

**Genetic resistance to fusariosis and base rot in interspecific F₁ progenies of passion fruit
(*Passiflora* spp.)**

**Resistência genética a fusariose e podridão do colo em progênes interespecíficas F₁ de
maracujazeiro (*Passiflora* spp.)**

**Resistencia genética a fusariosis y podredumbre de la base en progenies F₁
interespecíficas de maracuyá (*Passiflora* spp.)**

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Abstract

Fusariosis and base rot, caused by the fungi *Fusarium oxysporum* f. sp. *passiflorae* and *F. solani*, respectively, seriously damage the cultivation of sour passion fruit in Brazil. This work aims to obtain and evaluate F₁ hybrids of *Passiflora* spp. wild plants resistant to fusariosis and base rot using genotypes of *P. edulis* (commercial species) and certify interspecific hybridizations using microsatellite markers. Hybridizations were performed using a *P. edulis* female parent and *P. nitida* and *P. mucronata* male parents for crosses aiming fusariosis resistance, and *P. nitida*, *P. cincinnata* and *P. quadrangularis* for hybridizations aiming tolerance to base rot. 35 microsatellite markers were used to confirm hybridization. The washed roots method was used for fusarium resistance tests and inoculation procedure with a mycelium disk fixed on a small wound on the plant stem for base rot. The interspecific hybridizations provided 49 potentially hybrid genotypes. Confirmation of hybridization by microsatellite marker was verified for 57% of the analyzed genotypes. The hybrids 115-1, 115-3, 115-4, 115-5, 115-6, 115-7, 115-9 and 128 are indicated as promising genotypes for a new stage of the breeding program. In the resistance evaluation of 13 F₁ hybrids to *F. oxysporum* f. sp. *passiflorae*, the genotypes 142 and 143-2 were selected as the most resistant to continue the breeding program.

Keywords: *Passiflora*; *Fusarium oxysporum* f. sp. *Passiflorae*; *Fusarium solani*; Genetic improvement; SSR marker.

Resumo

A fusariose e a podridão do colo, causadas respectivamente pelos fungos *Fusarium oxysporum* f. sp. *passiflorae* e *F. solani*, vêm prejudicando seriamente o cultivo do maracujazeiro azedo no país. Este trabalho objetivou obter e avaliar híbridos F₁ entre espécies de *Passiflora* spp. silvestres resistentes a fusariose e podridão do colo com genótipos de *P. edulis* (espécie comercial) e certificar as hibridações interespecíficas por meio de marcadores microsatélites. As hibridações foram realizadas utilizando-se como genitor feminino *P. edulis* e genitores masculinos *P. nitida* e *P. mucronata* para os cruzamentos visando a resistência a fusariose, *P. nitida*, *P. cincinnata* e *P. quadrangularis* para hibridações visando

resistência a podridão do colo. Para a confirmação da hibridação foram utilizados 35 marcadores microssatélites. Utilizou-se para o teste de resistência da fusariose o método de raízes lavadas e para podridão do colo o procedimento de inoculação com um disco de micélio fixado sobre um pequeno ferimento no caule da planta. As hibridações interespecíficas proporcionaram a obtenção de 49 genótipos potencialmente híbridos. A confirmação da hibridação via marcador microssatélite foi verificada para 57% dos genótipos analisados. Para a resistência a podridão do colo são indicados como genótipos promissores em uma nova etapa do programa de melhoramento genético os híbridos 115-1, 115-3, 115-4, 115-5, 115-6, 115-7, 115-9 e 128. Na avaliação de resistência de 13 híbridos F1 ao *F. oxysporum* f.sp. *passiflorae* foram selecionados para prosseguir no programa de melhoramento visando a resistência à fusariose os genótipos 142 e 143-2, como os mais resistentes.

Palavras-chave: *Passiflora*; *Fusarium oxysporum* f. sp. *Passiflorae*; *Fusarium solani*; Melhoramento genético; Marcador ssr.

Resumen

Fusariosis y podredumbre de la base, causada por el hongo *Fusarium oxysporum* f. sp. *passiflorae* y *F. solani*, respectivamente, dañaron seriamente el cultivo de maracuyá ácida en Brasil. Este trabajo tiene como objetivo obtener y evaluar híbridos F1 de *Passiflora* spp. plantas silvestres resistentes a fusariosis y podredumbre de bases utilizando genotipos de *P. edulis* (especie comercial) y certificar hibridaciones interespecíficas utilizando marcadores microssatélites. Las hibridaciones se realizaron utilizando un progenitor hembra de *P. edulis* y progenitores masculinos de *P. nitida* y *P. mucronata* para cruces que apuntan a la resistencia a la fusariosis, y *P. nitida*, *P. cincinnata* y *P. quadrangularis* para hibridaciones que apuntan a la tolerancia a la pudrición de la base. Se utilizaron 35 marcadores microssatélites para confirmar la hibridación. El método de raíces lavadas se utilizó para las pruebas de resistencia al *Fusarium* y el procedimiento de inoculación con un disco de micelio fijado en una pequeña herida en el tallo de la planta para la pudrición de la base. Las hibridaciones interespecíficas proporcionaron 49 genotipos potencialmente híbridos. Se verificó la confirmación de hibridación por marcador de microssatélites para el 57% de los genotipos analizados. Los híbridos 115-1, 115-3, 115-4, 115-5, 115-6, 115-7, 115-9 y 128 están indicados como genotipos prometedores para una nueva etapa del programa de mejoramiento. En la evaluación de la resistencia de 13 híbridos F1 a *F. oxysporum* f. sp. *passiflorae*, los genotipos 142 y 143-2 fueron seleccionados como los más resistentes para continuar con el programa de

mejoramiento.

Palabras clave: *Passiflora*; *Fusarium oxysporum* f. sp. *Passiflorae*; *Fusarium solani*; Mejora genética; Marcador SSR.

1. Introduction

The cultivation of sour passion fruit (*Passiflora edulis* L.) has been gaining prominence in recent years in Brazil. However, the increase in production causes several phytosanitary problems. Among the most limiting diseases, fusariosis and base rot, caused by the fungi *Fusarium oxysporum* f. sp. *passiflorae* and *F. solani* (Amorim et al., 2016), respectively, both soil pathogens, stand out.

The symptoms of these diseases are similar. They begin with the withering of young branches, leaf yellowing and fall of fruits. Subsequently, the plant begins the senescence process and may die within two weeks in the summer months (Amorim et al., 2016). These fungi have a chlamydospore, a resistance structure that allows the pathogen to remain in the soil for up to eight years. Therefore, the control of these diseases is only preventive because once affected by the pathogen, the plant will certainly die (Roncetto et al., 2004).

The use of resistant cultivars is the most practical and efficient control measure in addition to being less costly for farmers (Pereira et al., 2011). However, there is no record of *P. edulis* cultivars resistant/tolerant to fusariosis and base rot registered at the Ministry of Agriculture, Livestock and Supply (MAPA, 2019).

In this sense, a strategy of genetic improvement to control these diseases is the incorporation of resistance genes to commercial cultivars of *P. edulis* by hybridization with resistant wild species, followed by a backcrossing program assisted by molecular markers (Bueno et al., 2010).

Molecular markers are important tools in a genetic improvement program as they help to estimate the genetic similarity/difference between genotypes and confirm hybridizations, enabling the choice of desirable materials with a greater accuracy (Millach, 1998).

Thus, this work aims to obtain and evaluate interspecific hybrids resistant to fusariosis and base rot and certify hybridizations using microsatellite markers.

2. Methodology

2.1 Obtaining Hybrids

The experiment was conducted at the experimental area of the Laboratory of Plant Genetic Improvement of the State University of Mato Grosso (UNEMAT), Cáceres, MT (16°11'42" S and 57°40'51" W, 118 m altitude). The region's climate is Aw, according to the Köppen classification, with an average annual temperature of 26.3°C and average annual rainfalls of 1,300 mm (Neves et al., 2011).

The wild genotypes selected for interspecific crosses were those described by Preisigke et al. (2015, 2017) as resistant to *F. solani* and *F. oxysporum* f. sp. *passiflorae*. They are deposited at the Germplasm Active Bank of the State University of Mato Grosso (BAG-UNEMAT). In order to optimize compatibility between interspecific crosses, a review of specific literature was carried out to select wild species with the same number of chromosomes as *P. edulis* (Melo & Guerra 2003; Melo et al., 2001; Soares-Scott et al., 1999). *P. nitida* and *P. mucronata* were selected for crosses aiming resistance to fusariosis and *P. nitida*, *P. cincinnata* and *P. quadrangularis* were selected for hybridizations aiming resistance to base rot.

The crossings were performed manually according to the methodology described by Bruckner and Otoni (1999). The anthesis period of the species involved in the crossings was considered since pollen release and stigma receptivity occurred on the same day but at different times. To overcome this temporal barrier of fertility, a pollen preservation technique was used in silica gel at 4°C for up to 24 hours (Almeida et al., 2011). The flower buds of *P. edulis* parents in pre-anthesis were protected with paper bags the day before hybridization. Buds belonging to the female parent were emasculated before anthesis. The next day, the anthers of wild species were collected, and the pollen grains were deposited using tweezers in the stigmas of *P. edulis*.

After artificial hybridization, the crosses were identified and the flowers were protected with a paper bag for 24 h (Bruckner & Otoni, 1999). The fruits resulting from hybridizations were stacked until complete ripening, when seeds of potential hybrids were collected and obtained.

The F₁ seeds were placed in 72-cell polystyrene trays containing Plantmax[®] substrate (Eucatex Mineral Ltda., Paulínia, SP). The seedlings were kept in a greenhouse until they reached the ideal size (\pm 40 cm) for transplantation to the field. Soil preparation and

cultivation followed methods normally adopted for cultivation: monthly fertilization with 25 g of N and 30 g of KCL and semiannual fertilization with 30 g of P₂O₅ per plant.

After six months in the field, all F₁ plants were cut. The cuttings contained three internodes. Then, they were planted in 72-cell trays containing Plantmax[®] substrate and kept in a greenhouse with controlled irrigation and two weekly applications of leaf fertilizer to stimulate plant rooting. After rooting, the cuttings were transplanted into 400-ml containers containing the substrate Plantmax[®].

2.2 Confirmation of hybridization by molecular marker

For the validation of interspecific hybridizations, the leaves at the intermediate stage of ripening of each of the supposed hybrids, the *Passiflora edulis* female parent and the *Passiflora* spp. male parent were collected, and the genomic DNA was extracted using the Wizard[®] DNA purification kit. Good quality DNA samples were diluted in H₂O milli-Q at a concentration of 3 ng/μL.

To confirm interspecific hybridization, 35 microsatellite markers were tested, as described by Araya et al. (2017). The amplification reaction was carried out in a total volume of 13 μL containing 5.49 μL H₂O milli-Q, 1.3 μL of buffer 10X, 1.04 μL of dNTP (0.25 mM), 1.04 primer forward (0.2 mM), 1.04 primer reverse (0.2 mM), 0.12 μl of Taq DNA polymerase (5 U/μl), and 3 μl of genomic DNA (3 ng/μl). The amplification program used was that proposed by Oliveira et al. (2006). An initial denaturation cycle was 94°C for five minutes, followed by 35 cycles of 94°C for 40 seconds; the temperature (T°) indicated for the primer for 40 seconds was 72°C for one minute and a final extension of 72°C for five minutes. The products of the PCR reaction were stained with 0.5 μl of Blue Green Loading Dye I and separated by electrophoresis in 2.5% agarose gel in a program of 70 V at 200 mA for five hours.

After electrophoresis, the gels were taken to a photo-documenter (Loccus Biotechnology), digitized, and then analyzed using L-Pix Image, version 2.7 (Loccus Biotechnology), to confirm polymorphism between parents.

2.3 Resistance evaluation

For the tests of resistance to fusariosis and base rot, the most aggressive isolates from the UNEMAT library were used according to the results obtained by Marostega et al. (2019).

To produce the inoculum, the isolates preserved on filter paper were grown in Petri dishes containing PDA culture medium (potato-dextrose-agar) and incubated in BOD at 25°C and a photoperiod of 12 h. After seven days, a slide of the isolate was mounted to verify the growth conditions and then proceed to inoculation. At the end of each experiment, the pathogen was re-isolated to comply with the assumptions of Koch's postulate.

2.4 Inoculation of *F. solani*

The inoculation of *F. solani* (FSUNEMAT40 isolate) was performed according to the procedure described by Fischer et al. (2003). The cuttings of F₁ hybrids inoculated with *F. solani* were arranged in a randomized block design with 45 treatments (40 F₁ hybrids + the parents and the positive control without inoculum), three replications and three plants per plot. Assessments of disease progress began five days after inoculation (DAI). They were carried out every two days until the 33rd DAI or until the death of the plant.

The levels of resistance to *F. solani* were determined by the characteristics described by Preisigke et al. (2015). Ten characteristics were used to estimate resistance to pathogen: lesion expansion, length and width (LL and LW), number of plants in which the lesion reached less than 50% of circumference (NPL-50%P), inoculation period until the lesion reached more than 50% of the injured plant stem circumference (PILA+50%), inoculation period until the lesion reached 100% of the plant injured stem circumference (PILA100%S), normalized area under the lesion area expansion curve (NAULEAC), normalized area under the lesion width expansion curve (NAULWC), normalized area below the length expansion curve (NAULEC), number of dead plants (NSP), and survival period (SP).

The lesions were measured as to length and width of necrotic area using a digital caliper. The injured area (IA, mm²) was estimated considering the formula for calculating the area of an ellipse ($\pi \cdot C \cdot L / 4$), where C is lesion length and L is lesion width.

2.5 Statistical analysis

The analysis of variance was performed based on a randomized blocks model with three replications. The analysis followed the statistical model $Y_{ij} = \mu + g_i + B_j + e_{ij}$, where:

Y_{ij} : i is the i -th genotype of j -th replication;

μ : general test average;

gi : effect of the genotype i

bj : effect of block j

ε_{ij} : experimental error or residue

For multivariate analysis, the distance matrix was obtained using the Mahalanobis method (D2ii'); subsequently, the genotypes were grouped by the Ward method (Mohammadi & Prasanna, 2003). To establish a cut-off point in the dendrogram and to define the number of groups, the Mojena (1977) procedure was used based on the relative size of the merging (distances) levels in the dendrogram. The relative importance of each character in the distinction of genotypes based on genetic resistance to the fungus was estimated through the method proposed by Sing (1981).

All analyses were performed using the software GENES (Cruz, 2016) with interaction of the "ape" package of the software R (R Development Core Team, 2016).

2.6 Inoculation of *F. oxysporum* f. sp. *passiflorae*

The FOUNEMAT22 isolate was used for the fusariosis resistance test. It was inoculated in the genotypes of the families of complete siblings 116, 128, 142, 143 and their respective parents (Table 1). For inoculation, the washed roots method was used. The seedlings were removed from the trays and the roots were washed in distilled water. Then, the roots were cut using sterile scissors and immersed in a conidia suspension (1×10^6 conidia/ml) for 24 hours (Preisigke et al., 2017). For the control, the procedure was the same, only replacing the suspension of conidia for distilled water. After the 24-hour period, the suspension was withdrawn and 100 mL of nutrient solution were added as proposed by Clark (1975). This solution was changed every three days.

Table 1. Genotypes of *Passiflora* spp. used in interspecific crossings to obtain families of complete siblings aiming resistance to fusariosis and base rot. Cáceres, Mato Grosso, 2019.

Parents/Crosses	Family	Genotypes/hybrids
<i>P. nitida</i> ♂		
<i>P. edulis</i> (51) x <i>P. nitida</i> (B3P2)	122	1
<i>P. edulis</i> (80) x <i>P. nitida</i> (B3P2)	124	1,2,3
<i>P. edulis</i> (113) x <i>P. nitida</i> (B3P2)	128	1
<i>P. edulis</i> (UFV50) x <i>P. nitida</i> (B3P2)	142	1
<i>P. edulis</i> (UFV50) x <i>P. nitida</i> (B3P1)	143	1,2
<i>P. quadrangularis</i> ♂		
<i>P. edulis</i> (22) x <i>P. quadrangularis</i> (B3P1)	113	1,2,3,4,5,6,7,8,9
<i>P. edulis</i> (51) x <i>P. quadrangularis</i> (B3P3)	115	1,2,3,4,5,6,7,8,9
<i>P. edulis</i> (113) x <i>P. quadrangularis</i> (B3P1)	125	1,2,3
<i>P. edulis</i> (51) x <i>P. quadrangularis</i> (B3P1)	126	1,2,3
<i>P. cincinnata</i> ♂		
<i>P. edulis</i> (22) x <i>P. cincinnata</i> (B3P2)	121	1,2,3,4,5,6,7,8
<i>P. mucronata</i> ♂		
<i>P. edulis</i> (51) x <i>P. mucronata</i> (B1P2)	116	1,2,3,4,5,6,7,8,9

Source: Authors (2019).

We evaluated the survival period (period in days from inoculation to plant death) and the number of live plants. Evaluations were carried out periodically up to 33 DAI. During this period, symptomatic seedlings had parts of their stem and roots disinfested and transferred to PDA medium to confirm the etiological agent. After collection, the data were submitted to Scott Knott test at 5% probability and graphic dispersion using the means of the characteristics. The statistical analysis was performed using the software Genes (Cruz, 2016).

3. Results and Discussion

The 411 flowers of *P. edulis* were pollinated manually to obtain interspecific hybrids: 88 flowers with pollen of *P. quadrangularis*, 161 flowers with pollen of *P. nitida*, 64 flowers with pollen of *P. cincinnata*, and 98 flowers with pollen of *P. mucronata*. However, only 4.54% of hybridizations with the species *P. quadrangularis* generated fruits with viable seeds: *P. nitida* had 3.10% of hybridizations. For *P. cincinnata* and *P. mucronata*, this percentage was below 2%. We observed especially for hybrids with *P. cincinnata* that many fruits aborted before they had even matured, and some fruits produced seeds without embryos.

Ocampo et al. (2016), working with crossings between six wild species of *Passiflora*, among them *P. cincinnata* and *P. mucronata*, and six cultivars of *P. edulis*, observed that there are pre-zygotic barriers, such as gametophytic and sporophytic incompatibility, that can influence the success of fertilization. In addition, they report that in 53.8% of interspecific crosses, the seeds presented flaws in the development of the endosperm, providing a low germination. In crossings of *P. cincinnata* with *P. edulis*, there was a high number of aborted fruits (81.2%) and a low percentage of germination (13.1%), similarly as those found in this work.

According to Bugallo et al. (2011), a common origin for many species of *Passiflora* is perhaps the answer to the presence of self-incompatibility genes, which were conserved during the evolution process and therefore prevented the formation of embryos in interspecific crosses.

However, several authors have reported success in interspecific hybridizations for the genus *Passiflora* (Junqueira et al., 2006; Bugallo et al., 2011; Coelho et al., 2015) and emphasized that for a successful hybridization to occur, it is necessary for parent species to be genetically close and present a certain chromosomal homology, minimizing the problems of incongruity or cross-incompatibility and thus making the use of the hybrid feasible (Pereira et al., 2005). In this sense, observing the results of this work, it appears that the interspecific hybridizations provided 49 viable hybrids (Table 1).

Santos et al. (2015), working with hybridization between *P. setacea* and *P. edulis*, found 100% of attachment for all crosses in which *P. edulis* was used as the female parent. According to Conceição et al. (2011), the attachment of fruits of interspecific hybridizations between wild species of *Passiflora* varied from 0 to 86% in the 24 crossings performed. In order to obtain ornamental and cold-tolerant hybrids, Bugallo et al. (2011) obtained 5.5 to

45.4% of attachment in interspecific crossings of *Passiflora*.

This variation in fertilization between the species studied may be related not only to genetic factors, but also to environmental factors such as temperature and photoperiod (Cordeiro et al., 2019). According to Sanzol and Herrero (2001), temperature, flower quality and chemical treatments can affect an effective pollination period in fruit species. According to Williams (1970), high temperatures (above 25°C) during anthesis can accelerate the growth of the pollen tube and prevent the fertilization of ovules that are still immature.

3.1 Confirmation of hybridization by molecular marker

From the 35 microsatellite primers (SSR) tested, the polymorphic markers that best discriminated the presence of information bands for confirmation of interspecific crosses were selected (Table 2).

Table 2. Microsatellite markers (SSR) with informative polymorphic bands for confirmation of interspecific hybrids of *Passiflora*. Caceres, Mato Grosso, 2019.

Parents	Family	Marker	Allele size range (bp)
<i>P.edulis</i> (22) x <i>P.quadrangularis</i> (B3P1)	113	Pe07	100-200
<i>P.edulis</i> (51) x <i>P.quadrangularis</i> (B3P3)	115	Pe25	100-250
<i>P.edulis</i> (113) x <i>P.quadrangularis</i> (B3P1)	125	Pe07	100-200
<i>P.edulis</i> (51) x <i>P.quadrangularis</i> (B3P1)	126	Pe07	100-200
<i>P.edulis</i> (22) x <i>P.cincinnata</i> (B3P2)	121	Pe12	150-200

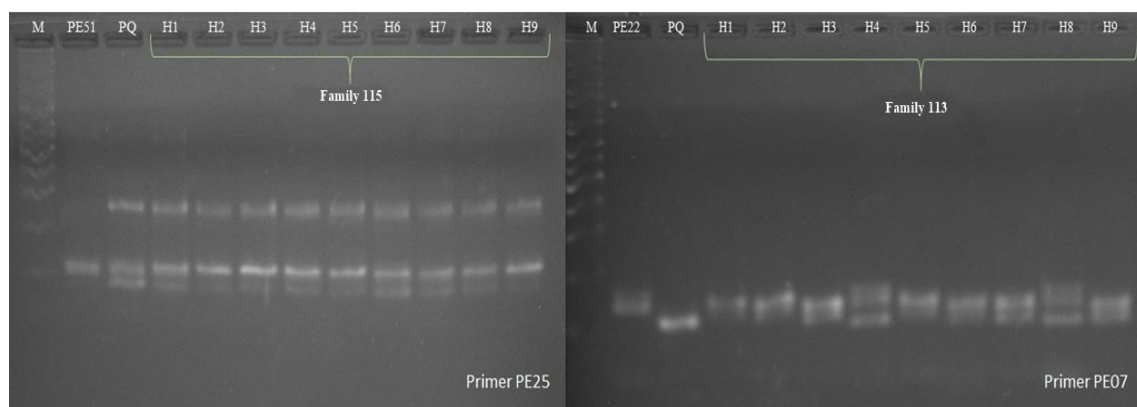
Source: Authors (2019).

The sizes of the amplified products, according to the pairs of initiators, varied from 100 to 250 bp. The size difference between the amplified fragments allows detecting polymorphism. According to Rallo et al. (2000), this difference is due to the number of replications within microsatellites. In addition, microsatellites appear to have a frequent and random distribution, allowing a complete coverage of the genome.

When comparing the alleles of individuals of each progeny with alleles of male parents (*Passiflora* spp. Wild) and female parents (*P. edulis*), 28 individuals (57% obtained

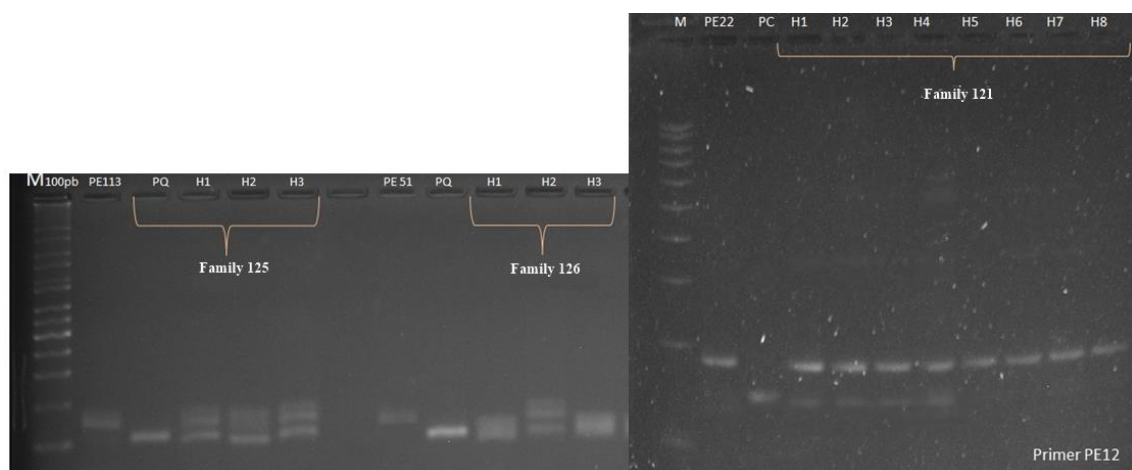
by interspecific hybridizations) presented alleles of both parents. All genotypes from crosses with the *P. quadrangularis* male parent (families 113, 115, 125 and 126) could be confirmed (Figure 1). In the family 121, hybridization was confirmed only for the genotypes 1, 2, 3 and 4, while the other individuals presented only the female parent allele (Figure 2).

Figure 1. 2.5% agarose gel with SSR loci of parents and genotypes of the families 115 (A) and 113 (B) resulting from interspecific hybridizations. M: 100 bp Ladder marker; FP: *P. edulis* female parent; MP: *P. quadrangularis* male parent; H: interspecific hybrid. Cáceres, Mato Grosso, 2019.



Source: Authors (2019).

Figure 2. 2.5% agarose gel with SSR loci of parents and genotypes of the families 125, 126 (A) and 121 (B) resulting from interspecific hybridizations. M: 100 bp Ladder marker; FP: *P. edulis* female parent; MP: *P. quadrangularis* male parent; MP: *P. cincinnata* male parent; H: interspecific hybrid. Cáceres, Mato Grosso, 2019.



Source: Authors (2019).

According to Faleiro et al. (2003), working with microsatellite markers to detect interspecific crossing between *Theobroma cacao* and *T. grandiflorum*, the use of one or two SSR primers or combinations of primers with at least one information band is enough to confirm the occurrence of cross-fertilization. However, for the genotypes originating from crosses with *P. nitida* and *P. mucronata* parents, it was not possible to detect hybridization because no polymorphic informative loci were found among the 35 studied primers. According to reports by Cerqueira-Silva et al. (2012), a low number of alleles per locus and few polymorphic microsatellite markers are characteristic of the genus *Passiflora*, which may suggest that these loci are concentrated in conserved regions with a low rate of mutation. Other authors, such as Cazé et al. (2012) and Paiva (2014), upon characterizing SSR loci created for *Passiflora* spp., detected allele numbers per locus ranging from 2 to 9 alleles.

In *Passiflora*, several authors have reported using SSR markers to confirm interspecific hybridizations. Santos et al. (2012) showed cross-pollination between twenty ornamental hybrids of *P. sub lanceolata* with *P. foetida* var. *foetida* using microsatellite markers. Belo et al. (2018) also confirmed interspecific hybridization between *P. gardneri* and *P. gibertii* using the SSR Pe75, a marker that amplified heterozygous bands of 300 and 350 pb.

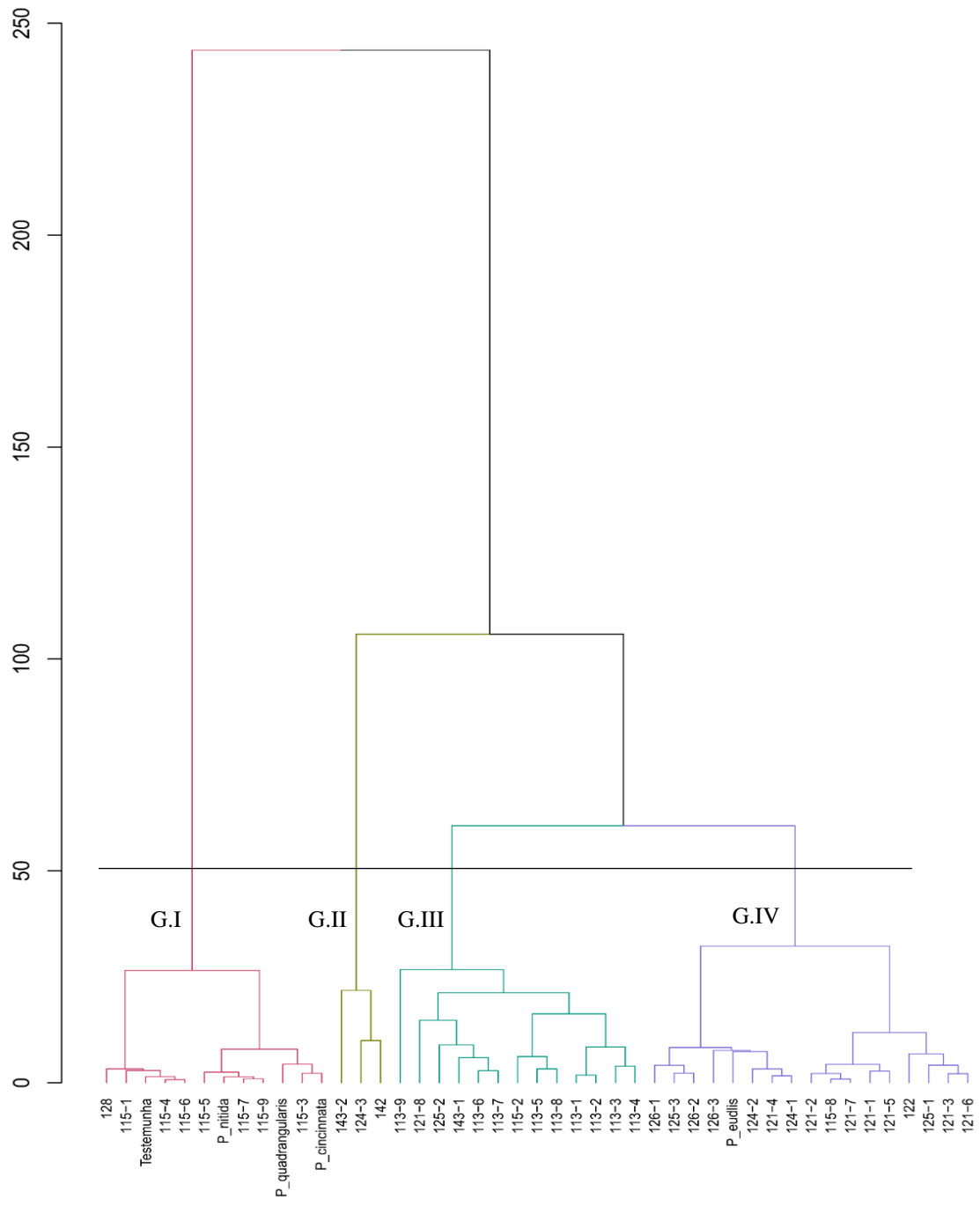
Confirmation of interspecific hybridization is necessary in plant breeding programs since, even with care in the hybridization procedure, such as removing anther before floral anthesis, the possibility of self-pollination cannot be ruled out. This is justified by the presence of autogamy in some cultivars of *P. edulis*, as highlighted by Araya et al. (2017) in their research with the cultivar BRS MJ, which was self-compatible with an autogamy rate of up to 99.9%.

3.2 Evaluation of resistance to *F. solani*

By performing ANOVA at 1 or 5% probability, there was a difference between interspecific passion fruit hybrids for the ten resistance characteristics. Since it is a cross between contrasting species aiming resistance, variability in progenies was expected.

This variability of genotypes through resistance can be observed in the multivariate analysis, making it possible to form four groups with a cut-off point at 50% (Figure 3).

Figure 3. Dendrogram representing the genetic divergence between forty interspecific passion fruit hybrids obtained by the Ward Hierarchical grouping method based on ten characteristics of resistance to base rot (cophenetic correlation coefficient = 0.71). Cáceres, Mato Grosso, 2019.



Source: Authors (2019).

Group I (GI) was formed by 12 genotypes, including the resistant parents involved in crosses (*P. nitida*, *P. quadrangularis*, *P. cincinnata*) and the control *P. edulis* without inoculum. The hybrids in this group come from crosses using *P. nitida* and *P. quadrangularis* male parents and presented the best values for all resistance characteristics evaluated, with higher SP at 27.39 days, lower number of dead plants (average 0.83 plants), lower CL (9.25 mm), LL (2.49 mm), and NAULEAC (499.17 mm). This group is composed of the most resistant hybrids to base rot: 115-1, 115-3, 115-4, 115-5, 115-6, 115-7, 115-9 and 128.

It is proven that the species that grouped with the hybrids present a genetic resistance to *F. solani* (Fischer et al., 2005; Freitas et al., 2016; Preisigke et al., 2015). However, the species *P. cincinnata* was not efficient in transferring its resistance gene to descendants. Studies on the introgression of genes resistant to *F. solani* in passion fruit are still incipient. Thus, the importance of this work, as it was possible to identify eight resistant hybrids. They can be recommended for use in rootstocks and for the improvement of generations in genetic improvement programs.

Group II (G.II) was formed by three genotypes (143-2, 124-3 and 142), all from crossing with the *P. nitida* male parent. This group was responsible for intermediate values of SP at an average of 19.65 days, and the second lowest value for NSP with an average of 2.38 plants. However, they presented a lower value for NPL-50%, with an average of 1.11 plant. Note that for these hybrids, base rot had a higher disease progression than group I, but lower than the other groups.

In the third group (G.III) are all genotypes of the family 113, in addition to the hybrids 121-8, 115-2, 125-2 and 143-1. These hybrids also have an intermediate resistance, differing from G.II, which presents lower values for SP (15.22 days) and higher values for NPL-50% (1.56 plants) and PILA+50% (23.28 days).

The fourth group gathered 17 genotypes: the negative control (*P. edulis* with inoculum), all hybrids of the families 121 and 126, and the genotypes 122, 124-1, 124-2, 125-1, 125-2 and 115-8. This group gathered the genotypes with less resistance to *F. solani*, obtaining a lower SP (11.28 days), higher NSP (2.82 plants), a higher CL average (25.03 mm), a LL of 15.79 mm and, consequently, a greater NAULEAC (4,016.65 mm).

Regarding the relative contribution of each resistance characteristic to *F. solani* to the genetic diversity among interspecific hybrids based on the criterion proposed by Singh, it is noted that the NSP variable contributed the most to the discrimination of genotypes (36.52%), followed by LL (23.42%), NAULEC (15.20%), and NAULEAC (10.71%). These four variables were responsible for 85.86% of the total distribution. They are considered the most

important in the present study for the characterization of hybrids resistant to base rot.

Similar data were reported by Preisigke et al. (2015) upon indicating the variables NAULEAC, NPL-50% and NSP among the four most important for the diversity of the 14 species of *Passiflora* with respect to resistance to *F. solani*.

3.3 Evaluation of resistance to *F. oxysporum* f. sp. *passiflorae*

The variables studied in the pathogen resistance test to *F. oxysporum* f. sp. *passiflorae* showed significant differences between genotype means at 1% probability. The survival period of the evaluated genotypes varied from 14.22 to 30.99 days. The hybrid 143-2 (*P. edulis* (UFV50) x *P. nitida* (B3P1)) had the longest survival period, with a mean of 24.66 days (Table 3). The genotypes 143-2 and 142 were the most resistant to fusariosis by crossing *P. edulis* with *P. nitida*.

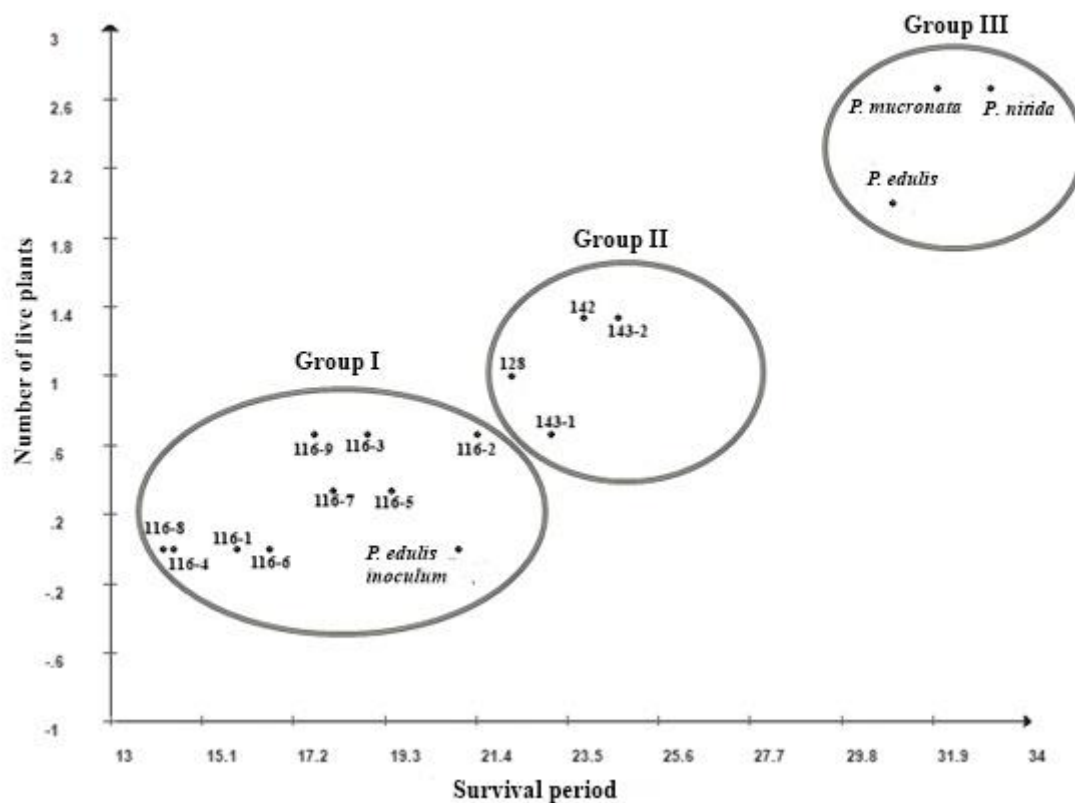
Table 3. Average of the two characteristics of resistance to *F. oxysporum* f. sp. *passiflorae* evaluated in 17 genotypes of *Passiflora*. Cáceres, Mato Grosso, 2019.

Genotype/hybrid	Variable	
	Survival period	No. of Live Plants
116-1	15.88 c	0.00 c
116-2	21.44 b	0.66 c
116-3	18.88 c	0.66 c
116-4	14.44 c	0.00 c
116-5	19.44 c	0.33 c
116-6	16.66 c	0.00 c
116-7	18.11 c	0.33 c
116-8	14.22 c	0.00 c
116-9	17.66 c	0.66 c
128	22.22 b	1.0 c
142	23.88 b	1.33 b
143-1	23.11 b	0.66 c
143-2	24.66 b	1.33 b
<i>P. mucronata</i>	32.00 a	2.66 a
<i>P. nitida</i>	33.22 a	2.66 a
<i>P. edulis</i> /inoculum	21.00 b	0.00 c
Control	30.99 a	2.00 a

* Means followed by the same lowercase letter in columns do not differ by Scott Knott test at 5% probability. Source: Authors, (2019).

From 17 genotypes, 13 of which are interspecific hybrids, three are parents (*P. edulis*, *P. mucronata* and *P. nitida*) and one is a positive control (*P. edulis* without inoculum). There was the formation of three groups according to the graphic dispersion of the genotypes in relation to the characteristics of resistance to fusariosis (Figure 4).

Figure 4. Graphical dispersion of genotypes for resistance to *Fusarium oxysporum* f. sp. *passiflorae* (number of live plants and survival period) for 13 interspecific hybrids of passion fruit, the parents (*P. edulis*, *P. mucronata* and *P. nitida*) and a positive control (*P. edulis* without inoculum). Cáceres, Mato Grosso, 2019.



Source: Authors, (2019).

In the first group are all genotypes of the family 116, which originated from the crossing of *P. edulis* (51) with *P. mucronata*, and the susceptible parent (inoculated *P. edulis*). The plants in this group showed a greater susceptibility to *F. oxysporum* f. sp. *passiflorae* because a maximum survival period of 21 days was found. Thus, the genotypes of the 116 family are not recommended for the next stage of the breeding program aiming resistance to fusariosis, considering that they presented a susceptibility similar as or greater than the species *P. edulis*. Although the species *P. mucronata* shows resistance to the pathogen, in this study it was not efficient for transferring this characteristic to the descendants, indicating that the inheritance of resistance in this crossing is of a complex nature.

Group II was formed by the most resistant hybrids (128, 142, 143-1 and 143-2), progenies from crossbreeding with the *P. nitida* male parent. According to Preisigke et al.

(2017), the species *P. nitida* is among the most resistant among the 14 evaluated in their studies. These genotypes, especially 142 and 143-2, have the potential to be used at subsequent stages of the breeding program, including as a rootstock. Data using *P. nitida* as rootstock for *P. edulis* evidenced positive results for the control of fusariosis (Chaves et al., 2004; Junqueira et. al., 2006); however, productivity was lower than conventional planting. Thus, using an interspecific hybrid (*P. nitida* x *P. edulis*) as a grafting horse could restore this productivity.

Finally, group III gathered wild species involved in interspecific crossings: *P. nitida*, *P. mucronata* and the control. This grouping was already expected, since wild species are more resistant than interspecific hybrids and *P. edulis*. This was because progenies inherited only 50% of the paternal genomic material and 50% of the maternal material (Griffiths, 2009). This may suggest the action of more than one gene conferring resistance.

In plants identified as resistant, there was the formation of adventitious roots. This reaction of plants, according to Ortiz et al. (2014), is associated with an important defense function and is accompanied by hypertrophy of the vascular parenchyma, which helps to produce ethylene.

Obtaining hybrids 142 and 143-2 (*P. nitida* x *P. edulis*) provides great advances to passion fruit culture as these genotypes are resistant to fusariosis and moderately resistant to base rot, limiting diseases. These hybrids can be used as rootstock and for improvements in breeding programs, which will culminate in a resistant cultivar.

4. Conclusions

It was possible to obtain viable hybrids among the species *P. nitida*, *P. quadrangularis*, *P. cincinnata* and *P. mucronata* with *P. edulis*, confirming a 57% paternity of the genotypes analyzed. All genotypes from crosses with the *P. quadrangularis* male parent could be confirmed.

The hybrids 115-1, 115-3, 115-4, 115-5, 115-6, 115-7, 115-9 and 128 are recommended to breeding programs aiming resistance to soil pathogens and base rot, and the hybrids 142 and 143-2 are recommended for resistance to fusariosis.

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