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**RESEARCH ARTICLE / *ARTIGO***

# Identification of passion fruit genotypes resistant to *Fusarium oxysporum* f. sp. *passiflorae*

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**ABSTRACT**

This study aimed to characterize the resistance of 31 passion fruit (*Passiflora* sp.) genotypes to *Fusarium oxysporum* f. sp. *passiflorae* (FOP). About 20 seedlings of each genotype were inoculated by immersing the roots in a suspension of $10^6$ macroconidia $mL^{-1}$ for five minutes and then transplanting them into pots containing a mixture of soil and sterile substrate. They were evaluated daily for 120 days for the occurrence of wilt symptoms and death. These data were used to calculate the area under the disease progress curve, for survival analysis and grouping. The mortality rate ranged from 0 to 100% and the AUDPC ranged from 0 for genotypes without symptoms of the disease to 6,650.63 for the hybrid HFOP-01. Genotypes were classified into four subgroups: resistant, moderately resistant, susceptible and highly susceptible. The resistance was genotype dependent, but no significant variation was found between the purple and the yellow *P. edulis*.
**Key words**: *Passiflora*, germplasm, genetic improvement, passion fruit withering, survival analysis.

## INTRODUCTION

The passion fruit belongs to the genus *Passiflora*, which is considered the most important genus of the family *Passifloraceae* (Bernacci et al., 2003; Nunes & Queiroz, 2006) with the greatest number of cultivated species. The passion fruit originates in tropical and subtropical America. In Brazil, there are about 131 species, 88 of them are endemic (Cervi et al., 2011). *Passiflora edulis*, the yellow passion fruit, is the most widely commercially cultivated species (Ganga et al., 2004).

Brazil is the largest producer and consumer of passion fruit in the world, and the cultivation area is expanding. As a consequence, pest problems have become apparent or have worsened. Diseases caused by soilborne pathogens are economically important, as they reduce the useful life of the passion fruit and turn it into a transient crop, with orchards renewed every two years or less (Alabouvette, 1999). Among these diseases, stands out the fusariosis or the passion fruit wilt caused by the fungus *Fusarium oxysporum* f. sp. *passiflorae* (FOP) (McKnight, 1951). FOP occurs in several producing regions of Brazil, affecting the crop yield (Liberato & Costa, 2001) and causing economic losses and social problems. The importance of fusariosis is enhanced by the pathogen ability to survive for long periods in the soil as chlamydospores (Agrios, 2005).

The fungus penetrates the roots and its hyphae invade the xylem vessels, preventing the transport of water and nutrients to other organs of the plant. Wilted branches are the first visible symptoms and can occur in any season or phenological stage of the plant. Longitudinal sections of the wood show streaks of a red-brown discoloration, indicating the presence of chromatic cells that waterproof the timber vessels (Santos Filho, 1998).

The main measures to control the disease are preventive and cannot eliminate the pathogen if it is already in the soil (Roncatto et al., 2004). The use of resistant cultivars is the most practical and efficient method of control for most diseases caused by soilborne fungi, including the *F. oxysporum* complex (Navas-Cortés et al., 2008). Unfortunately, attempts to find passion fruit resistant varieties have been unsuccessful (Yamashiro & Cardoso, 1982).

In South Africa, Grech & Rijkenberg (1991) reported that *Passiflora caerulea* is resistant and can be used as a rootstock for purple passion fruit; such findings have not been explored in Brazil. Purss (1958) evaluated the resistance of various *Passiflora* species and found the yellow passion fruit to be the most promising material. Gardner (1989) reached the same conclusion, also identifying *Passiflora suberosa* as a resistant species. In Brazil, however, the yellow passion fruit is highly susceptible to fusariosis. Nevertheless, in field experiments, variation in symptoms expression has been observed, regardless the studied *P. edulis*, some purple selections show lower proportions of dead plants than the yellow ones.

Considering the great genetic variability within the genus *Passiflora* (Bernacci et al., 2005), coupled with the wide variation in resistance to fusariosis among species from the genus *Fusarium*, it is necessary a better understanding of how the passion fruit germplasm reacts to FOP infections. This study aimed to characterize the reaction of 31 *Passiflora* accessions to Fusarium wilt after inoculation under controlled conditions.

## MATERIAL AND METHODS

### Plant material

Thirty one accessions from the Active Germplasm Bank of Embrapa Mandioca e Fruticultura (code BGP) were used, including six purple passion fruit, 11 yellow passion fruit, five accessions of *P. cincinnata* Mast., one accession of *P. alata* Curtis, and eight yellow passion fruit hybrids. A commercial variety of yellow passion fruit, FB 100, was used as a susceptible control (Table 1). The seedlings were grown in tubes containing vermiculite and maintained in greenhouse. The seedlings were inoculated when they reached the three-leaves stage, 60 days after planting. The experimental design was completely randomized; in average, twenty plants per accession were evaluated, with each plant considered to be an observation.

### Isolates and inoculation

The isolate of F. *oxysporum* f. sp. *passiflorae* - FOP002 was collected from passion fruit plants with typical wilting symptoms in Cruz das Almas, BA. After single spore cultivation, the species was identified by morphological characterization at the Universidade Federal de Lavras and stored in the mycology collection of the Phytopathology Laboratory of Embrapa Mandioca e Fruticultura.

To produce the inoculum, mycelial disks were transferred to Petri dishes containing potato-dextrose-agar (PDA) media (41 g $L^{-1}$). The dishes were kept in BOD for seven days continuously at 25°C, under light. The spore suspension was prepared some minutes before inoculation: 10 mL of sterile distilled water was added to the Petri dishes, and the fungal colonies were scraped off to release the spores. The resulting suspension was filtered in a double layer of sterile gauze, and the spore concentration was adjusted to $10^6$ macroconidia $mL^{-1}$ using a Neubauer chamber.

The seedlings were removed from the sterile soil in which they were grown and their roots were washed with sterile water. The root system was then immersed for 5 min in the conidial suspension before being replanted in 15 cm pots containing 3:1 soil:sterilized vermiculite (w:w). Plants whose roots were soaked only in sterile water were used as a control.

### Disease evaluation and data analysis

Seedlings were evaluated daily from the third day after inoculation (DAI) until 120 DAI and the occurrence of wilting or death was recorded. Symptomatic seedlings had parts of their stem and roots sterilized and transferred to PDA media. After five days, the colonies were evaluated for macro- and microconidia to confirm the etiologic agent.

The percentage of dead plants and the area under the disease progress curve (AUDPC) (Campbell & Madden, 1990) was calculated for each genotype every day. In addition, the time between inoculation and the appearance of wilt symptoms was determined for each plant, and the average incubation period (IP) for the genotype was calculated.

From the AUDPC of each accession, genotype clustering was performed using the Ward method with Euclidean distance as a dissimilarity measure. All tests were performed using the R statistical program, version 2.12.0 (R Development Core Team, 2008). To confirm the consistency of the grouping in each resistance group, the genotypes with

**TABLE 1 -** List of passion fruit accessions from the Active Germplasm Bank (PF-AGB) of Embrapa Mandioca e Fruticultura, hybrid and commercial variety used to identify sources of resistance to fusariosis

| Accession | Scientific and common name |
|---|---|
| BGP018 | *Passiflora edulis* (yellow passion fruit) |
| BGP020 | *Passiflora edulis* (yellow passion fruit) |
| BGP023 | *Passiflora edulis* (purple passion fruit) |
| BGP028 | *Passiflora edulis* (yellow passion fruit) |
| BGP029 | *Passiflora edulis* (purple passion fruit) |
| BGP033 | *Passiflora edulis* (purple passion fruit) |
| BGP037 | *Passiflora edulis* (yellow passion fruit) |
| BGP168 | *Passiflora edulis* (purple passion fruit) |
| BGP177 | *Passiflora edulis* (yellow passion fruit) |
| BGP179 | *Passiflora edulis* (yellow passion fruit) |
| BGP181 | *Passiflora edulis* (yellow passion fruit) |
| BGP185 | *Passiflora edulis* (yellow passion fruit) |
| BGP188 | *Passiflora edulis* (yellow passion fruit) |
| BGP207 | *Passiflora edulis* (yellow passion fruit) |
| BGP208 | *Passiflora edulis* (purple passion fruit) |
| BGP224 | *Passiflora edulis* (yellow passion fruit) |
| BGP229 | *Passiflora edulis* (purple passion fruit) |
| BGP235 | *Passiflora alata* (sweet passion fruit) |
| BGP268 | *Passiflora cincinnata* (crato passion fruit) |
| BGP290 | *Passiflora cincinnata* (crato passion fruit) |
| BGP296 | *Passiflora cincinnata* (crato passion fruit) |
| BGP297 | *Passiflora cincinnata* (crato passion fruit) |
| BGP298 | *Passiflora cincinnata* (crato passion fruit) |
| HFOP-01 | *Passiflora edulis* (hybrid of yellow passion fruit) |
| HFOP-04 | *Passiflora edulis* (hybrid of yellow passion fruit) |
| HFOP-05 | *Passiflora edulis* (hybrid of yellow passion fruit) |
| HFOP-07 | *Passiflora edulis* (hybrid of yellow passion fruit) |
| HFOP-08 | *Passiflora edulis* (hybrid of yellow passion fruit) |
| HFOP-09 | *Passiflora edulis* (hybrid of yellow passion fruit) |
| HFOP-10 | *Passiflora edulis* (hybrid of yellow passion fruit) |
| HFOP-13 | *Passiflora edulis* (hybrid of yellow passion fruit) |
| FB 100 | *Passiflora edulis* (yellow passion fruit) |

lower and higher final mortality were compared, using the proportions comparison test (Zar, 1996). The groups were considered consistent when statistical differences were not detected (P>0.05). Survival analysis was used to test the adequacy of the classification of groups. Kaplan-Meier curves were produced for each resistance group, which were compared by Cox's F test. The classification was considered appropriate when the groups survival curves were statistically different (P<0.001). The consistency and adequacy analyses were performed with the Statistica 7 software (Statsoft, 2005).

## RESULTS

### Resistance detection

In a total of 900 plants, 262 plants wilted and died. The pathogen was reisolated from 97% of these plants, confirming the cause of death. Accession BGP181 had the first symptomatic plants at 7 DAI. However, on average the incubation period for all accessions that showed symptoms was 34 days.

All yellow passion fruit BGP018 plants died by 120 DAI. In contrast, eight genotypes had no mortality, including one accession of yellow passion fruit (BGP020), one *P. alata* (BGP235), five accessions of *P. cincinnata* (BGP268, BGP290, BGP296, BGP297, and BGP298) and a hybrid (HFOP-07) (Table 2).

The AUDPC ranged from 0 for genotypes without symptoms to 6.350,63 for the HFOP-01 hybrid. This hybrid, despite having the second highest mortality rate, had an incubation period lower than the BGP018 accession (Table 2).

### Cluster and survival analyses

The dissimilarity matrix allowed for grouping the genotypes into two main groups: resistant (Group 1) and susceptible (Group 2) (Figure 1). Each cluster was divided into two subgroups, resulting in a total of four subgroups

**TABLE 2 -** Results of evaluations from the inoculation of *Fusarium oxysporum* f. sp. *passiflorae* 120 days after inoculation

| Access | PI[1] | % Mortality | AUDPC[2] | Level of resistance[3] |
|---|---|---|---|---|
| BGP020 | - | 0.00 | 0.00 | R |
| BGP235 | - | 0.00 | 0.00 | R |
| BGP268 | - | 0.00 | 0.00 | R |
| BGP290 | - | 0.00 | 0.00 | R |
| BGP296 | - | 0.00 | 0.00 | R |
| BGP297 | - | 0.00 | 0.00 | R |
| BGP298 | - | 0.00 | 0.00 | R |
| HFOP-07 | - | 0.00 | 0.00 | R |
| BGP029 | 41 | 5.88 | 358.68 | MR |
| BGP037 | 72 | 28.57 | 791.20 | MR |
| BGP188 | 85 | 19.04 | 609.28 | MR |
| BGP207 | 95 | 27.77 | 419.33 | MR |
| BGP208 | 49 | 9.09 | 301.48 | MR |
| FB 100 | 15 | 12.76 | 612.20 | MR |
| HFOP-13 | 39 | 17.77 | 759.60 | MR |
| BGP023 | 13 | 22.00 | 1397.00 | S |
| BGP028 | 18 | 27.58 | 1861.37 | S |
| BGP033 | 8 | 17.77 | 1590.56 | S |
| BGP168 | 22 | 26.92 | 1270.68 | S |
| BGP177 | 26 | 31.57 | 2317.74 | S |
| BGP179 | 31 | 56.25 | 2990.39 | S |
| BGP181 | 7 | 17.94 | 1332.79 | S |
| BGP185 | 34 | 28.57 | 1599.59 | S |
| BGP224 | 32 | 33.32 | 2565.64 | S |
| BGP229 | 67 | 80.95 | 2546.19 | S |
| HFOP-05 | 37 | 36.84 | 1665.43 | S |
| HFOP-08 | 30 | 16.66 | 1457.75 | S |
| HFOP-09 | 12 | 42.00 | 2595.00 | S |
| HFOP-10 | 43 | 19.04 | 1204.28 | S |
| HFOP-01 | 11 | 85.41 | 6350.63 | HS |
| HFOP-04 | 10 | 62.71 | 3756.21 | HS |
| BGP018 | 14 | 100.00 | 5040.05 | HS |

[1]PI= incubation period; [2]Area under the disease progress curve; [3]R = resistant; MR = moderately resistant; S = susceptible; and HS = highly susceptible

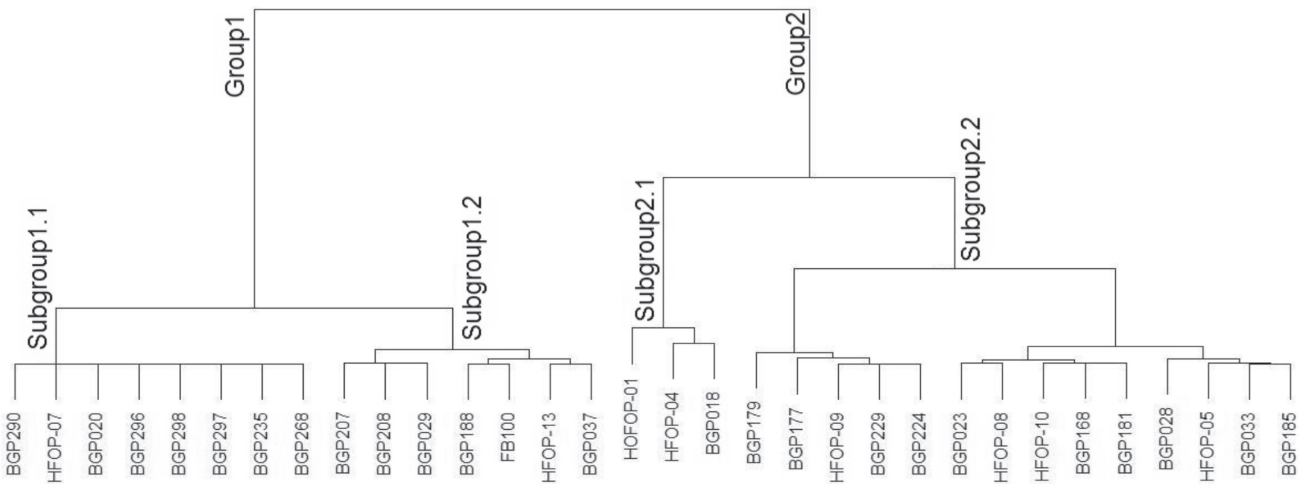


**FIGURE 1 -** Clustering of passion fruit genotypes evaluated for resistance to fusariosis based on the area under the disease progress curve.

based on AUDPC: resistant, moderately resistant, susceptible and highly susceptible.

Among the 32 evaluated genotypes, 25% (eight accessions) were included in the resistant subgroup 1.1, which was composed of an accession of yellow passion fruit (BGP020), one sweet passion fruit (BGP235), five accessions of *P. cincinnata* (BGP268, BGP290, BGP296, BGP297, and BGP298) and one hybrid (HFOP-07). Another seven accessions were classified in subgroup 1.2 as moderately resistant, with a mean incubation period of 56 DAI and a mean mortality rate of 14.73%. These included three accessions of yellow passion fruit (BGP037, BGP118 and BGP207), two accessions of purple passion fruit (BGP208 and BGP029), one hybrid of yellow passion fruit (HFOP-13) and the commercial control FB100.

In the susceptible subgroup 2.2, with a mean incubation period of 27 DAI and a mean mortality rate of 32.67%, six accessions of yellow passion fruit (BGP028, BGP177, BGP179, BGP181, BGP185, and BGP224), four accessions of purple passion fruit (BGP023, BGP033, BGP168 and BGP229) and four hybrids of yellow passion fruit (HFOP-05, HFOP-08, HFOP-09 and HFOP10) were included. In the highly susceptible subgroup 2.1, one accession of yellow passion fruit (BGP018) and two hybrids (HFOP-01 and HFOP-04) were classified, with a mean incubation period of 11 DAI and a mean mortality rate of 82.71%.

The survival curves estimated by the Kaplan-Meier method showed a clear separation for the four groups (Figure 2). The highly susceptible and susceptible subgroups had a lower survival and lower incubation period compared with the moderately resistant and resistant subgroups. All curves were statistically different (Cox's F-test, $p<0.001$). The proportions test showed no statistical differences within the groups.

## DISCUSSION

Fusarium wilt symptoms typically occur between the 2nd and 4th DAI for several species (Alexopoulos et al., 1996), but depend on the host. In this work, the first symptoms were observed 7 DAI for the most susceptible genotypes, extending up to 117 DAI for genotypes with some level of resistance. The initiation and continuation of symptoms varied greatly for each accession, which indicates that the selection of resistant plants appears to be a long-term and genetically dependent process, even under conditions controlled for fungal growth and symptoms onset. The seedlings used in this study originated from crossings, with controlled crossings for almost all of the accessions. Because there was no inbreeding in the parents of these plants, high levels of genetic variability were generated among the accessions, even though they belong to the same species. Ssekyewa et al. (1999) and Fischer et al. (2005) observed differences in resistance for yellow passion fruit genotypes that were inoculated with *Fusarium solani.* This variability should be further explored for the selection of genotypes that are resistant to fusariosis.

The differences in the resistance between and within each genotype may be associated with the high level of heterozygosity of passion fruit, an allogamous plant with gametophytic self-incompatibility (Suassuna et al., 2003). Considering the resistance of the passion fruit to fusariosis as a quantitative trait, the mortality of plants within each genotype would be directly related to the frequency of allelic resistance genes. The use of only one isolate of the pathogen may have also influenced the results, as the reaction of plants to different isolates can be individualized (Cavalcanti et al., 2002).

A long incubation period is an important component for host plants to have a partial resistance to a given pathogen (Van der Plank, 1963). The BGP207 accession, classified as

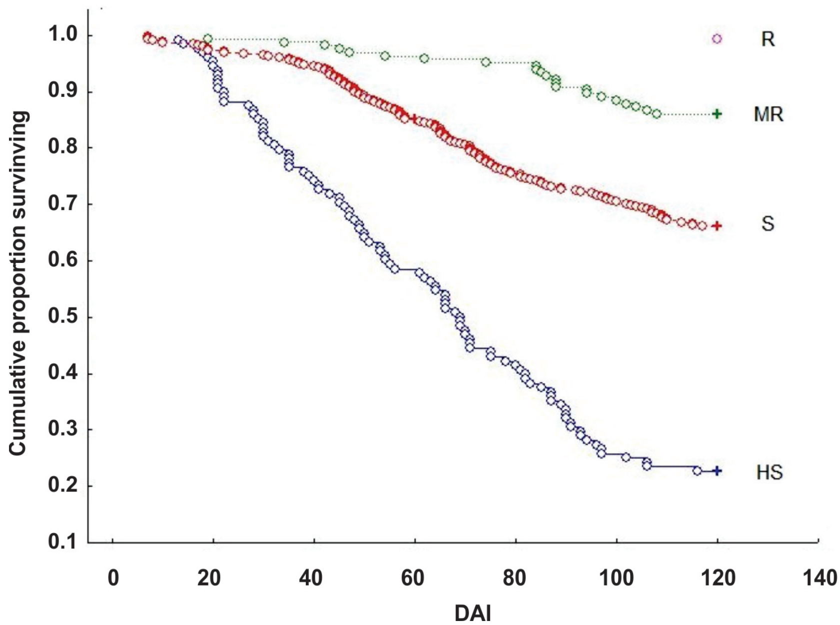


**FIGURE 2** - Kaplan-Meier curves of survival of groups of resistance to *Fusarium oxysporum* f. sp. *passiflorae*. The survival profiles of the groups were statistically different from each other by the F-Cox test (P <0.001).

moderately resistant, had a higher mortality than the HFOP-08, which was grouped as susceptible. This fact is explained by the AUDPC of the first being lower than the second as a result of the incubation period, which was 95 days for BGP207 and 30 days for HFOP-08. Within the moderately resistant group, a large proportion of plants survived and could be taken to the field for further studies. It is possible that they are resistant, because of the high variability of passion fruit within the accessions.

The AUDPC allowed for the distinction between resistance among the accessions with reliability, because of the inclusion of both the time of assessment and the severity of the disease. Although the purple passion fruit is considered to be susceptible in some regions, the results of this study revealed that there are different forms of purple germplasm with differing levels of resistance to fusariosis, i.e., BGP029 and BGP208 (moderately resistant). This finding demonstrates, once again, that the resistance is possibly dependent on genotype.

Among the 20 yellow passion fruit genotypes evaluated, five of them were classified as moderately resistant, with one accession (BGP020) and one hybrid of yellow passion fruit (HFOP-07) deemed resistant. These observations are very relevant because the HFOP-07 hybrid already has highly competitive agronomic traits in relation to the commercially available cultivars. Accessions BGP020 (R), BGP029, BGP037, BGP188, BGP207 and BGP208 (MR) are not competitive from an agronomic point of view, with traits that do not allow for their use as cultivars. However, because they belong to the *P. edulis* species, they share phenotypic characteristics that facilitate transferring resistance genes.

The search for resistance in *P. edulis* is important in order to add this trait to materials with commercial potential in a short period of time (Roncatto et al., 2004). However, Laranjeira et al. (2005) quantified the incidence of Fusarium wilt in passion fruit plants grafted to *P. edulis, P. alata, P. gibertii* and *P. cincinnata* using a survival analysis and revealed that the yellow passion fruit showed a worse survival profile than the others. Nevertheless, they evaluated only one accession of *P. edulis*; as the resistance to FOP seems to be genotype-dependent, that particular accession may be susceptible to fusariosis.

Among the genotypes classified as resistant, five accessions of *P. cincinnata* and one of *P. alata* were included. *P. cincinnata* is considered to be a strong species with wide adaptation, which are important characteristics for breeding programs (Ruggiero & Oliveira, 1998). Field evaluations have shown that this species shows considerable resistance to fusariosis (Laranjeira et al., 2005). *P. alata* can be crossed with other *Passiflora* species (Coppens-D'eeckenbrugge, 2005) to obtain interspecific hybrids that expand the possibilities of the species even further.

Because *P. edulis* is considered susceptible in general, genetic improvement has been directed to obtain interspecific hybrids. This strategy is not always successful because of the incompatibility between species and hybrid sterility that is generated. The results presented herein can contribute to generate resistant genotypes in less time than is necessary with interspecific crossings.

Survival analysis provides tools that are specifically designed to handle data in which the response variable is a time until the occurrence of an event (Cox & Oakes, 1984; Kleinbaum, 1995; Scherm & Ojiambo, 2004). For this study, the event was the death of each plant. The Kaplan-Meier curves allowed for the evaluation of the pace at which events occurred in the different study groups. The survival curves of the four groups indicate that most deaths occurred between 45 and 100 days and suggest that the evaluation period may need to be extended for longer periods. This fact may explain why some species, such as *P. cincinnata*, that were considered to be resistant became susceptible to the pathogen when in infested fields for one or two years (Laranjeira et al., 2005).

The variation in the resistance of yellow and purple forms and within other species of passion fruit can be attributed to experimental conditions, differences between field and controlled conditions, and soil microbes. Another important factor is the isolate's aggressiveness, which is linked to its region of origin. Therefore, it is necessary to conduct studies to characterize the isolates so that the possible genetic differences can be known.

The study of genetic resistance must be directed toward understanding the inheritance of resistance to FOP. The *Fusarium oxysporum*-plant interaction is well studied, and genes for resistance to *Fusarium* have been identified in other species (Diener et al., 2005; Jiménes-Díaz et al., 1991; Rep et al., 2005; Sela-Buurlage et al., 2001). Most plants have the genetic machinery to defend themselves; the susceptibility of plants is the result of their failure to rapidly detect the presence of the pathogen or the lack of an important gene for resistance (Hammershimidt, 2001). Alternatively, some genetic change in the fungus may not allow for the interaction of signals between the host and the pathogen, thus preventing the reaction of the plant. For beans and chickpeas, the resistance to *Fusarium oxysporum* is polygenic (Cross et al., 2000; Jiménes-Díaz et al., 1991; Salgado, 1995). In contrast, genes that govern the reaction to *Fusarium* are dominant and independent for each physiological race of the pathogen, for the tomato plant (Cirulli & Alexander, 1996). The genetic control for resistance to passion fruit Fusarium wilt is still unknown. Therefore, it must be evaluated whether the genetic control is monogenic, polygenic (Agrios, 2005), or even an integration of the two types of resistance.

The passion fruit plant has large natural genetic variability for different characteristics of the plant and fruit spread in the Brazilian territory, placing the country among the major centers of genetic diversity of this species (Oliveira et al., 2008). It is possible to explore this variability with genetic improvement programs, aiming to increase the knowledge of the germplasm of wild passion fruit with significant genetic gains (Meletti & Brukner, 2001). These gains would allow for the expansion of its commercial cultivation, as well as identify sources of resistance to diseases, including fusariosis.

The evaluation of resistance sources under controlled conditions minimizes the environmental effects so that differences in the manifestation of symptoms are almost exclusively of a genetic origin. However, resistance observed in such conditions should be confirmed in the field under a natural infestation of FOP. Laranjeira and Oliveira (unpublished) evaluated accessions of *P. edulis, P. cincinnata* and *P. alata* in infested fields and found variations in the levels of intra-specific resistance; however, the species with the highest mortality was *P. edulis* in yellow form, as in this study.

The success of a genetic improvement program is connected to the efficient evaluation of a large number of accessions in a short time with little use of physical and financial resources. From this perspective, such screenings ensure the results reliability and reduce the number of accessions to be evaluated under field conditions. This work enabled the pre-selection of eight accessions that can be classified as resistant, which will be taken to FOP infested fields for further investigation. In this area of research, it is essential to establish a constant monitoring system for the variability of the pathogen, including the potential emergence of new variants.

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