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RESISTANCE TO DISEASES OF SOUR, SWEET AND WILD PASSION FRUIT GENOTYPES

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Abstract
The cultivation of passion fruit is important for Brazil, since the country is currently the largest producer and consumer of fruit in the world. However, the fields of passion fruit still face important problems due to the incidence and severity of diseases in the field. Thus, the present study aimed to assess resistance to bacterial and fungal diseases in 13 genotypes of sour, sweet and wild passion fruit, in field conditions in the Distrito Federal, Brazil. For this, a field experiment was installed in a randomized block design, with four replications and 13 treatments (genotypes). The characteristics of incidence, severity and degree of resistance for bacteriosis, septoriosis, scab and anthracnose diseases were evaluated in 5 fruits per plot of each genotype. Genetic parameters of the evaluated traits were also estimated. High heritability values and CVg/Cve ratio were observed for most of the evaluated characteristics. The genotypes presented mean values of incidence and severity of bacteriosis, septoriosis, scab and anthracnose different among them, and the one that presented the best results in the degree of resistance for all diseases was F1 (MAR20 # 24 x ECL7 P1 R4).

Keywords: *Cladosporium herbarum. Colletotrichum gloeosporioides. Passiflora* spp. Resistance. *Septoria passiflorae. Xanthomonas* spp.

1. Introduction

Brazil stands out as the world's largest producer of passion fruit, however with the expansion of the planted area there was the appearance and / or worsening of a large number of diseases. These phytosanitary problems have reduced the time of economic exploitation of the crop and even made its cultivation unviable in certain regions (Fischer et al. 2010).

Passion fruit is grown on small farms, most with orchards of 3 to 5 hectares. Although it is a high-risk culture, due to its high susceptibility to diseases, as it uses resources with high purchasing value and it is necessary to meet the quality requirements of the destination markets, it has been a very attractive activity, due to its high production value (Meletti 2011).

There is a constant change in the cultivated area, attributing an itinerant characteristic to the passion fruit culture. In part, this is due to the high number of diseases that accumulate over time in traditional growing regions and the intensity of the resulting damage, due to the lack of resistant varieties (Meletti 2011).

Therefore, it became necessary to obtain cultivars with resistance to diseases, either by incorporating resistance genes in the current elite cultivars, or in the development of new cultivars. In addition, there is a reduction in environmental impacts, such as a decrease in the use of pesticides, due to the incorporation of multiple resistance to diseases, in addition to reducing waste, resulting in the improvement and optimization of the use of natural resources culminating in a greater production per area (Meletti 2011).

Currently, genetic improvement programs aim mainly at increasing productivity and resistance to the main diseases of passion fruit (virus, bacteriosis, anthracnose, septoriosis and scab) occurring in the cultivated species (Faleiro et al. 2014). It is known that some uncultivated species can contribute substantially to genetic improvement, as they are resistant to diseases or pests (Jesus et al. 2014).

The Passion fruit plant (*Passiflora* spp.) has a wide genetic diversity, essentially Brazilian, assuming great importance, considering its potential for supplying useful genes for the genetic improvement of passion fruit. Therefore, it is necessary to explore the potential of wild passion fruit species and relatives involving basic research work in the areas of conservation and characterization of genetic resources and applied research aimed at genetic improvement. The integration between basic research and applied research is allowing the practical use of genetic resources, effectively contributing to the development of varieties, hybrids and other technological products (Faleiro et al. 2015).

The present study aimed to evaluate 13 sour, sweet and wild passion fruit genotypes resistance to bacterial and fungal diseases, to identify promising materials in field conditions in the Distrito Federal, Brazil.

2. Material and Methods

The study started in 2016 and was conducted in an experimental field at University of Brasília farm (FAL/UnB), Brasília, Distrito Federal, Brazil, located at 1.000 m of altitude between 15°56'550.6"S latitude and 47°56'2.5" longitude. According to the Köppen classification, the Distrito Federal climate is Aw, as also found in almost all territory of Goiás, which characterizes the climate as tropical with a dry winter season (Cardoso et al. 2014). The meteorological conditions were verified daily from the climatological database of the meteorological station of the University of Brasília, with an average monthly rainfall of 108 mm, an average temperature of 20.5 °C and an average relative humidity of 74% (UnB 2017).

Passion fruit seedlings of the species *P. edulis* Sims were obtained by sowing in polystyrene trays (72 cells) containing artificial substrate based on vermiculite and *Pinus* sp. bark, and kept in a protected environment. Approximately 25 days after sowing, the seedlings were transplanted into polystyrene bags (1.5 liters) containing sterile soil. The transplant to the field was carried out on October 4, 2016, when the seedlings were approximately 40 cm long and 2.5 months old. The seedlings of *Passiflora alata* Curtis. and *Passiflora cincinnata* Mast. were provided by EMBRAPA Cerrados. *Passiflora setacea* D.C. seedlings were acquired in a nursery licensed for sale by Embrapa Cerrados.

The experiment was installed using a randomized block design with 13 treatments, four replications and six plants in each plot, totaling 52 plots and 312 plants. The plants were conducted in a vertical trellis with posts spaced 6.0 m apart and two strands of smooth wire (nº12) at 1.60 m and 2.20 m in relation to the soil, spacing of 2.8 m between lines and 2 m between plants.

The irrigation system used was drip for 3 hours per day. Fertilization was carried out at planting (1 kg of simple superphosphate; 30g of Fertilizer FTE-BR 12 per pit; 300g of limestone per pit) and cover fertilization every two weeks (15 g of ammonium sulfate and 10 g of potassium chloride per plant). Eleven months after transplanting, fertigation started with twice a week frequency (25 kg of urea - 44% N, 15 kg of monoammonium phosphate and 15 kg of white potassium chloride - 60% K and 47% Cl). Weed control was carried out by manual weeding in the lines and mechanized (brushcutter) between the lines. Artificial pollination and chemical control of pests and diseases were not performed.

Eight genotypes of passion fruit (*Passiflora edulis* Sims) were used, seven of which were hybrid genotypes from the genetic improvement experimental field of passion fruit from UnB, and one BRS Gigante Amarelo (BRS GA1). In addition, three wild cultivars of passion fruit (BRS Pérola do Cerrado, BRS Sertão Forte, BRS Mel do Cerrado) and two hybrid genotypes of wild passion fruit from the germplasm bank of Embrapa Cerrados were used. Table 1 describes the 13 genotypes used, four of which are commercial cultivars and the other genotypes from the Germplasm Bank (BAG) of UnB and Embrapa Cerrados.

Table 1. Commercial cultivars and hybrid genotypes from the passion fruit germplasm bank (BAG) of UnB and Embrapa Cerrados. FAV/UnB, 2020.

Cultivars and Genotypes
BRS Pérola do Cerrado (*Passiflora setacea* D.C.)
BRS Sertão Forte (*Passiflora cincinnata* Mast.)
BRS Mel do Cerrado (*Passiflora alata* Curtis)
BRS Gigante Amarelo (*Passiflora edulis* Sims)
CPAC MJ-02-09 – Maternal progeny of BRS Mel do Cerrado (*Passiflora alata* Curtis)
CPAC MJ-02-17 – Paternal progeny of BRS Mel do Cerrado (*Passiflora alata* Curtis)
F_1 (MAR20#44 R4 x ECL7 P2 R4) (*Passiflora edulis* Sims)
F_1 (MAR20#21 P2 x FB 200 P1 R2) (*Passiflora edulis* Sims)
F_1 (MAR20#19 ROXO R4 x ECRAM P3 R3) (*Passiflora edulis* Sims)
F_1 (MAR20#24 x ECL7 P1 R4) (*Passiflora edulis* Sims)
F_1 (MAR20#24 P1 R4 x Rosa Claro P2 R4) (*Passiflora edulis* Sims)
F_1 MAR20#100 R2 x MAR20#21 R1 (*Passiflora edulis* Sims)
F_1 (MAR20#100 R2 x MAR20#21 R2) (*Passiflora edulis* Sims)

The disease resistance evaluation was carried out weekly, after the fruit harvest, with each plot harvest being individually placed in plastic boxes and identified according to the sketch of the experimental area. Only the fruits that reached the point of full maturity were harvested, that is, the fruits that fell in the soil after the natural abscission of the plant. There was no inoculation of pathogens, considering the natural inoculum pressure, under field conditions.

After harvesting, five fruits per plot were selected at random in each treatment and the evaluation of incidence (percentage of fruits with symptoms) and severity (percentage of injured area in the fruits) to bacteriosis (INC BAC and SEV BAC) (*Xanthomonas axonopodis* pv *passiflorae*), septoriosis (INC SEP and SEV SEP) (*Septoria passiflorae*), scabs (INC VERR and SEV VERR) (*Cladosporium* sp.) and anthracnose (INC ANT and SEV ANT) (*Colletotrichum gloeosporioides*).

The evaluation of diseases in the fruits was done through visual identification of the symptom, based on the perception and quantification of lesions on the fruit surface, and the characteristics of the degree of resistance were measured, using a scale of grades proposed by Junqueira et al. (2003) (Table 2).

Table 2. Classification of incidence (%) and severity (%) for the evaluation of bacteriosis, septoriosis, scabs and anthracnose in 13 genotypes of passion fruit (*Passiflora* spp.), Proposed by Junqueira et al. (2003). FAV/UnB, 2020.

Grade	Symptoms	Resistance degree
1	Absence of symptoms	Resistant (R)
2	Up to 10% of the fruit surface damaged	Moderately susceptible (MS)
3	Between 10 and 30% of the fruit surface damaged	Susceptible (S)
4	More than 31% of the fruit surface damaged	Highly susceptible (AS)

The data obtained were subjected to analysis of variance using the F test at the 1 and 5% level of probability, to the Tukey means comparison test at 1 and 5% probability and the estimate of genetic parameters and Pearson correlation using the GENES software (Cruz 2013).

As for genetic parameters, heritability was measured in the broad sense (ha2), the coefficient of genetic variation (CVg), the coefficient of environmental variation (CVe) and the relationship between the coefficient of genetic and environmental variation (CVg/CVe).

3. Results and Discussion

The evaluated characteristics showed significance in the F test at 1% probability, indicating that there are differences between the evaluated genotypes for incidence and/or severity for bacteriosis, septoriosis, scabs and anthracnose (Table 3). In addition, the results for coefficients of variation, ranged from 0.89% (INC ANT) to 7.18% (SEV SEP) demonstrating good experimental precision (Pereira et al 2016), which is important for the validation of the results found.

All the characteristics evaluated referring to bacterial and fungal diseases of the passion fruit culture presented important genetic parameters, with high and very high values for broad sense heritability and a relationship between the coefficient of genetic and environmental variation above one unit for all characteristics, except for anthracnose incidence (CVg/Cve = 0.80 - INC ANT) (Table 3). According to Vencovskay (1987), the understanding of the CVg/CVe relationships in the studied characteristics is important since these relationships do not have an influence on the average of the variables in question. Thus, they reveal the real improvement of a characteristic in the group of studied individuals. This facilitates the breeder's job in choosing the best breeding method for each characteristic under evaluation.

The average incidence of bacterial and fungal diseases ranged from 2% to 23% in the 13 genotypes evaluated (Table 3). The diseases that had the lowest mean incidence values were bacteriosis and anthracnose, indicating a lower appearance of these diseases in the analyzed experimental fields. However, when verifying the general averages of disease severity in the 13 genotypes of sour, sweet and wild passion fruits, it was possible to verify a variation of 30% in severity of anthracnose to 56% in severity of septoriosis. In this sense, according to the degree of resistance classification proposed by Junqueira et al. (2003), these materials, in general, would be classified as susceptible or highly susceptible.

However, to assess the degree of resistance for each genotype under evaluation, and to identify the differences between the studied genotypes, the results in Table 4 show the averages for the characteristics measured and the result of the Tukey means comparison test at 5% probability.

The incidence for the bacteriosis characteristic ranged from 0.005% in the F1 genotypes (MAR20 # 24 x ECL7 P1 R4) and F1 MAR20 # 100 R2 x MAR20 # 21 R1 to 0.232% for the genotype CPAC MJ-02-17 – Paternal progeny of BRS Mel Cerrado, these differ from each other (Table 4). Bacteriosis (*Xanthomonas campestris* pv. *Passiflorae*) is a major problem in many passion fruit producing regions in Brazil. According to Carvalho et al. (2015), the losses in passion fruit orchards affected by this disease can be total due to the disease's development speed. The biggest problems are observed in the hottest and humid periods of the year (Junqueira and Junqueira, 2007), often coinciding with the periods of crop peak production.

Thus, developing resistant materials to bacteriosis has been the subject of studies by many passion fruit breeders, since this is the best way to control the disease today (Costa et al. 2018). Of the genotypes/cultivars studied, BRS Sertão Forte and CPAC MJ-02-17 – Paternal progeny of BRS Mel do Cerrado presented higher average values for SEV BAC (Table 4) among the others (0.850% and 0.843%, respectively). These two cultivars showed a highly susceptible degree of resistance (AS) (Junqueira et al. 2003). The genotypes that presented the lowest averages of bacteriosis severity (SEV BAC) were F1 (MAR20 # 24 x ECL7 P1 R4), with 0.066%, and the cultivar BRS Gigante Amarelo, with 0.071% severity, which were considered moderately susceptible according to the degree of resistance (Table 4). In the present study, no genotype was considered resistant to bacteriosis, differently from what Viana et al. (2014) observed, who observed cultivars resistant to bacteriosis in studies developed in the Distrito Federal region.

The results demonstrate that there were significant differences in the Tukey means comparison test, at 5% probability, for the septoriosis incidence and severity characteristics (Table 4). The genotypes with the highest means values for septoriosis incidence were CPAC MJ-02-17 – Paternal progeny of BRS Mel do Cerrado and F1 (MAR20 # 21 P2 x FB 200 P1 R2), respectively, differing from BRS Gigante Amarelo, F1 (MAR20 # 19 ROXO R4 x ECRAM P3 R3), F1 (MAR20 # 24 x ECL7 P1 R4) and F1 (MAR20 # 100 R2 x MAR20 # 21 R1) which had the lowest average incidence among the others. Regarding the severity of septoriosis, the cultivar BRS Sertão Forte had the highest average (SEV SEP), 1.198%, differing from the genotypes F1 (MAR20 # 24 x ECL7 P1 R4) and F1 (MAR20 # 100 R2 x MAR20 # 21 R1) which were more resistant, with a moderately susceptible degree of resistance, as shown in Table 4. Similar to these results, Kudo et al. (2012) also found genotypes with greater resistance to septoriosis in a work developed in the region of the Distrito Federal.

Table 3. Summary of analysis of variance and genetic parameters of eight treatments measured in 13 genotypes of sour, sweet and wild passion fruits in the experimental passion fruit field of Água Limpa Farm - UnB. Brasília-DF, 2020**.**

	SEV BAC	SEV SEP	SEV VERR	SEV ANT	INC BAC	INC SEP	INC VERR	INC ANT
Treatments	0.06 **	0.09 **	0.02 **	0.03 **	0.00 **	0.02 **	0.00 **	0.00 **
Means	0.44	0.56	0.35	0.30	0.08	0.23	0.14	0.02
CV(%)	6.36	7.18	6.21	4.77	2.21	3.73	3.36	0.89
CVg(%)	9.75	11.96	6.7	7.25	3.36	6.61	3.96	0.71
CVg/CVe	1.53	1.67	1.08	1.52	1.52	1.77	1.18	0.8
Heritability (%)	90.4	91.74	82.32	90.24	90.25	92.61	84.71	71.79

* and ** = Significant at 1 and 5% probability in the F test, respectively. ns = Not significant in the F test. Caption: SEV BAC = severity of bacteriosis, SEV SEP = severity of septoriosis, SEV VERR = severity of scabs, SEV ANT = severity of anthracnose, INC BAC = incidence of bacteriosis, INC SEP = incidence of septoriosis, INC VERR = incidence of scabs and INC ANT = incidence of anthracnose.

The septoriosis has the characteristic of presenting small lesions on leaves, branches and fruits, which can coalesce with the development of the disease, leading to severe defoliation, directly influencing the production of photoassimilates, and can influence the yield of the fruit production fields (Kudo et al. 2012). In addition, septoriosis can also facilitate the development of other opportunistic diseases, such as bacteriosis, which develops much faster if there is a mechanism that facilitates the entry of bacteria into the plant tissues. Thus, identificating the materials with higher levels of resistance to this disease, such as those with the lowest septoriosis severity averages in Table 4, can help in the development of promising cultivars for the cultivation of passion fruit.

With regard to scabs, a disease that affects branches, leaves, flower buds and passion fruits, in this study, it presented different degrees of resistance between cultivars, being moderately susceptible, susceptible and highly susceptible, as shown in Table 4. The cultivar that showed the highest incidence and severity of the disease was BRS Sertão Forte (INC VERR = 0.300% and SEV VERR = 0.651%), differing from cultivar F1 (MAR20 # 24 x ECL7 P1 R4) with the severity lowest average and incidence for scabs (INC VERR = 0.015% and SEV VERR = 0.071%), with a moderately susceptible degree of resistance.

The results presented for anthracnose showed that the evaluated genotypes did not differ in the Tukey means comparison test, at 5% probability, for the incidence of the disease (Table 4).

Of all the diseases evaluated in the present study, resistant genotypes were identified only for anthracnose, according to the disease severity data, as is the case of the cultivars/genotypes: BRS Gigante Amarelo, F1 (MAR20 # 24 x ECL7 P1 R4) and F1 (MAR20 # 100 R2 x MAR20 # 21 R1, with 0% incidence and severity of the disease. The other genotypes were susceptible or highly susceptible to anthracnose (Table 4).

Observing all evaluated diseases (Table 4), the genotype F1 (MAR20 # 24 x ECL7 P1 R4) was highlighted because it was considered moderately susceptible to bacteriosis, septoriosis and scabs and was resistant to anthracnose. This genotype comes from the passion fruit breeding program at the University of Brasília and can be used in new hybridizations, or in successive self-fertilizations with the purpose of developing a true passion fruit hybrid with multiple resistance to crop diseases. In addition to this genotype, the cultivar BRS Gigante Amarelo and the F1 genotype (MAR20 # 100 R2 x MAR20 # 21 R1) also showed higher degrees of resistance to more than one disease evaluated.

Table 4. Results of the Tukey means comparison test, at 5% probability, for eight characteristics measured in 13 genotypes of sour, sweet and wild passion fruit in the experimental passion fruit field of Água Limpa Farm - UnB. Brasilia-DF, 2020.

Genotypes	SEV BAC	GR	SEV SEP	GR	SEV VERR	GR	SEV ANT	GR	INC BAC	INC SEP	INC VERR	INC ANT
BRS Pérola do Cerrado	0.357^{bcdef}	AS	0.569^{bcdef}	AS	0.328^{abcde}	AS	0.294^{abcd}	S	0.056^{cd}	0.172^{cd}	0.134^{abc}	0.015^{a}
BRS Sertão Forte	0.850^{a}	AS	1.198^{a}	AS	0.651^{a}	AS	0.575^{a}	AS	0.199^{ab}	0.446^{ab}	0.300^{a}	0.046^{a}
BRS Mel do Cerrado	0.684^{abc}	AS	0.850^{abc}	AS	0.488^{abcd}	AS	0.513^{a}	AS	0.134^{abc}	0.387^{abc}	0.221^{ab}	0.046^{a}
BRS Gigante Amarelo	0.071^{f}	MS	0.118^{ef}	S	0.082^{de}	MS	0.000^{d}	R	0.020^{cd}	0.040^{d}	0.030^{bc}	0.000^{a}
CPAC MJ-02-09 - MPG of BRS Mel do Cerrado	0.381^{abcdef}	AS	0.519^{bcdef}	AS	0.277^{abcde}	S	0.26^{abcd}	S	0.076^{bcd}	0.216^{bcd}	0.129^{abc}	0.010^{a}
CPAC MJ-02-17 - PPG of BRS Mel do Cerrado	0.843^{a}	AS	1.074^{ab}	AS	0.569^{abc}	AS	0.513^{a}	AS	0.232^{a}	0.464^{a}	0.277^{a}	0.025^{a}
F_1 (MAR20#44 R4 x ECL7 P2 R4)	0.594^{abcd}	AS	0.816^{abcd}	AS	0.452^{abcde}	AS	0.428^{abc}	AS	0.118^{abcd}	0.363^{abc}	0.216^{ab}	0.020^{a}
F_1 (MAR20#21 P2 x FB 200 P1 R2)	0.789^{ab}	AS	1.103^{ab}	AS	0.632^{ab}	AS	0.531^{a}	AS	0.194^{ab}	0.458^{a}	0.294^{a}	0.040^{a}
F_1 (MAR20#19 ROXO R4 x ECRAM P3 R3)	0.183^{def}	S	0.266^{def}	S	0.205^{bcde}	S	0.129^{cd}	S	0.030^{cd}	0.108^{d}	0.076^{bc}	0.005^{a}
F_1 (MAR20#24 x ECL7 P1 R4)	0.066^{f}	MS	0.082^{f}	MS	0.071^{e}	MS	0.000^{d}	R	0.005^{d}	0.030^{d}	0.015^{c}	0.000^{a}
F_1 (MAR20#24 P1 R4 x Rosa Claro P2 R4)	0.569^{abcde}	AS	0.645^{abcde}	AS	0.351^{abcde}	AS	0.446^{ab}	AS	0.051^{cd}	0.188^{cd}	0.118^{abc}	0.005^{a}
F_1 (MAR20#100 R2 x MAR20#21 R1)	0.134^{ef}	S	0.108^{f}	MS	0.150^{cde}	S	0.000^{d}	R	0.005^{d}	0.01^{d}	0.056^{bc}	0.000^{a}
F_1 (MAR20#100 R2 x MAR20#21 R2)	0.243^{cdef}	S	0.387^{cdef}	AS	0.254^{abcde}	S	0.188^{bcd}	S	0.056^{cd}	0.161^{cd}	0.113^{abc}	0.010^{a}
SMD	0.1905		0.226		0.180		0.136		0.058	0.104	0.090	0.023

Averages followed by the same letter do not differ by the Tukey test at 5% probability. Caption: SEV BAC = severity of bacteriosis, SEV SEP = severity of septoriosis, SEV VERR = severity of scabs, SEV ANT = severity of anthracnose, INC BAC = incidence of bacteriosis, INC SEP = incidence of septoriosis, INC VERR = incidence of scabs and INC ANT = incidence of anthracnose, SMD: Significant minimun difference.

The high values of heritability and CVg/CVe ratio for the severity of the diseases evaluated (Table 4), with the responses observed on the degrees of resistance for bacterial and fungal diseases of the studied genotypes (Table 4), strategies that prioritize increasing resistance to these diseases can be developed in a simple way, in new cycles of mass selection, with high possibilities of progress in the development of a promising cultivar to be launched to producers of sweet and wild sour passion fruit for the Distrito Federal region.

4. Conclusions

High heritability values and CVg/Cve ratio above one were observed for the incidence and severity of the studied diseases, except for anthracnose.

The evaluated genotypes presented average values for the incidence and severity of bacteriosis, septoriosis, scabs and anthracnose different among them, and the one that presented the best results in the degree of resistance for all diseases was F1 (MAR20 # 24 x ECL7 P1 R4). In addition to this genotype, the BRS Gigante Amarelo and the genotype F1 (MAR20 # 100 R2 x MAR20 # 21 R1) also showed higher degree of resistance in more than one evaluated disease.

These genotypes showed possibility of being used in new selection cycles, or in hybridization or self-fertilization studies aimed at the development of promising materials for disease control in fields of sour, sweet and wild passion fruit in the Distrito Federal.

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