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Plant protection

Identification of sources of resistance of *Passiflora* rootstocks to fusariosis in areas with disease outbreaks in Mato Grosso state, Brazil

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Abstract - The aim of the study was to identify sources of resistance of *Passiflora* rootstocks to *Fusarium* sp. in two experimental areas with histories of disease foci in Mato Grosso state, Brazil. The experiment was carried out from June 2012 to December 2015 and was of randomized block design with eight treatments in which susceptible cultivar *P. edulis* was grafted onto hybrids CPAC MJ-H-87, CPAC MJ-H-76, CPAC MJ-H-86 and CPAC MJ-H-88, the commercial cultivar and native *P. edulis*, *P. nitida* and *P. alata.* Mortality rates of grafted plants were evaluated up to 640 days after transplantation. All *Fusarium* sp. isolates were identified as *F. oxysporum* f. sp. *passiflorae*. Plant mortality occurred earlier in the clay area than in the area with sandy clay loam. Grafted plants involving *P. edulis* as rootstocks were highly susceptible to *Fusarium* sp. with overall mortality rates above 56.25% considering both soil types, while plants with CPAC MJ-H-76 or native *P. nitida* rootstocks were more resistant with mortality rates below 12.5%. Grafted plants with CPAC MJ-H-86 as rootstock were highly resistant in clay soil but highly susceptible in soil with high proportion of sand. Only grafted plants involving CPAC MJ-H-76 as rootstock showed moderate resistance.
Index Terms: *Passiflora* sp., *Fusarium oxysporum* f. sp. *passiflorae*, grafting, fusariosis resistance.

Identificação de fontes de resistência de porta-enxertos de *Passiflora* à fusariose em áreas com focos da doença em Mato Grosso, Brasil

Resumo - O objetivo do estudo foi identificar fontes de resistência de porta-enxertos de *Passiflora* ao *Fusarium* sp. em duas áreas experimentais, com registro de focos da doença em Mato Grosso, Brasil. O experimento foi conduzido de junho de 2012 a dezembro de 2015 e delineado em formato de blocos casualizados com oito tratamentos, nos quais a cultivar suscetível *Passiflora edulis* (copa) foi enxertada nos híbridos CPAC MJ-H-87, CPAC MJ-H-76, CPAC MJ-H-86 e CPAC MJ-H-88, na própria cultivar comercial e nas espécies nativas *P. edulis*, *P. nitida* e *P. alata*. As taxas de mortalidade das plantas enxertadas foram avaliadas até 640 dias após o transplante. Todos os isolados de *Fusarium* sp. foram identificados como *F. oxysporum* f. sp. *passiflorae*. A mortalidade ocorreu mais cedo na área com solo argiloso do que naquele argilo-arenoso. Plantas enxertadas com *P. edulis* foram altamente suscetíveis ao *Fusarium* sp. com taxas de mortalidade superior a 56,25%, considerando ambos os tipos de solo, enquanto plantas enxertadas com CPAC MJ-H-76 ou *P. nitida* foram mais resistentes com taxas de mortalidade inferiores a 12,5%. Plantas enxertadas com CPAC MJ-H-86 foram altamente resistentes em solo argiloso, mas altamente suscetíveis em solo com alta proporção de areia. Somente plantas enxertadas com CPAC MJ-H-76 mostraram ter resistência moderada.
Termos para Indexação: *Passiflora* sp., *Fusarium oxysporum* f. sp. *passiflorae*, enxerto, resistência à fusariose.

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Introduction

Passiflora is the largest genus in the family Passifloraceae and most of its members are native to tropical regions of the American continent. The genus comprises more than 500 species, some 70 of which produce edible passion fruit, and more than a dozen are grown commercially in various countries in the world for food or ornamental purposes (FALEIRO et al., 2017). Owing to the rich biodiversity of the genus, various species have been targeted by breeding programs for genetic improvement and for expanding existing production systems (FALEIRO et al., 2019a).

In Brazil, the most cultivated species is *Passiflora edulis* Sims. (known locally as maracujá-azedo or maracujá-amarelo), although commercialization of cultivars of *P. alata* Curtis (maracujá-doce), *P. setacea* DC. (maracujá-do-cerrado) and *P. cincinnata* Mast. (maracujá-da-caatinga) has been approved by the Brazilian Ministry of Agriculture, Livestock and Food Supply (MACHADO et al., 2017; RONCATTO et al., 2011). Nevertheless, *P. edulis* represents more than 95% of the national production intended for consumption *in natura* and for agroindustry. Even though Brazil is one of the world's leading producers of passion fruit, with some 41,800 ha under cultivation yielding 593,429 t of fruit in 2019 (IBGE, 2020), production is confined mainly to small-scale family-based units and is, therefore, of considerable socioeconomic importance to the country (FALEIRO et al., 2016).

Passion fruit orchards are vulnerable to various diseases including fusariosis and collar rot caused by soil-borne *Fusarium* spp., particularly *Fusarium solani* and *F. oxysporum* f. sp. *passiflorae*, that can reduce significantly the useful productive life of orchards and bring about substantial economic losses (SILVA et al., 2017; MACHADO et al., 2015). In the semiarid region of Brazil, *Fusarium* sp. can cause 70 to 100% mortality of passion fruit plants within a short period of time and, in some cases, even before an orchard has become productive (SÃO JOSÉ et al., 2011).

The control of *Fusarium* wilt is difficult not only because of the lack of efficient agrochemicals, but also because the pathogen can survive in the soil without a host for extended periods of time (MACHADO et al., 2015; MELETTI, 2011; FISCHER et al., 2005). The fungus enters through the rhizome via natural or artificial wounds including those caused by agricultural implements and phytonematodes such as *Meloidogyne incognita* and *Rotylenchulus reniformis* (GARCIA et al., 2007). Thus, symptoms such superficial root rot and/or the presence of lesions at the plant collar serve to distinguish *Fusarium* wilt from other diseases (MACHADO et al., 2015). As infection spreads up into the stems and leaves, water flow is restricted causing wilting and yellowing of the foliage and eventually plant death.

Fusariosis occurs initially as localized outbreaks of disease deriving from contaminated seeds and seedlings or the remains of infected crops and fruits, and spreads rapidly to neighboring plants through direct contact with the roots or by water present in the soil (MELETTI, 2011; SÃO JOSÉ et al., 2011). In the absence of efficient chemical control, the only alternatives for preventing the spread of *Fusarium* wilt are the adoption of appropriate management practices and the introduction of genetic resistance by developing resistant cultivars or by grafting onto resistant rootstocks (HURTADO-SALAZAR et al., 2015; MACHADO et al., 2015; MORGADO et al., 2015). Commercial cultivars of passion fruit present low genetic variability with regards to disease-resistance. Species of *Passiflora* that are native to Brazil represent valuable resources for genetic improvement owing to their rich biodiversity and, in many cases, compatibility with *P. edulis* (JUNQUEIRA et al., 2005). Reports are available concerning the yields, under commercial conditions, of *P. edulis* grafted onto native passion fruit species as rootstocks including, for example, *P. phoenicea* Lindl. (syn. *P. alata*) in Rio de Janeiro state, *P. nitida* Kunth and *P. alata* in Mato Grosso state, *P. gibertii* N.E. Br. in Bahia state and *P. foetida* L. in Rio Grande do Norte state (MACHADO et al., 2015; PREISIGKE et al., 2015; CAVICHIOLI et al., 2011a,b).

In the municipality of Terra Nova do Norte, Mato Grosso, *Fusarium* wilt was detected for the first time in 2005 (ARAUJO et al., 2012) and has now spread to 50 out of the 160 ha planted with *P. edulis*. There is, therefore, an urgent need for action to control the disease in the region, and the most plausible solution is the use of grafting techniques (AMBROSIO et al., 2018; SEMPREBOM et al., 2012). Considering that properly characterized and registered *Fusarium*-resistant rootstocks are currently unavailable (FALEIRO et al., 2019b), the aim of the present study was to identify sources of resistance of *Passiflora* rootstocks to *Fusarium* sp. in two experimental areas with histories of disease foci in Mato Grosso state, Brazil.

Material and Methods

The experiments were performed between June 2012 and December 2015 in two private farms situated in the municipality of Terra Nova do Norte, Mato Grosso, located about 150 km from the major regional town of Sinop. Both farm A (-55.103 W, -10.537 S; 292 m altitude) and farm B (-55.126 W, -10.575 S; 307 m altitude) had related occurrence of *F. oxysporum* f. sp. *passiflorae*. According to the Secretaria de Estado de Planejamento e Coordenação Geral do Mato Grosso (2011) the local soil is classified as Dystrophic Typic Hapludults clay (area A) and sandy clay loam (area B).

The experiment was of randomized block design and involved eight treatments with four repetitions each of four plants, totalizing 16 plants per treatment. The treatments comprised the commercial cultivar *P. edulis* 'BRS Gigante Amarelo' (control) as scion with the hybrids CPAC MJ-H-87 (*Passiflora alata* Curtis. x *Passiflora maliformis* L.), CPAC MJ-H-76 'BRS Gigante Amarelo' x (*Passiflora quadrifaria* Vanderpl. x *Passiflora setacea* DC.) x *Passiflora incarnata* L., CPAC MJ-H-86 (*Passiflora setacea* DC. x *Passiflora coccinea* Aubl. x *P. speciosa* Gardener) and CPAC MJ-H-88 (*Passiflora katsshbachu* x (*P. vitifolia* Kunth x *P. setacea* DC.), the cultivar *P. edulis* 'BRS Gigante Amarelo', and the native *P. edulis* Sims, *P. nitida* Kunth and *P. alata* Curtis.

Preparation of seedlings was carried out in the experimental nursery of Cooperativa Agrícola Mista Terra Nova Ltda (Coopernova) according to the method described by Nogueira-Filho et al. (2010). In the case of *P. nitida* Kunth, seeds were stored at 10ºC for 6 months prior to sowing in order to break dormancy. Seeds were soaked in distilled water for 12 h and then sown in 72-cell polystyrene seed trays (120 mL per cell) containing a mixture of light soil, fully-matured cattle manure and commercial Plantmax® substrate (peat, pine bark and vermiculite; Plantmax® Sementes) in the proportion of 3:1:1. Germinated seedlings remained under greenhouse conditions until required for grafting.

The cleft graft method with a full slit on the hypocotyl of the rootstocks (NOGUEIRA-FILHO et al., 2010) was employed in grafting seedlings that were 6 to 8 cm in height and with around three definitive leaves, a stage attained around 30 days after sowing for vigorous growing seedlings or 90 days for slower plants. Grafted seedlings were maintained in the nursery and irrigated daily with the aid of a micro sprinkler system until required for transplantation to the field at 30 days after grafting.

The experimental areas were laid out with 4 m spaces between the 0.40 x 0.40 x 0.40 m plant pits and 3 m spaces between the training wires that were fixed 2 m above ground on support posts. Prior to the commencement of experiments, the chemical and physical properties of the soil in experimental areas A and B were analyzed in 0 - 20 cm layer (Table 1) and soil acidity was corrected by liming each of the areas with dolomitic limestone (2.5 t ha^{-1}) as well as the individual pits (0.225 t ha^{-1}) as recommended by Lima (2009). The plant pits also received localized fertilizing with 5 L of chicken manure, 1.0 kg of superphosphate, 200 g of limestone, 200 g of gypsum and 100 g of monoammonium phosphate to provide a nutrient pool as recommended by Borges (2004). Grafted seedlings were transferred to the prepared pits on 24th July 2012 and the vines were direct to grow up towards the support wires with the aid of string lines. Standard cultural management, including drip irrigation, was carried out throughout the experimental period.

Vegetative growth was determined by measuring stem diameter (cm) above the grafting point using a digital caliper at 90 days after planting (DAP) during the juvenile stage of the grafted plants. The mean diameter values of the grafted plants in the eight different treatments were compared using Scott-Knott test at 5% significance. The survival and mortality rates (%) were determined by collecting the plants that had died at 10 evaluation times, namely, 60, 120, 266, 320, 330, 360, 440, 520, 600 and 640 days after planting (DAP). The responses of the scion-rootstocks combinations to *Fusarium* wilt were categorized as proposed in Table 2. Samples of dead plants that had root lesions were transferred to the Laboratory of Phytopathology at the Universidade Federal de Mato Grosso (UFMT) for isolation and identification of the genus of the disease-causing fungus.

In order to identify the species of *Fusarium* present in fungal isolates, DNA profiling was performed in the Phytopathology Laboratory at Embrapa Agrossilvipastoril. Briefly, mycelia of fungal isolates were diluted with Milli-Q water, spread onto water agar medium and incubated at 25 ºC for 24 h under a 12 h photoperiod. Single spores were selected with the aid of a hand lens, transferred to potato dextrose agar (PDA) medium and incubated under the conditions described above for 5 days. Monosporic colonies were subcultived onto new plates containing PDA medium in order to eliminate any contaminants and maintained in a biochemical oxygen demand incubator under the conditions described above for approximately 7 days, enough time for the fungus to cover the culture medium.

Table 1. Chemical and physical attributes of the soil (0 - 20 cm layer) in experimental areas A and B located in Terra Nova do Norte, Mato Grosso, Brazil

Area	Chemical composition											Granulometry and organic matter			
	pH		Ca	Mg	Al	H	K	P	SB	CEC	BS	Sand	Silt	Clay	OM
	H_2O	$CaCl_2$	($cmol_c$ dm^{-3})				(mg dm^{-3})		($cmol_c$ dm^{-3})		(%)	(g kg^{-1})			(g dm^{-3})
A	5.8	5.1	1.9	0.8	0.0	2.9	45	3.3	2.8	5.7	49.4	390	153	457	18.9
B	5.6	4.8	1.6	0.5	0.0	3.3	62	3.9	2.3	5.6	41.1	625	84	291	22.0

SB, sum-of-bases; CEC, cation exchange capacity; BS, base saturation; OM, organic matter

Table 2. Classification of passion fruit rootstocks according to resistance to fusariosis (*F. oxysporum* f. sp. *passiflorae*) on the basis of mortality rate as proposed in this study

Mortality rates (%)	Classification
0	resistant
1.1 - 12.5	moderately resistant
12.6 - 25.0	moderately susceptible
25.1 - 50.0	susceptible
> 50.0	highly susceptible

DNA was extracted according to the method described by Raeder and Broda (1985) and the integrity of extracted DNA evaluated by 1.5% agarose gel electrophoresis and quantitative assessment using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Polymerase chain reaction (PCR) was performed using a PCR thermal cycler (Bio-Rad, T100, Hercules, CA, USA) and the fungi-specific primer pairs ITS1-F (5'TCCGTAGGTGAACCTGCGG3') and ITS4A-R (5'TCCTCCGCTTATTGATAT-GC3') (WHITE et al., 1990). The reaction mixture contained 2.5 µL of 10 X reaction buffer, 2.0 µL of $MgCl_2$ (25 mM), 0.5 µL of dNTP mixture (10 mM), 1.5 µL each of the forward and reverse primers (10 µM), 1 µL of Taq DNA polymerase (5 U/µL), 100 ng of DNA template and deionized water to a final volume of 25 µL. The amplification procedure involved an initial denaturation at 94 °C for 5 min, annealing at 55 °C for 30 s and extension at 72 °C for 30 s, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s, with a final extension step at 72 °C for 10 min. The PCR products of each sample were submitted to 1% agarose gel electrophoresis together with a 50 – 700 bp DNA ladder to detect the expected 600 bp fragment. PCR products were purified by suspension in 100% isopropanol and centrifugation at 9,000 rpm for 15 min, followed by suspension of the pellet in 70% ethanol and centrifugation under the same conditions. After complete evaporation of the 70% ethanol, the pellet was resuspended in 20 µL of sterile distilled water and dispatched to Myleus Biotecnologia (Belo Horizonte, MG, Brazil) for sequencing.

Three composite soil samples containing 500 g of soil and 50 g of roots were taken from each experimental area in March 2013 for analysis of nematodes. The samples were placed in plastic bags, labeled and transported to the Laboratory of Nematology at Embrapa Agrossilvipastoril for extraction and identification of nematodes.

Results and Discussion

The scion (*P. edulis* 'BRS Gigante Amarelo') was compatible with all tested rootstocks during the experimental period as shown by the growth of the grafted plants (Table 3) despite the occurrence of disease. Some of the scion-rootstock combinations had a higher longevity than the control plants in which scion and rootstock originated exclusively from *P. edulis* 'BRS Gigante Amarelo'.

Table 3. Mean diameter values of commercial cultivar *Passiflora edulis* 'BRS Gigante Amarelo' grafted onto various rootstocks and grown in two different experimental areas in Terra Nova do Norte, Mato Grosso, Brazil.

Rootstocks	Diameter above the grafting point (cm)[1]	
	Area A	Area B
Hybrid CPAC M5-H-87 (*P. alata* x *P. maliformis*)	13.8 a	14.6 a
Hybrid CPAC MJ-H-76 ['BRS Gigante Amarelo' x (*P. quadrifaria* x *P. setacea*) x *P. incarnata*]	14.2 a	11.6 a
Hybrid CPAC MJ-H-86 [*P. setacea* x (*P. coccinea* x *P. speciosa*)]	12.3 a	11.4 a
Hybrid CPAC MJ-H-88 [*P. katsshbachu* x (*P. vitifolia* x *P. setacea*)]	15.4 a	14.9 a
Commercial cultivar *P. edulis* 'BRS Gigante Amarelo' (control)	16.0 a	14.1 a
Native *P. edulis*	18.6 a	14.8 a
Native *P. nitida*	12.4 a	11.2 a
Native *P. alata*	13.1 a	10.9 a
Mean	14.5	12.9
Coefficient of variance (%)	18.1	22.6

[1] Mean values followed by dissimilar lowercase letters in the column are significantly different according to the Scoot-Knott test at 5% significance

The isolates of *Fusarium* sp. collected from experimental areas A and B were all identified as *F. oxysporum* f. sp. *passiflorae*. The mortality of plants occurred earlier in the clay area A (39% sand) in comparison with area B with a higher proportion of sand (63%) in the 0-20 cm layer (Table 1). However, plant mortality occurred more abruptly and at a considerably higher rate in area B (Figure 1a and 1b).

Since *P. edulis* 'BRS Gigante Amarelo' and native *P. edulis* are highly susceptible to *Fusarium* wilt, the finding that grafts involving these rootstocks had overall mortality rates (considering both areas) of over 56% was expected (Table 4). Grafted plants with hybrids CPAC M5-H-87 and CPAC MJ-H-88 as rootstock were moderately susceptible in area A and susceptible in area B. The overall mortality rates of plants with CPAC MJ-H-76 and *P. nitida* as rootstocks were even lower (≤12.5%), thus being considered moderately resistant, indicating that the hybrid and the native species probably presented a certain source of resistance to fusariosis.

P. alata was moderately resistant in area A and susceptible in area B. Interestingly, grafted plants with hybrid CPAC MJ-H-86 as rootstock exhibited somewhat discrepant results since they were resistant in area A but highly susceptible in area B (62.5% mortality). Hence, the moderately resistant CPAC MJ-H-76**,** *P. nitida* and *P. alata*, along with the potentially resistant cultivar CPAC MJ-H-86, constitute promising rootstocks for improving the resistance of *P. edulis* 'BRS Gigante Amarelo' to attack by *Fusarium* sp.

The differences in susceptibilities between grafted plants grown in the two experimental areas could be attributed to various intrinsic and environmental factors. The occurrence of intra-species or intra-cultivar variation and the extent of such genetic variability in the rootstocks could account for the differences, and this factor needs to be investigated further in order to improve *Fusarium*-resistance traits. The occurrence of variation between seeds could also be a contributing factor since each rootstock was derived from a single seed. In this study, the dissimilar soil texture accentuated differences in susceptibility to *Fusarium* sp. since area B was sandier than area A (Table 1). Sandy soils favor fungal dissemination (FISCHER; REZENDE, 2016), while clay soils suppress fungal disease. Languasco et al. (2000) observed that the incidence of *Fusarium* wilt in melon was higher in sandy soils compared with clay soils owing to the increase in pathogen population. Similar result was reported by Rodrigues et al. (1998), i.e. the severity of *Fusarium* wilt in tomatoes grown in sandy soil was higher than in clay soils. *Fusarium solani* is another species that occurs in passion fruit, although it is more common in clay soils with poor drainage, mainly when rainwater or irrigation water accumulates around the plants (FALEIRO; JUNQUEIRA, 2016).

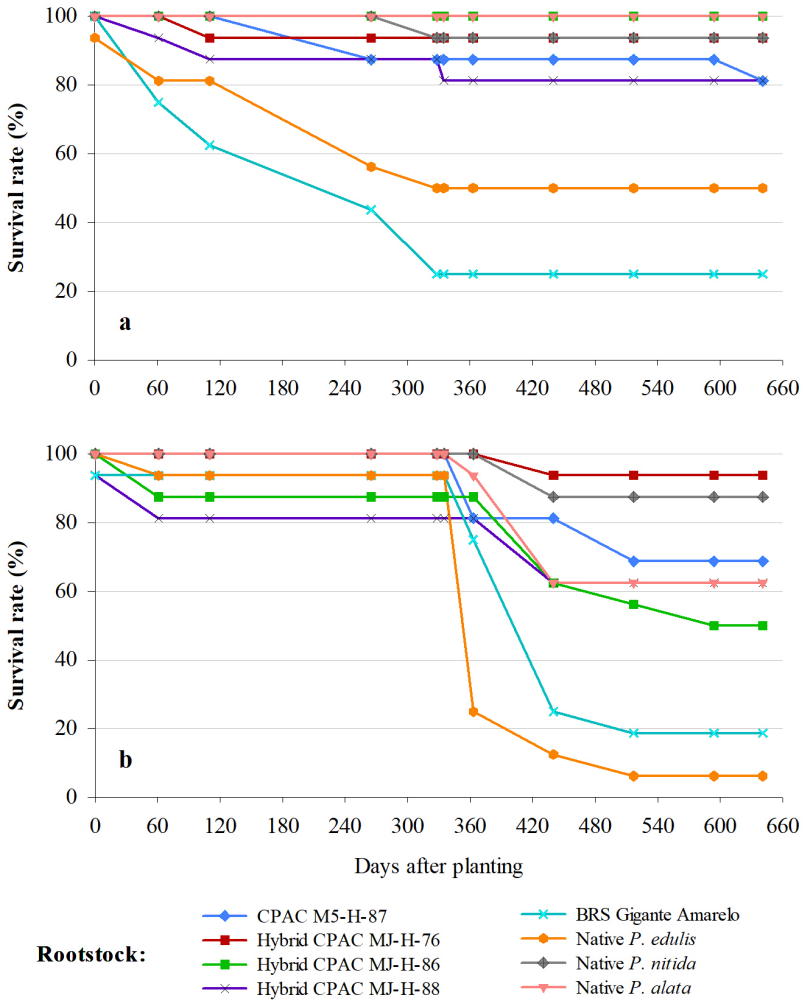

Figure 1. Survival rates (%) in area A (a) and B (b) of cultivar *Passiflora edulis* 'BRS Gigante Amarelo' grafted onto different rootstocks.

The mycelia or spores of *Fusarium* sp. penetrate the plant roots, advance through the root cortex into the sap stream and are transported upwards. Eventually the spores and the mycelia clog the vascular vessels preventing the plant from taking up and translocating nutrients. Disruption of the transport of xylem sap towards the aerial parts and phloem sap towards the sugar sinks leads to a decrease in plant turgidity. This situation is aggravated in sandy soils because the availability of water in such soils is lower than in predominantly clay soils, so that water and nutrients become even more limited under these circumstances (KRAMER; BOYER, 1995).

An additional factor relates to the different densities of phytonematodes detected in the two experimental areas. In area B, the two major nematodes of *Passiflora* cultures, namely *R. reniformis* and *Meloidogyne* spp., together with the pathogenic nematode *Helicotylenchus dihystera*, were identified, whereas only the reniform nematode was detected in area A and at low densities. Although many species of nematodes have been associated with passion fruit, members of the genera *Rotylenchulus* and *Meloidogyne* are predominantly responsible for reduced longevity, decreased productivity and economic losses (GARCIA et al., 2007). Considering the above, it is likely that the sandy soil and the high nematode density in experimental area B contributed to the increased susceptibility of grafted plants.

Table 4. Mortality rates and susceptibility to *Fusarium* sp. of commercial cultivar *Passiflora edulis* 'BRS Gigante Amarelo' grafted onto various rootstocks and grown in two different experimental areas in Terra Nova do Norte, Mato Grosso, Brazil, each with a history of fusariosis

Rootstocks	Area A		Area B	
	Mortality (%)[1]	Classification[2]	Mortality (%)[1]	Classification[2]
Hybrid CPAC M5-H-87 (*P. alata* x *P. maliformis*)	18.75 ± 11.97	Moderately susceptible	37.50 ± 21.65	Susceptible
Hybrid CPAC MJ-H-76 ['BRS Gigante Amarelo' x (*P. quadrifaria* x *P. setacea*) x *P. incarnata*]	6.25 ± 6.25	Moderately resistant	6.25 ± 6.25	Moderately resistant
Hybrid CPAC MJ-H-86 [*P. setacea* x (*P. coccinea* x *P. speciosa*)]	0.00 ± 0.00	Resistant	62.50 ± 16.14	Highly susceptible
Hybrid CPAC MJ-H-88 [*Passiflora katsshbachu* x (*P. vitifolia* x *P. setacea*)]	18.75 ± 6.25	Moderately susceptible	43.75 ± 6.25	Susceptible
Commercial cultivar *P. edulis* 'BRS Gigante Amarelo' (control)	81.25 ± 6.25	Highly susceptible	75.00 ± 17.68	Highly susceptible
Native *P. edulis*	56.25 ± 25.77	Highly susceptible	93.75 ± 6.25	Highly susceptible
Native *P. nitida*	6.25 ± 6.25	Moderately resistant	12.50 ± 12.50	Moderately resistant
Native *P. alata*	6.25 ± 6.25	Moderately resistant	37.50 ± 21.65	Susceptible

[1] Data presented as mean percentage values ± standard error [2] Classification based on the mortality rate 640 days after planting: resistant (0%); moderately resistant (0.1 - 12.5%); moderately susceptible (12.6 – 25%); susceptible (25.1 - 50 %); highly susceptible (> 50%)

Conclusions

Of the four rootstocks observed to confer any source of *Fusarium* sp. resistance to the commercial cultivar *P. edulis* 'BRS Gigante Amarelo', only grafts employing the hybrid CPAC MJ-H-76 showed some source of resistance in both experimental areas and resulted in comparable mortality rates. Grafted plants employing hybrid CPAC MJ-H-86 exhibited some resistance to *F. oxysporum* f. sp. *passiflorae* when grown in predominantly clay soil but were highly susceptibility in soil containing higher levels of sand. The influence of soil properties and structure on the susceptibility of the commercial passion fruit cultivar requires further investigation since the virulence of *Fusarium* sp. could be modulated by soil characteristics.

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