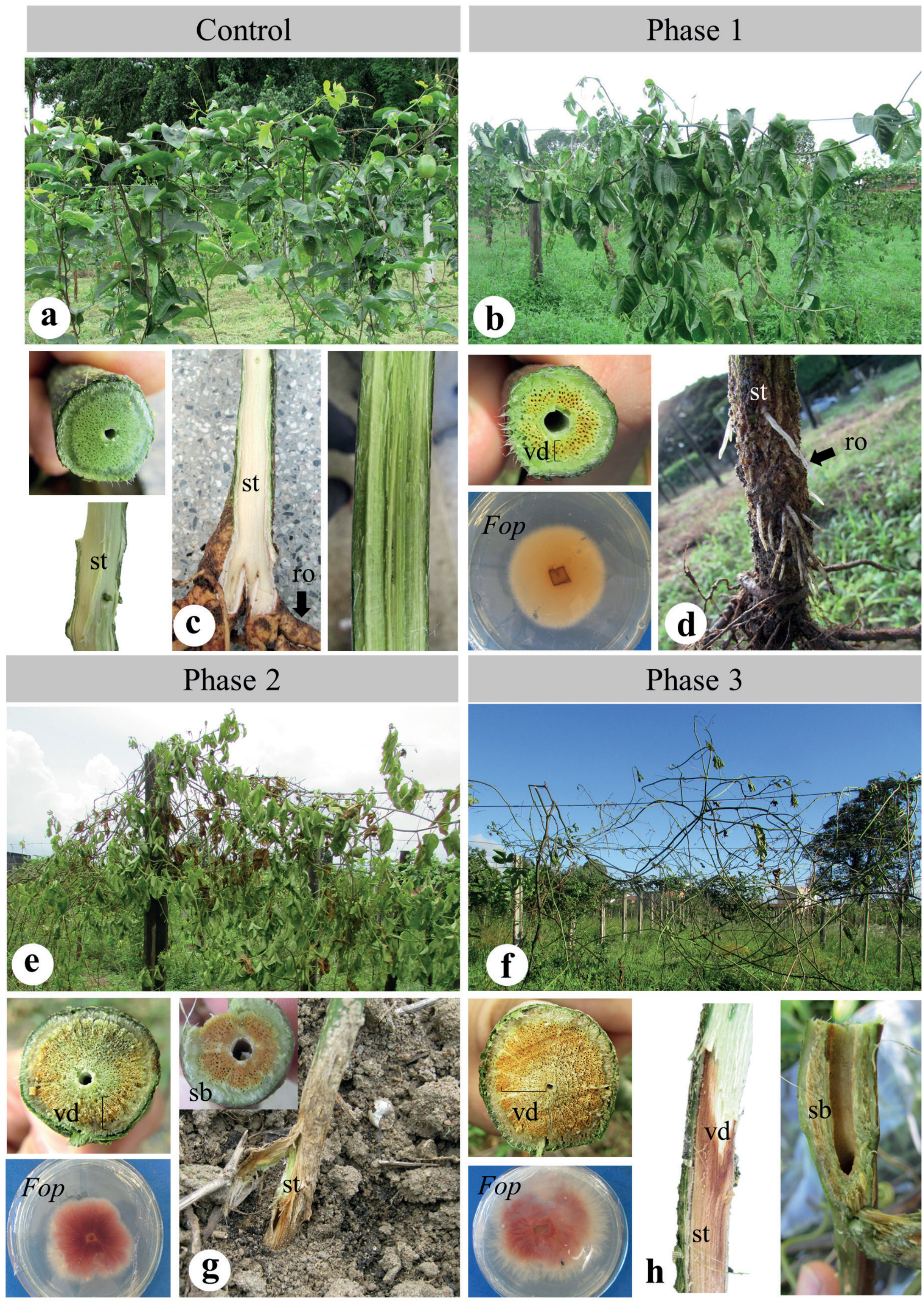

Figure 2 - Morphological aspects of non-grafted plants of yellow passion fruit (*Passiflora edulis* Sims.) at different stages of *Fusarium* wilt. **a-c**: healthy plant. **b-d**: first stage of wilt. **e- g**: second stage of wilt. **f-h**: third stage of wilt. st: stem, vd: vascular discoloration, sb: secondary branch, ro: root, *Fop*: *Fusarium oxysporum* f. sp. *passiflorae*.

nonparametric log-rank, Peto and Peto's Wilcoxon, and Cox tests ($p \leq 0.05$). In addition to the survival analysis, the average life expectancy at 122 days (coinciding with the initial reproductive stage of the plants) was estimated using the lifetime table and the number of plants affected by *Fusarium* wilt using the boxplot statistical algorithm.

RESULTS

EVALUATION IN GREENHOUSE

Biometric variables and percentage of graft take presented significant differences ($p \leq 0.05\%$) in relation to the scion-rootstock combinations and grafting heights (Figure 3). There was interaction between the scion-rootstock vs. grafting height factors for the variables graft length and stem diameter (Figures 3a, d).

P. gibertii/P. edulis presented higher graft length than the *P. edulis/P. gibertii* combination at all grafting heights except for 30 cm (Figure 3a). Plants with grafts at 5 cm showed the least growth, especially when *P. edulis* was grafted on *P. gibertii* (Figure 3a). In relation to non-grafted plants, *P. gibertii* presented greater plant height with 76.0 cm, superior to *P. edulis* with 41.0 cm (Figure 3b). Mean plant height of non-grafted plants (58.4 cm), was higher than average graft length of all scion-rootstock combinations with 45.8 cm (Figure 3c).

For the stem diameter, there were different responses between the combinations and grafting heights (Figure 3d). *P. gibertii/P. edulis* presented the largest stem diameters at all grafting heights except 5 cm, at which the reciprocal combination (*P. edulis/P. gibertii*) had wider stem diameter (Figure 3d). The stem diameter of non-grafted *P. edulis* was 3.66 mm, 27% thicker than *P. gibertii* with 2.68 mm (Figure 3e). The grafted and non-grafted plants did not differ significantly for stem diameter (Figure 3f).

Regarding the graft take of *P. edulis/P. gibertii*, the grafting height at 10 cm provided the highest percentage (92%), although it did not differ significantly from plants grafted at 5 cm, with 87% (Figure 3g). In contrast, the lowest percentages were observed at 20 cm and 30 cm, both with 62% (Figure 3g). In general, the non-grafted plants showed higher plant survival (93%) than the grafted plants with 75% (Figure 3i). This behavior was not maintained among non-graft plants (Figure 3h).

EVALUATION IN FIELD CONDITIONS

In field conditions there was no effect of grafting height on the plant growth index (GRI 7.57) (Figure 4a). When the *P. gibertii* scion was grafted on the *P. edulis* rootstock, a higher GRI 6.72 ($p = 0.01$) occurred, 20% higher than the inverse combination (Figure 4b). Regarding the non-grafted plants, opposite behavior was observed, with higher GRI for *P. edulis* (9.53) than *P. gibertii* (5.89) (Figure 4c). There were no differences between the grafted and non-grafted GRI plants of 7.57 and 7.71, respectively (Figure 4d).

The number of fruit per plant and the productivity were not influenced by the evaluated grafting heights, and there was no difference in these variables between the grafted plants and non-grafted *P. edulis* plants (Figures 4e-f).

The Peto and Peto's Wilcoxon, log-rank and Cox tests indicated that the survival curves generated by the Kaplan-Meier nonparametric method differed significantly ($p < 0.0001$) between the non-grafted *P. edulis* and *P. gibertii* seedlings and the combinations *P. gibertii/P. edulis* and *P. edulis/P. gibertii* (Figure 5a).

The use of *P. gibertii/P. edulis* grafting affected the susceptibility to *Fusarium* wilt of *P. gibertii,* which is resistant to the disease. The manifestation of symptoms by *P. gibertii/P. edulis* plants started at 150 days after planting (DAP), and, at the end of the study, only 32% of plants were still alive (Figure 5a).

The non-grafted seedlings of *P. edulis* were more susceptible to *Fusarium* wilt, with the onset

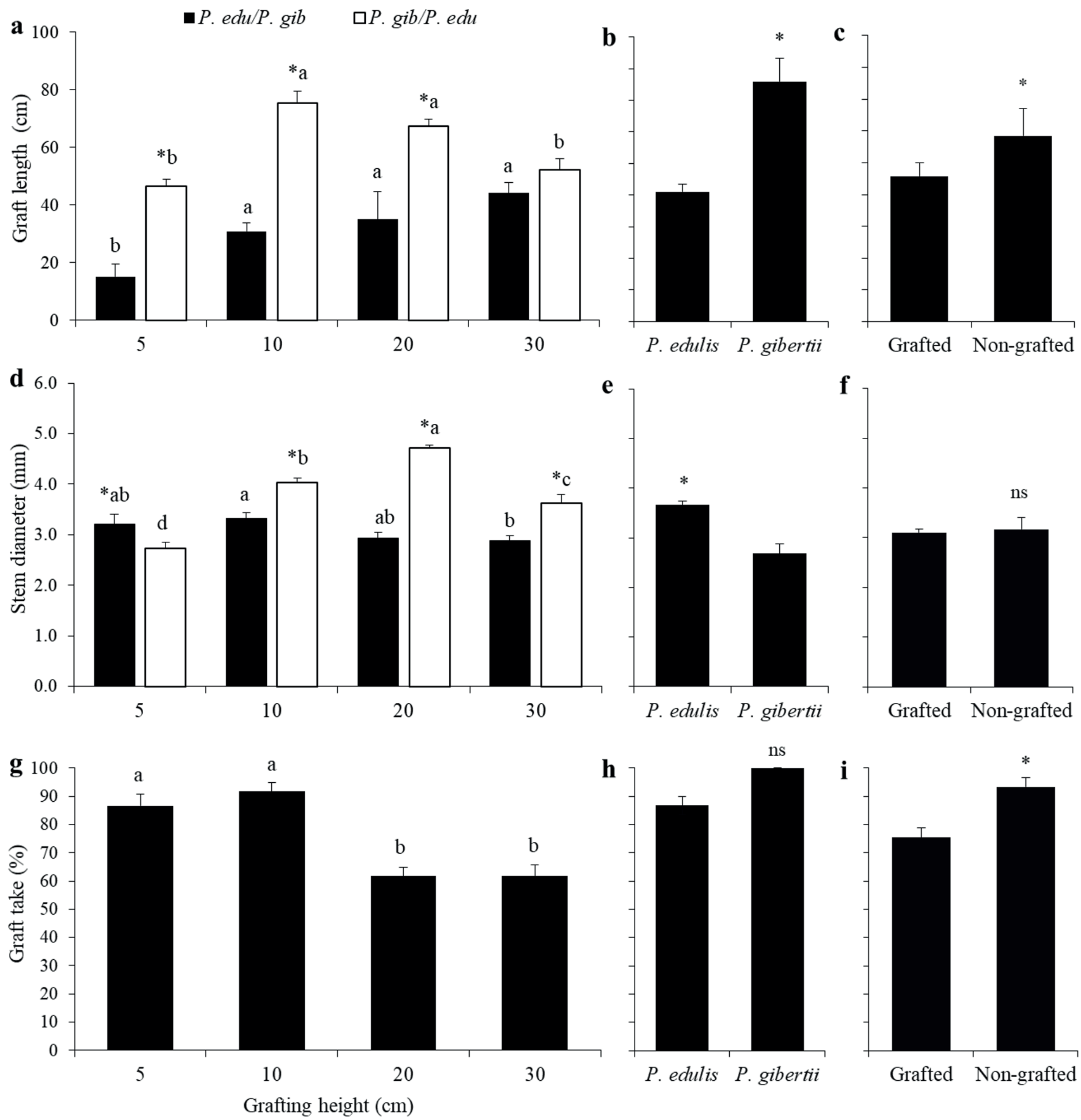

Figure 3 - Biometric variables of *Passiflora edulis* grafted on *P. gibertii* their reciprocal grafting (*P. gibertii/P. edulis*) non-grafted *P. edulis* and *P. gibertii* plants and the grafted plants and non-grafted plants. (**a-c**): graft length. (**a**) scion-rootstock combination x grafting height. (**b**) comparison between non-grafted plants and (**c**) comparison between grafted and non-grafted plants. (**d-f**): stem diameter. (**d**) scion-rootstock combination x grafting height. (**e**) comparison between non-grafted plants and (**f**) comparison between grafted and non-grafted in general. (**g**) graft take as a function of grafting height of *P. edulis/P. gibertii.* (**h**) plant survival (%) of the non-grafted plants and (**i**) survival between grafted and non-grafted plants. Error bar = standard error of the mean different letters indicate significant differences by the Tukey test ($p \leq 005$). * Indicates significant difference by the F-test ($p \leq 005$).

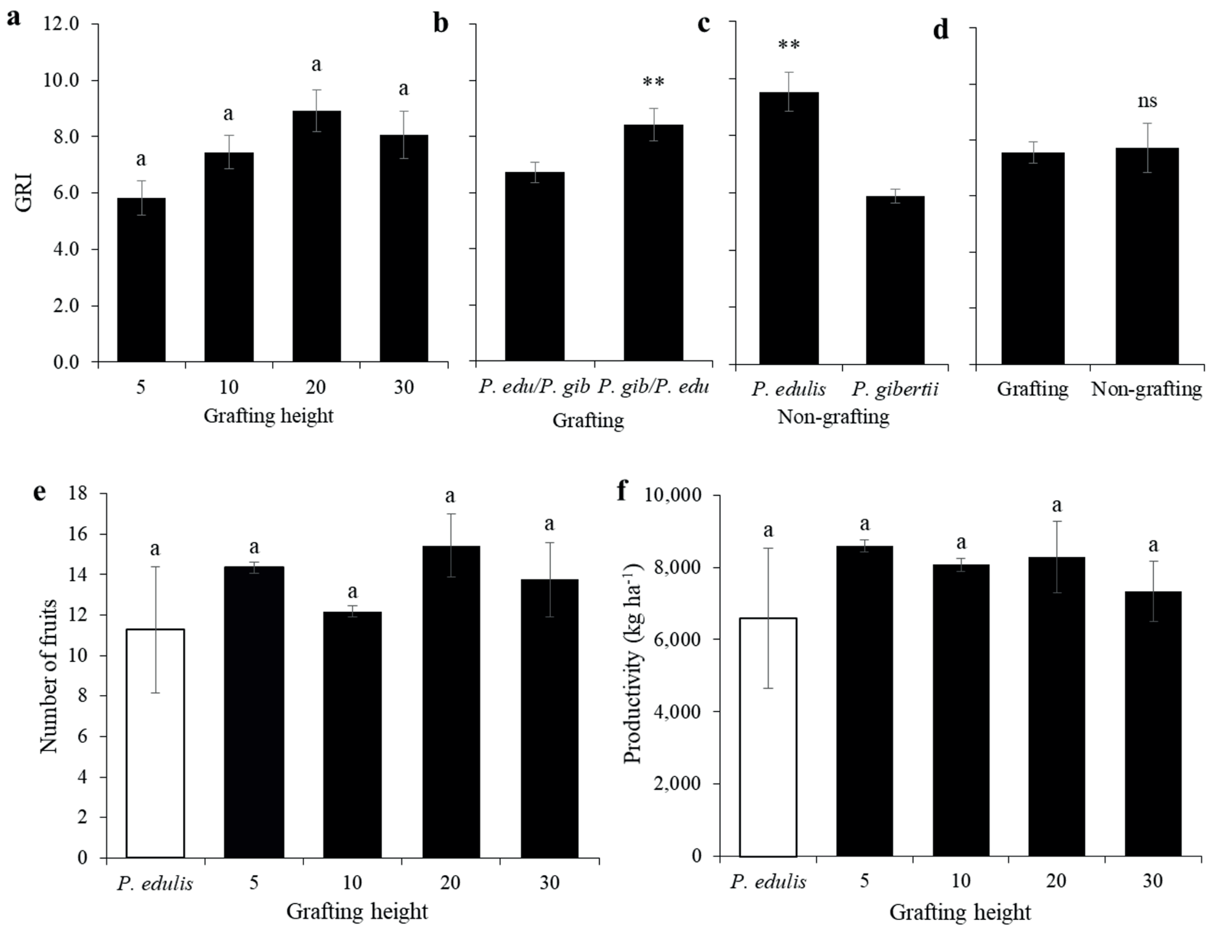

Figure 4 - Growth rate index (GRI) number of fruits per plant and productivity. (**a**) GRI at different grafting heights. (**b**) comparison of *P. edulis* grafted on *P. gibertii* and their reciprocal grafting. (**c**) comparison between non-grafted *P. edulis* and *P. gibertii* plants. (**d**) comparison between grafted and non-grafted plants in general under field conditions. (**e**) Number of fruits and (**f**) Productivity at different grafting heights. Error bar = standard error of the mean different letters indicate difference of the means by the Tukey test ($p \leq 005$). **Indicates significant difference by the F-test ($p \leq 001$).

of symptoms at 450 DAP and only 20% of survival at the end of the study (Figure 5a). Based on the box plot analysis, the manifestation of *Fusarium* wilt symptoms occurred earlier in non-grafted *P. edulis*, with the first quartile between 110 and 200 DAP, while for the combination of *P. gibertii/P. edulis* the first quartile was between 175 and 248 DAP (Figure 5b). The average life expectancy also followed the same trend, with 168 and 245 days for *P. edulis* and *P. gibertii/P. edulis*, respectively (Figure 5c). *P. edulis* plants grafted on *P. gibertii* and *P. gibertii* seedlings did not present typical symptoms of *Fusarium* wilt (Figure 5a), and had mean life expectancy of 327 days (Figure 5c).

Survival to *Fop* of the *P. gibertii/P. edulis* combination was significantly lower independent of the grafting height used, and no variation was observed between the survival curves by the three tests (Peto and Peto's Wilcoxon, p = 0.31964, Cox, p = 0.45898, and log-rank, p = 0.16047) (Figure 5d). Lower dispersions attributed to *Fop* were obtained at 5 and 20 cm grafting heights, with the first quartile between 220-260 and 180-210 days at these grafting heights, respectively, when compared to grafts at 10 and 30 cm (Figure 5e).

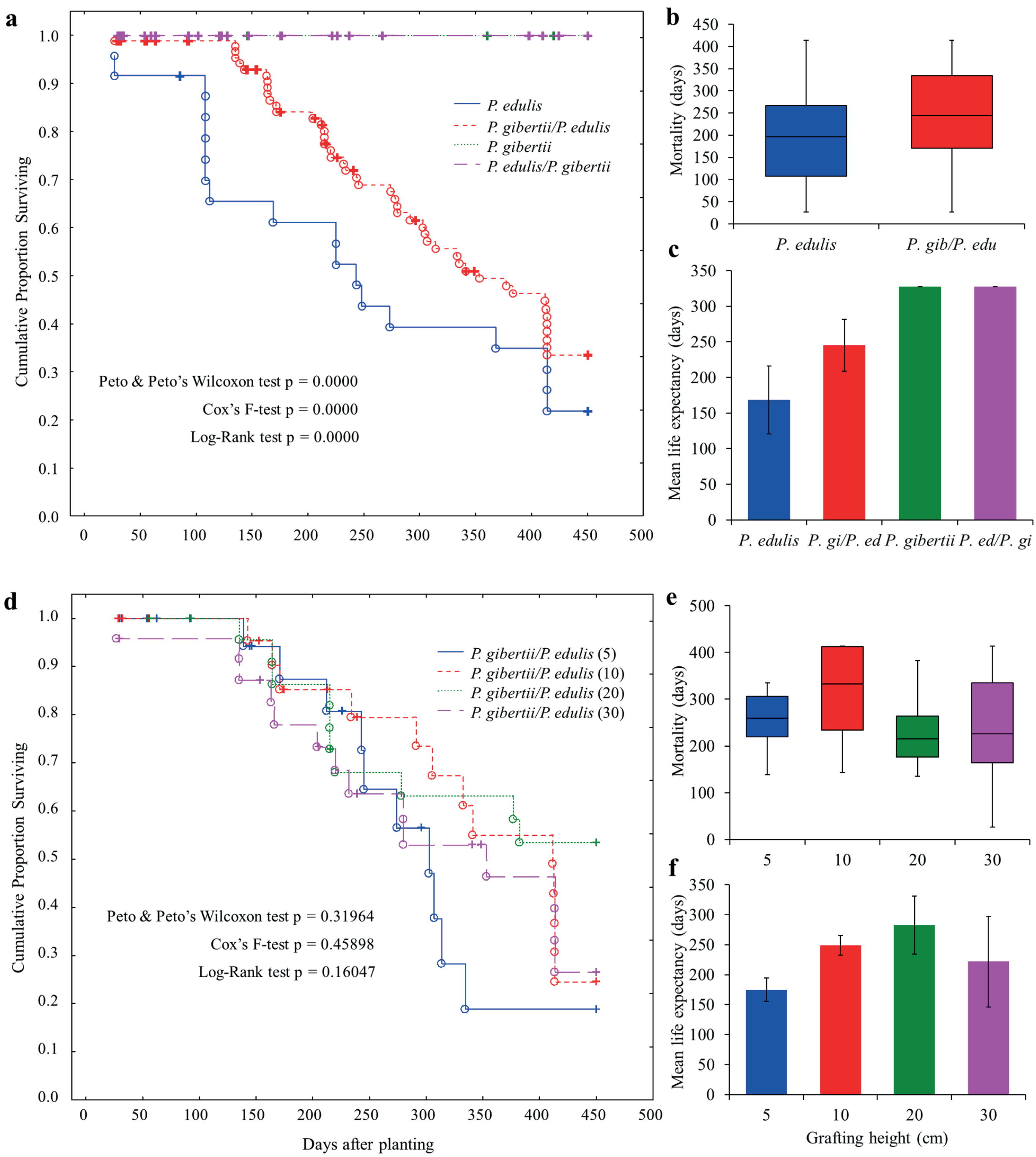

Figure 5 - (**a**) Cumulative survival rate of *P. edulis* (non-grafted) *P. gibertii P. gibertii/P. edulis* and *P. edulis/P. gibertii* to *Fusarium* wilt (*Fop*). (**b**) boxplot of *Fop* survival (days) of *P. edulis* (non-grafted) and *P. gibertii/P. edulis*. (**c**) average survival time to *Fop* of the non-grafted *P. edulis* and *P. gibertii* and combination *P. gibertii/P. edulis* and *P. edulis/P. gibertii*. (**d**) cumulative survival ratio to *Fop* of *P. gibertii/P. edulis* at grafting heights of 5 cm, 10 cm, 20 cm and 30 cm. (**e**) boxplot of *Fop* survival (days) of *P. gibertii/P. edulis* at different grafting heights. (**f**) mean survival time to *Fop* (days) of *P. gibertii/P. edulis* with grafting heights of 5 cm, 10 cm, 20 cm and 30 cm.

The mean survival time was lower (175 days) for the grafting performed at 5 cm (Figure 5f) and the intensification of mortality occurred from 250 days after planting (Figure 5d).

Based on the physicochemical characteristics of the fruits of *P. edulis* grafted on *P. gibertii*, there were no differences ($p \leq 0.05$) between the grafting heights for all the evaluated characteristics (Table I). The grafting height did not influence the average number of fruit per plant when compared to that of non-grafted *P. edulis* (Figure 4e).

HISTOPATHOLOGICAL ANALYSIS OF ROOTS

The fluorescence microscopy images of the *P. edulis* seedling (non-grafted) plants without visual symptoms of *Fusarium* wilt revealed the presence of fibers in the cortical parenchyma of the root with light staining and deposition of starch in the same region extending to the xylem vessels (Figures 6a, e). In the first wilting stage, there was a marked reduction in the presence of starch in comparison to the control, in addition to a smaller volume of fibers (Figures 6b, f). In the second wilt stage, complete absence of starch was observed in both the cortex and xylem (Figures 6c, g), and in the third wilt stage, this behavior was maintained, with further deterioration of vascular tissues (Figures 6d, h). No callus deposition was observed in the xylem vessels in any of the wilt stages, indicating that the species *P. edulis* does not have this metabolite compound, which constitutes a physical barrier that prevents the colonization of the pathogen (Figures 6f-h).

The presence of starch in the cortical parenchyma (Figures 6i, m) was identified in the transversal sections of the root of healthy plants stained with Lugol's iodine, while in the fungus-infected plants, low starch concentration was observed in the first wilt stage (Figures 6j, n) in comparison to the control, and complete absence of this polysaccharide in the other wilt stages (Figures 6k, l, o, p).

With the root clearing technique, it was possible to identify the presence of hyphae inside cells in secondary roots in the first wilt stage (Figure 6r) and the presence of chlamydospores in the last wilt stage (Figure 6t). No pathogen structures were

TABLE I
Physicochemical characteristics of fruits of *Passiflora edulis* plants grafted on *P. gibertii* at four grafting heights compared with non-grafted *P. edulis*.

Factor of variation	FM (g)	FL (mm)	FD (mm)	ST (mm)	SM (g)	PM (g)	SS (°Brix)	TA (%)	SS/TA
Block	1.66^{ns}	0.36^{ns}	2.96^{ns}	0.29^{ns}	2.40^{ns}	1.88^{ns}	4.21*	0.71^{ns}	0.88^{ns}
Grafting height	1.27^{ns}	1.66^{ns}	2.23^{ns}	2.01^{ns}	2.78^{ns}	0.38^{ns}	0.69^{ns}	1.09^{ns}	0.12^{ns}
Error	18.256	51.86	48.41	1.44	51.42	629.51	2.50	0.54	1.07
CV (%)	21.65	8.07	9.67	17.68	22.84	33.22	11.64	23.28	23.06
Combinations									
P. edulis non-grafted	189.33a	88.45a	70.06a	6.31a	90.48a	77.65a	13.55a	3.10a	4.49a
P. edu/P. gib (5)	194.15a	87.26a	72.43a	6.54a	95.56a	75.90a	13.88a	3.39a	4.43a
P. edu/P. gib (10)	214.79a	92.27a	74.88a	7.24a	113.37a	76.00a	13.33a	2.95a	4.61a
P. edu/P. gib (20)	174.00a	97.50a	74.70a	8.30a	112.63a	59.00a	15.50a	3.90a	3.97a
P. edu/P. gib (30)	172.66a	86.13a	63.90a	7.16a	91.59a	61.00a	13.16a	3.12a	4.26a

^{ns}not significant *significant at 5% by the F-test of analysis of variance Means followed by the same letter in the column do not differ by the Tukey test ($p \leq 005$). FM: fruit mass, FL: fruit length, FD: fruit diameter, ST: skin thickness, SM: skin mass, PM: pulp mass, SS: soluble solids (°Brix), TA: titratable acidity, Ratio: SS/TA. *P. edu/P. gib* (5) (10) (20) (30): *P. edulis* grafted on *P. gibertii* at heights of 5 cm 10 cm 20 cm and 30 cm, respectively.

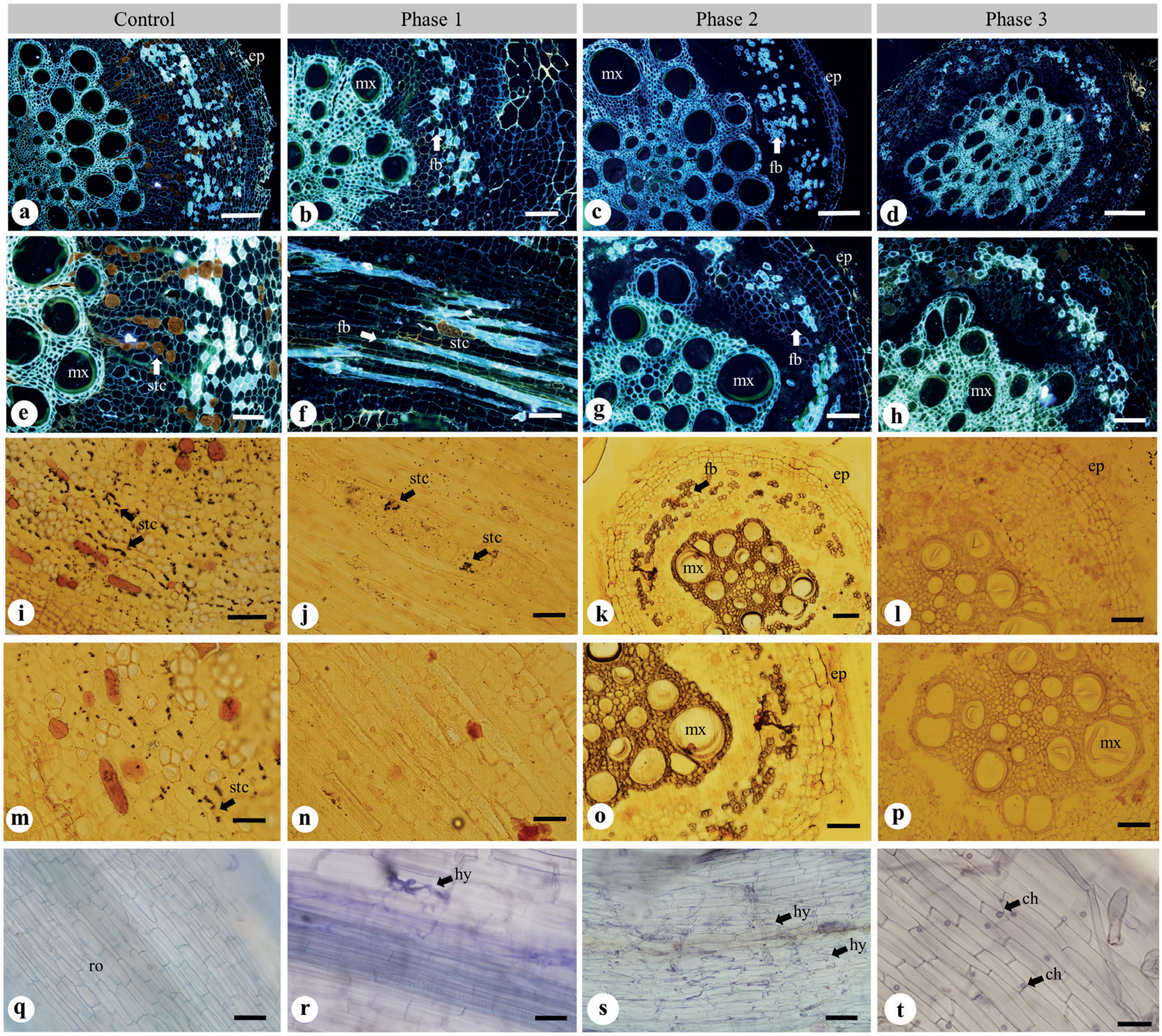

Figure 6 - Anatomical aspects of non-grafted *P. edulis* in three stages of *Fusarium* wilt. (**a-e**): fluorescence microscopy of the root of control plants not inoculated showing the presence of fibers and starch. (**b-f**): first wilt stage with marked reduction in the presence of starch and absence of callus. (**c-g**): absence of starch and callus and lower fluorescence of the fibers. (**d-h**): absence of starch with disintegration of tissues. (**i-m**): root control with presence of starch in the cortical parenchyma. (**j-n**): low starch concentration in the first wilt stage (**k-l, o-p**): absence of starch in the parenchyma of the cortex and vascular system. (**q**): control plant root with absence of pathogen. (**r-t**): presence of intracellular hyphae and chlamydospores. hy: hyphae, ch: chlamydospores, ro: root, ep: epidermis, stc: starch, mx: metaxylem, fb: fibers. Bars: (**a, c, d**) = 250 µm (**k**) = 125 µm (**b, e-i, l, o, p, s**) = 100 µm (**j, m, n, q, r, t**) = 50 µm.

observed in control plants, which had healthy roots with well-delimited cells (Figure 6q).

DISCUSSION

The definition of grafting techniques for yellow passion fruit is important to obtain vigorous plants associated with resistance to the main soil-borne diseases that constrain cultivation in several regions of Brazil. In the present study, results indicated that as the grafting height increases, there is a reduction in the graft take in the nursery.

In field conditions, no differences in the plant growth were observed regarding grafting heights, indicating that the effect is probably temporary in the greenhouse, due to the healing time for the graft between the scion and the rootstock, and the reestablishment of the vascular connections (Lima et al. 2017). However, the grafting height influenced on the number of leaves and shoots of sweet cherry (Yazdani et al. 2016), and on the plant height and branching of apple (Karlidag et al. 2016). Four grafting heights were evaluated on different cultivars of apple, pear, quince and sweet cherry, and the results showed significant effects of the parameters evaluated in all species and cultivars (Karlidag et al. 2016).

On the other hand, the growth rate of *P. gibertii* when grafted on *P. edulis* was higher than the inverse combination. These results are very interesting, since they indicate that the reduction in the vegetative vigor of *P. edulis* when grafted on *P. gibertii* is associated with the developmental limitation of the root system of the rootstock and not with the vascular connection process. This assumption is supported by the larger GRI of *P. gibertii/P. edulis* combination in relation to the non-grafted *P. gibertii* plants. *P. edulis* grafted on *P. gibertii* showed lower growth speed compared to *P. edulis*. A lower root dry mass in *P. edulis/P. gibertii* was observed in potted plants in greenhouse in relation to the self-graft of *P. edulis* (Morgado et al. 2015). These results corroborate the size of the root system of *P. gibertii* as the limiting factor for the development of *P. edulis* scion. The root system of the rootstock is of fundamental importance, since the water flow to the scion controls many plant processes, such as growth, mineral nutrition, photosynthesis, transpiration and, consequently, productivity (Martínez-Ballesta et al. 2010, Gambetta et al. 2012).

All plants of *P. edulis/P. gibertii* and seedlings of *P. gibertii* survived throughout the evaluation period in an area with a *Fop* history, regardless the grafting height, indicating that the resistance of *P. gibertii* occurs at the root level. Therefore, the lower survival of *P. edulis/P. gibertii* reported by Santos et al. (2016) may be associated with the crop management, climatic conditions or genetic variation within the rootstock seedlings, among other factors. There are few studies on the effect of the grafting height on the incidence of soil-borne diseases of fruit crops. The incidence of *Pseudomonas* sp. was reduced in apricot and Prune due to grafting height of 180 cm in relation to 30 cm above the root collar (Prunier et al. 1999, Sayler et al. 2002). This range of grafting height is obviously of no practical use in vine crops such as passion fruit.

On the other hand, the use of *P. gibertii* as a scion grafted on *P. edulis* resulted in high mortality of plants associated with *Fop* throughout the same period. These results confirm the existence of inhibitory compounds of infection and/or colonization of the pathogen in the roots of *P. gibertii,* and that there is no flow of these substances from the scion to the roots of the susceptible species, *P. edulis*. Recent studies indicate the movement of miRNA via phloem from shoot to root in vines, which, however, is strongly dependent on the environment (Yang et al. 2015).

The maintenance of the yield and quality of the yellow passion fruit in grafted plants is also fundamental for the acceptance of rootstocks by growers. The results obtained showed that there were no changes in the physical and chemical attributes as a function of the grafting heights tested in relation to the non-grafted *P. edulis* plants. The number of fruit and productivity were also not influenced by the grafting height or in comparison with non-grafted seedlings of *P. edulis*. The productivity in the first production cycle in the grafted plants observed in this study was below (39%) the national average which is of 14.1 t ha^{-1} (IBGE 2017), which is calculated on the basis of at least two production cycles over the year. Nevertheless, the average life expectancy of non-grafted *P. edulis* was only 168 days, indicating that the number of live plants in the second production cycle will be less than 40%. Cavichioli et al. (2011a) found a production of 3,517 kg ha^{-1} in *P. edulis/P. gibertii* from December 2006 to February 2007, which did not differ from self-grafted *P. edulis*.

Grafting is an important alternative for living with *Fusarium* wilt until obtaining a variety of *P. edulis* with resistance to the pathogen. The grafting should only be used in areas infested with *Fop*, because although there is no compromise of the physical and chemical characteristics of the fruits, the vigor and productivity of grafted plants, when compared with those of seedling plants, might be reduced in areas without the disease. Cavichioli et al. (2011b), when evaluating the quality of passion fruit fruits from plants of *P. edulis* grafted on three rootstocks (*P. edulis, P. alata* and *P. gibertii*), verified that grafted plants produced fruits satisfying marketing standards, despite being shorter in length than the non-grafted plants. No significant rootstock effects on antioxidants contents or fruit quality of *P. edulis* were observed in the graft combinations of *P. edulis/P. mucronata* and *P. edulis/P. gibertii* (Salazar et al. 2016). Grafting did not affect the fruit quality of peach (Picolotto et al. 2010), whereas rootstocks are major factors for the fruit yield and/or quality of pear (Ikinci et al. 2014), watermelon (Turhan et al. 2012), sweet cherry (López-Ortega et al. 2016) and citrus (Castle 1995). Such diferences are also associated to the interaction between the scion/rootstock combination x environmental and management conditions (Al-Jaleel et al. 2005).

The histopathological evaluations of roots of non-grafted *P. edulis* plants at different *Fusarium* wilt stages demonstrated that as disease progresses, there is a significant reduction of starch in the roots, and the presence of hyphae and chlamydospores, especially in the last wilt stage. The fiber distribution and lignification of the cortical walls in the secondary roots was low, both in control plants and in the different wilt stages, with reduction in the fluorescence of the fibers in the infected plants. These physical structures represent an important barrier to pathogen infection and the their fragility may be associated with the susceptibility of yellow passion fruit to *Fop*, since the cell wall is considered the first barrier to penetration or colonization of the pathogen. Furthermore, changes in this membrane can occur that make it impossible for the pathogen to colonize the plant (Underwood 2012, Miedes et al. 2014).

The available information in the literature on starch dynamics in the plant-pathogen interaction is scarce, and the few studies that have been carried out show that pathogen-affected plants present a reduction in the deposition of this polysaccharide in roots (Keunen et al. 2013, Manila and Nelson 2014). In the healthy plants, greater starch accumulation was observed in the root cortex, and as the wilt stage intensified, the starch concentration declined or was too low to detect. This reduction may also be associated with the stage of wilt observed in plants regardless of the pathogen, since studies show that starch degradation is associated with osmotic adjustment to water deficit (Ponce et al. 2008). Nevertheless, the most important anatomical and histopathological changes due to *Fop* infection were the concentration of starch, hyphae and chlamydospores as a function of the wilt stage.

CONCLUSION

Graft take of *P. edulis* on *P. gibertii* in greenhouse was higher at 5 and 10 cm height. In field conditions, the grafted *P. gibertii*/*P. edulis* plants presented higher growth than the reciprocal grafting and non-grafted *P. gibertii*. However, they were susceptible to *Fop*, indicating that resistance occurs at the root level. Grafting height from 5 cm up to 30 cm above the root collar did not influence the *Fusarium* wilt incidence of *P. edulis* grafted on *P. gibertii*, thus this combination is considered resistant to *Fop*. Fruit yield and quality were also not influenced by the grafting height or in relation to the *P. edulis* seedlings. Based on the fluorescence and histopathological analyses of the roots, it was possible to identify changes in starch concentration and presence of hyphae and chlamydospores as a function of the *Fusarium* wilt stage in non-grafted *P. edulis* plants. These phenomena are possibly related to the infection and colonization processes in this pathosystem.

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