Original Article

REACTION OF YELLOW PASSION FRUIT TO PASSION FRUIT WOODINESS DISEASE AND TO BACTERIAL SPOT

REAÇÃO DE MARACUJAZEIRO AZEDO À VIROSE DO ENDURECIMENTO DOS FRUTOS E À BACTERIOSE

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ABSTRACT: The use of resistant varieties is a promising strategy for passion fruit woodiness disease (PWD) (*Cowpea aphid-borne mosaic virus* - CABMV) and bacterial spot (*Xanthomonas axonopodis* pv. *passiflorae* - Xap) control in yellow passion fruit (*Passiflora edulis* Sims). This study aimed at evaluating the reaction of nine genotypes of yellow passion fruit to both mechanically inoculated CABMV and Xap, under protected cultivation. The experiment was conducted using a randomized block design with subdivided parcels comprised of nine treatments, four repetitions, six replications per genotype, and five evaluations. Disease incidence (% plants infected) and severity (% of total leaf area with necrotic lesions or leaf symptoms) were calculated at 7-day intervals. All genotypes evaluated were classified as moderately susceptible to PWD. MAR20#10, MAR20#41, and Rosa Intenso were classified as moderately resistant to bacterial spot whereas the remaining genotypes were classified as moderately susceptible. Genotypes Rosa Intenso, MAR20#41, MAR20#15, and MSCA stood out for presenting the lowest PWD mean severity scores as well as the greatest numbers of plants presenting resistance to PWD after five evaluations. MAR20#10, MAR20#41, and Rosa Intenso demonstrated the lowest bacterial spot mean severity scores. Among the genotypes selected, Rosa Intenso and MAR20#41 were the most promising genotypes for presenting the lowest severity scores for both PWD and bacterial spot disease.

KEYWORDS: *Cowpea aphid-borne mosaic virus*; *Xanthomonas axonopodis* pv. *passiflorae*; Genetic breeding

INTRODUCTION

Brazil is the largest passion fruit producer in the world with an estimated planted area of 51,187 ha and fruit yield of 14.1 t ha^{-1} (IBGE, 2016). Yellow passion fruit (*Passiflora edulis* Sims) stands out as the predominant species cultivated and commercialized due to its fruit quality and yield (FALEIRO et al., 2011). However, this species is susceptible to a variety of diseases, such as the passion fruit woodiness disease (PWD), caused by *Cowpea aphid-borne mosaic virus* (CABMV), and bacterial spot disease, caused by *Xanthomonas axonopodis* pv. *passiflorae* (Xap). Such diseases are widespread in all productive regions, threatening fruit quality and crop's productive cycle (FISCHER; REZENDE, 2008).

The use of resistant cultivars associated with other integrated management techniques is the most efficient, economic, and ecologically correct means for disease control (JUNQUEIRA et al., 2003). Therefore, identification of disease resistance sources is an important strategy in a breeding program aiming at developing resistant cultivars (FALEIRO et al., 2005). Some genotypes have been reported to be resistant to bacterial spot (VIANA et al., 2014a), but to date there are no reports on yellow passion fruit genotypes showing resistance to PWD (SANTOS et al., 2015). For this reason, the objective of this study was to evaluate the reaction of nine yellow passion fruit genotypes to both mechanically inoculated CABMV and Xap, under protected cultivation.

MATERIAL AND METHODS

The experiment was conducted under protected cultivation (14 - 30ºC; 61- 82% ARH) at the University of Brasilia's (UnB) Experiment Station (16ºS and 48ºW, 1010 m above sea level), located in Brasilia, DF, Brazil. The genotypes evaluated were obtained from a research program developed by Embrapa (Empresa Brasileira de Pesquisa Agropecuária – Brazilian Agricultural Research Corporation) and UnB, which used yield, fruit quality, and disease resistance as selection criteria. Genotypes MAR20#10, MAR20#15, MAR20#19, MAR20#41, MAR20#44, and MAR20#2005 were originated from mass selection from nine superior genotypes: Maguary Mesa 1, Maguary Mesa 2, Havaiano, MSC (Marília Seleção Cerrado), Seleção DF, EC-2-0, F1 (Marília x Roxo Australiano), F1 (Roxo Fiji x Marília), and RC1 [F1 (Marília x Roxo Australiano) x Marília (recurrent

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parent)]. Rosa Intenso was originated through recurrent selection based on half-sibling family, whereas AR02 was originated through individual selection from anthracnose resistant plants of Roxo Australiano family. MSCA is derived from cultivar Marília Seleção Cerrado.

Seeds were sown in November 2013, in polystyrene trays (120 mL cell^{-1}) containing Vivatto Slim Plus® (Technes Agrícola Ltda, São Paulo, SP, Brazil) artificial substrate. Seedlings were transplanted in July 2014 into 2 L plastic bags filled with soil. They were irrigated (400 mL) daily and fertilized every two weeks with urea (0.1 g plant^{-1} at each fertilization event), until experiment initiation. The fertilizer was dissolved in water prior to application directly to the substrate. No pest control was performed during the trial.

The experiment consisted of inoculating passion fruit plants with CABMV on 30 January 2015, followed by inoculation with Xap on 02 June 2015, on the same plants. The CABMV isolate was collected from yellow passion fruit plants at UnB's Água Limpa Farm (FAL). Three young leaves per plant were inoculated with the CABMV isolate, by gently rubbing the adaxial foliar surface with a vegetable extract obtained from the maceration of leaves of yellow passion fruit showing severe symptoms of CABMV infection, including mosaic, leaf deformations, and blisters. The leaf macerate was diluted 1:2 (W:V) with a buffer solution (0.1 M potassium phosphate + 0.1 M sodium sulphite), pH 7.0, with addition of a few grams of silica (Celite® 503; Sigma-Aldrich Co.), as described by Viana et al. (2014b). Plants were washed for 10 min after inoculation in order to avoid leaf burn due to silica abrasiveness. Disease incidence (% of plants infected) and disease severity (leaf symptoms) were recorded at 7-day intervals after disease symptoms first appeared. The first of five evaluations was performed 21 days after inoculation. For disease severity assessment, a 1 to 4 scale (VIANA et al., 2014b) was used, as follows: 1 – no symptoms; 2 – mild mosaic and no leaf deformation; 3 – mild mosaic, blisters and leaf deformation; 4 – severe mosaic, blisters and leaf deformation. The score was defined per plant according to the most severe symptoms observed in the leaves. Based on the average disease severity score (DS) obtained from this scale, plants were classified as: Resistant (R), $1 \leq DS \leq 1.5$; Moderately Susceptible (MS), $1.5 < DS \leq 2.5$; Susceptible (S), $2.5 < DS \leq 3.5$; Highly Susceptible (HS), $3.5 < DS \leq 4$ (adapted from VIANA et al., 2014b).

Bacteria inoculation was performed using the Xap strain registered as UnB-1393, which was obtained from the plant pathology laboratory at UnB. The UnB-1393 strain was multiplied using 523 culture media (KADO; HESKETT, 1970) at 28 - 30ºC, for 72 h (FRANCO; TAKATSU, 2004). The bacterial suspension concentration (~1 x 10^6 CFU mL^{-1}) was adjusted on a spectrophotometer to an optical density of 0.145 at 550 nm wavelength, previously determined by the calibration curve. For inoculation, four needles were simultaneously immersed in the bacterial suspension and then used to pierce the adaxial leaf surface of three leaves per plant (adapted from VIANA et al., 2014a). After inoculation, plants were kept in a humid chamber for 72 h. Disease incidence was calculated as the percentage of plants infected and disease severity was calculated as the percentage of total leaf area with necrotic lesions. Disease incidence and severity were recorded at a 7-day interval after disease symptoms first appeared. The first evaluation was performed 25 days after inoculation. For disease severity assessment, a 0 to 5 scale (adapted from VIANA et al., 2014a) was used, as follows: 0 – no symptoms; 1 – 1.0 to 10.0% of total leaf area with necrotic lesions; 2 – 11.0 to 25.0% of total leaf area with necrotic lesions; 3 – 26.0 to 50.0% of total leaf area with necrotic lesions; 4 – more than 50.0% of total leaf area with necrotic lesions; and 5 – leaf drop. Based on the average of the DS obtained from this scale, plants were classified as: Resistant (R), $0 \leq DS < 1$; Moderately Resistant (MR), $1 \leq DS < 2$; Moderately Susceptible (MS), $2 \leq DS < 3$; Susceptible (S), $3 \leq DS < 4$; and Highly Susceptible (HS), $DS \geq 4$ (VIANA et al., 2014a). At the end of the bacterial spot disease assessments, plants were pruned and they were fertilized every two weeks until CABMV inoculation.

The experiment consisted of a randomized block design (RBD) with subdivided parcels comprised of nine treatments (genotypes), four repetitions, five replications per genotype, and five evaluations. Analysis of variance was performed to evaluate possible interactions between genotype and evaluation date. Regression analysis was performed to evaluate linear and quadratic responses of genotypes to evaluation dates. The means were grouped by the Scott-Knott´s test ($P \leq 0.05$). Disease severity and incidence heritability, genetic and environmental coefficient of variation ratio (GCV/ECV), and phenotypic correlations between disease severity and incidence were calculated. Correlation intensity was classified according to the magnitude of the values, as suggested by Carvalho et al. (2004): $r = 0$ (null); $0 < |r| \leq 0{,}30$ (weak); $0{,}30 < |r| \leq 0{,}60$ (medium); $0{,}60 < |r| \leq 0{,}90$ (strong); $0{,}90 < |r| \leq 1$ (very strong); and $|r| = 1$ (perfect). The

area under the disease progress curve (AUDPC) was calculated using AS score data collected in the five evaluation dates (CAMPBELL; MADDEN, 1990). The means were grouped by the Scott-Knott´s test ($P \leq 0.05$). All analyses were performed using Genes software (CRUZ, 2013).

RESULTS AND DISCUSSION

PWD severity assessments did not identify any difference among genotypes ($P > 0.05$) and they were classified as MS, in accordance with the mean number obtained from the grading scale (Table 1). Our results are in conformity with Nogueira (2016), who also reported that genotypes Rosa Intenso and MSCA were moderately susceptible to PWD, showing the lowest severity scores among the genotypes tested when inoculated with the same CABMV isolate, but on a distinct evaluation date. Although our study did not differentiate genotypes for PWD severity, it is important to recognize that MAR20#41(1.6) and Rosa Intenso (1.6) exhibited 11.1% less mean PWD severity than the highest value identified (1.8), demonstrating a slightly greater resistance to PWD.

Table 1. Passion fruit woodiness disease (PWD) incidence (DI), severity (DS), resistance reaction (RR), and percentage of resistant plants (%RP) in yellow passion fruit (*Passiflora edulis* Sims) mechanically inoculated with *Cowpea aphid-borne mosaic virus* (CABMV), in Brasilia, DF, Brazil.

Genotype	DI (%)					DS	RR	%RP
	E1	E2	E3	E4	E5			
MAR20#10	32.5 aB	50.0 aA	62.5 aA	62.5 aA	62.5 aA	1.8 a	MS	44.4
MAR20#41	21.3 bA	31.3 bA	36.3 bA	36.3 bA	36.3 bA	1.6 a	MS	63.2
Rosa Intenso	30.0 aA	35.0 bA	30.00bA	35.0 bA	35.0 bA	1.6 a	MS	63.2
MAR20#44	43.8 aA	53.8 aA	53.8 aA	53.8 aA	53.8 aA	1.8 a	MS	50.0
AR2	21.3 bB	52.5 aA	52.5 aA	52.5 aA	52.5 aA	1.8 a	MS	47.4
MAR20#2005	20.0 bB	50.0 aA	50.0 aA	50.0 aA	50.0 aA	1.8 a	MS	50.0
MAR20#19	37.5 aA	42.5 bA	42.5 bA	47.5 aA	52.5 aA	1.8 a	MS	50.0
MSCA	40.0 aA	40.0 bA	40.0 bA	40.0 bA	45.0 bA	1.7 a	MS	60.0
MAR20#15	33.8 aA	38.8 bA	38.8 bA	38.8 bA	38.8 bA	1.7 a	MS	61.1

E = evaluations; DI and DS = Average of incidence and severity scores, respectively, of five evaluations; %RP = % of resistant plants at the end of the study, 49 days after inoculation. Different lowercase letters within columns and uppercase letters within rows indicate significant differences (Scott-Knott´s test, $P \leq 0.05$).

Since yellow passion fruit exhibit low variability to disease resistance, distinguishing genotypes is often found to be difficult. For this reason, not only statistical but also genetic analyses are very useful tools in breeding programs with focus on disease resistance. In this context, any minimal differences between and within genotypes are useful in providing information for resistance selection and should not be underestimated or discarded (JUNQUEIRA et al., 2003; KUDO et al. 2012, FUHRMANN et al. 2014).

Furthermore, variability for disease resistance may occur within plants of the same genotype (PINTO et al., 2008). After five PWD severity assessments, resistant plants could be observed in all genotypes (Table 1). MAR20#41 (63.2%), Rosa Intenso (63.2%), MAR20#15 (61.1%), and MSCA (60.0%) presented the greatest numbers of resistant plants at the end of the evaluations. AR2 (47.4%) and MAR20#10 (44.4%) exhibited the lowest numbers of resistant plants.

There was an interaction between genotype and evaluation dates for PWD incidence ($P \leq 0.05$) (Table 1). Genotypes Rosa Intenso, MSCA, and MAR20#15 presented the lowest increase in disease incidence over time. In contrast, AR2 and MAR20#2005 showed the lowest percentage of plants with symptoms during evaluation 1, but quickly achieved high incidence scores during evaluation 2. To date, no immunity to CABMV has been detected in yellow passion fruit and variability on resistance to PWD is low (SANTOS et al., 2015). The resistance levels detected are often unsatisfactory for virus control as genotypes usually present some level of susceptibility to the disease (JUNQUEIRA et al., 2003; MACIEL et al., 2009) and, consequently, an expected progress in disease severity and incidence with time, as observed in this study ($P \leq 0.01$). This lack of immunity would ultimately lead to 100.0% disease incidence over a certain period. Nonetheless, it is expected that plants with greater resistance degrees could have PWD

symptom expression delayed. For this reason, along with severity assessments, evaluations of PWD incidence over time could be useful in genotype screening for disease resistance during early stages of disease development.

There were differences among all five bacterial spot severity and incidence assessments ($P \leq 0.01$), revealing a progressive increase in disease with time. Bacterial spot severity assessments identified an interaction between genotypes and evaluation dates ($P \leq 0.01$) (Table 2). MSCA and MAR20#15 proved to be more susceptible, reaching high severity scores early during evaluation 2. In contrast, MAR20#10 reached maximum disease severity only during evaluation 5 and exhibited 51.2% less mean disease severity (1.3) as compared to the highest value observed (2.7). Based on the mean number obtained from the grading scale, MAR20#10, MAR20#41, and Rosa Intenso were classified as MR. The remaining genotypes were classified as MS (Table 2). Our findings are corroborated by Costa et al. (2018) who also verified that MAR20#2005 (S) and MSCA (S) presented higher severity scores as compared to other genotypes when inoculated with UnB-1393 Xap strain during the wet season. Conversely, Nogueira (2016) found greater susceptibility of Rosa Intenso (MS), MAR20#41 (S), and MSCA (S) when 90-day-old plants were inoculated with UnB-1393 Xap strain during the same study period of this report.

After five bacterial spot severity assessments, resistant plants could be observed in all genotypes. MAR20#10 (37.5%) and MAR20#41 (25.0%) were the genotypes with the greatest numbers of resistant plants at the end of the study, contrasting with MAR20#19 (12.5%) and MAR20#2005 (5.9%). Differently from our findings, Costa et al. (2018) did not observed a single resistant plant to Xap strain UnB-1393 after five evaluations in a distinct season. According to Fuhrmann et al. (2014), these results point to the existence of diverse genetic resistance levels to bacterial spot, including within plants of the same genotype.

Even though no differences were observed among genotypes regarding their response to bacterial spot incidence, MAR20#10 (59.7%) presented 22.2% less disease symptoms as compared to MAR20#2005 (76.7%). MAR20#10 has been selected in our passion fruit breeding program over the years due to the lower bacterial spot severity and incidence scores demonstrated as well as to the higher percentage of resistant plants at the end of the evaluations, even when inoculated with higher concentrations of other Xap isolates (VIANA et al., 2014a).

Pathogen's virulence and, consequently, the expression of disease symptoms may be influenced by environmental conditions (GARCÍA-GUZMÁN et al., 2016), plant variables, such as genetic variability, age at inoculation, growth condition, nutritional status (FUHRMANN et al., 2014; WANGUNGU et al., 2014), and pathogen characteristics, such as genetic variability, isolate aggressiveness, and inoculum concentration (NAKATANI et al., 2009; CAMERA et al., 2012). Some of these variables could possibly explain divergences between our results and those reported in the literature. Therefore, these divergences strengthen the importance of performing disease assessments over time in order to obtain reliable data on disease progress and genotype resistance (KOSOSKI et al., 2008; VIANA et al., 2014a).

AUDPC is a measurement of disease progress over time. It has been used to assess quantitative disease resistance in crop cultivars, integrating disease progress features with host development and growth (JEGER; VILJANEN-ROLLINSON, 2001). In this study, AUDPC was calculated for disease severity as an attempt to differentiate genotypes regarding their resistance to PWD and to bacterial spot. However, no differences were found among genotypes for PWD or bacterial spot.

Our results demonstrate that genotypic variance was greater than environmental variance for both bacterial spot severity ($\sigma_g^2 = 0.09$; $\sigma_e^2 = 0.07$) and AUPDC ($\sigma_g^2 = 85.58$; $\sigma_e^2 = 68.28$). These data are satisfactory since it is important that the environment presents the lowest interference as possible in genotype response for genetic breeding studies. Medium heritability values were observed for bacterial spot severity (55.40%) and AUDPC (55.61%). Heritability values of this magnitude are valuable to genetic breeding programs when screening for disease resistance, given the disease polygenic inheritance. Both bacterial spot severity and AUDPC showed a GCV/ECV equal to 0.56. GCV/ECV values equal or close to one demonstrate that phenotypic variances among individuals are triggered exclusively by genetic differences between them (ALLARD, 1971). Moreover, Silva et al. (2012) stated that high ECV values, instead of revealing experimental inaccuracy, might indicate that the trait under study is of polygenic inheritance and, as a result, is highly affected by the environment. In circumstances like this, the adoption of traditional breeding methods could

result in low efficiency on improving passion fruit resistance to bacterial spot. Thus, breeding methods based on family performance would be more appropriate than those based on individual plant performance (FREITAS et al., 2015).

Table 2. Bacterial spot disease incidence (DI), severity (DSE and DS), resistance reaction (RR), and % resistant plants (%RP) in yellow passion fruit (*Passiflora edulis* Sims) mechanically inoculated with *Xanthomonas axonopodis* pv. *passiflorae*, in Brasilia, DF, Brazil.

Genotype	DI(%)	Severity at each evaluation date (DSE)					DS	RR	%RP
		E1	E2	E3	E4	E5			
MAR20#10	59.7 a	0.2 aC	1.2 cB	1.3 dB	1.6 cB	2.5 bA	1.3 a	MR	37.5
MAR20#41	62.4 a	0.2 aC	1.4 cB	1.8 cB	2.5 bA	2.8 bA	1.7 a	MR	25.0
Rosa Intenso	66.4 a	0.2 aD	1.4 cC	2.0 cB	2.5 bA	2.9 bA	1.8 a	MR	16.7
MAR20#44	72.4 a	0.4 aC	1.9 bB	2.4 bB	2.7 bA	3.0 bA	2.1 a	MS	23.5
AR2	72.3 a	0.2 aD	1.8 bC	2.1 cC	3.1 aB	3.7 aA	2.2 a	MS	14.3
MAR20#2005	76.7 a	0.2 aD	1.8 bC	2.6 bB	3.1 aA	3.3 aA	2.2 a	MS	5.9
MAR20#19	74.4 a	0.2 aD	1.6 bC	2.5 bB	3.3 aA	3.7 aA	2.3 a	MS	12.5
MSCA	72.2 a	0.2 aD	2.2 aC	2.7 bB	3.0 aA	3.5 aA	2.3 a	MS	21.4
MAR20#15	72.2 a	0.3 aC	2.7 aB	3.2 aB	3.6 aA	3.9 aA	2.7 a	MS	16.7

E = evaluations; DI and DS = Average of incidence and severity scores, respectively, of five evaluations; %RP = % of resistant plants at the end of the study, 53 days after inoculation. Different lowercase letters within columns and uppercase letters within rows indicate significant differences (Scott-Knott´s test, $P \leq 0.05$).

PWD severity was strongly and positively correlated to PWD incidence (0.82; $P \leq 0.01$). The trait bacterial spot severity was positively correlated to bacterial spot incidence at a magnitude of 0.81 ($P \leq 0.01$). Strong correlations between disease severity and incidence have also been reported for PWD and bacterial spot (COSTA et al., 2018; VIANA et al., 2014b). Therefore, this information emphasizes the importance of analysing these variables during plant selection. Additionally, Costa et al. (2018) distinguished a contrasting response from genotypes regarding bacterial spot disease and PWD. The authors found that genotypes with greater resistance to bacterial spot showed greater susceptibility to PWD. Our study evaluated plant response to the same isolates used by those authors when inoculated in distinct dates from that experiment. We found no correlation between PWD and bacterial spot when CABMV was inoculated before Xap. Hence, further studies are necessary in order to better understand plant response to different diseases and possible existing correlations between bacterial spot and PWD.

The PWD and bacterial severity and incidence degrees observed in this study reveal the existence of variability within genotypes. As a result, genotypes Rosa Intenso, MAR20#41, MAR20#15, and MSCA were selected for presenting the lowest PWD mean severity scores as well as the greatest numbers of individual plants presenting resistance to PWD. MAR20#10, MAR20#41, and Rosa Intenso were also selected as the genotypes with the lowest bacterial spot mean severity scores. MAR20#41 and Rosa Intenso are especially promising genotypes to be used in breeding programs with focus on disease resistance due to their superior performance to both PWD and bacterial spot. The selected genotypes will be cloned and, again, assessed for CABMV and Xap resistance, providing means of following up with the breeding program on disease resistance.

RESUMO: O uso de variedades resistentes é uma estratégia promissora para o controle da virose do endurecimento dos frutos (VEF) (*Cowpea aphid-borne mosaic virus* - CABMV) e da bacteriose (*Xanthomonas axonopodis* pv. *passiflorae* - Xap) no maracujazeiro amarelo (*Passiflora edulis* Sims). Este estudo objetivou avaliar a reação de nove genótipos de maracujazeiro amarelo à CABMV e Xap, ambos inoculados mecanicamente, sob cultivo protegido. O experimento foi conduzido em delineamento de blocos casualizados com parcelas subdivididas, composto por nove tratamentos, quatro repetições, seis plantas por genótipo e cinco avaliações. A incidência (% plantas infectadas) e a severidade (% da área foliar total com lesões necróticas ou sintomas foliares) das doenças foram calculadas em intervalos de sete dias. Todos os genótipos avaliados foram classificados como moderadamente suscetíveis à VEF. MAR20#10, MAR20#41 e Rosa Intenso foram classificados como moderadamente resistente à bacteriose enquanto os demais genótipos foram classificados como moderadamente suscetíveis. Os genótipos Rosa Intenso, MAR20#41, MAR20#15 e MSCA se

destacaram por apresentarem menores severidades médias da VEF bem como pelo maior número de plantas apresentando resistência à virose após as cinco avaliações. MAR20#10, MAR20#41 e Rosa Intenso demonstraram as menores severidades médias de bacteriose. Entre os genótipos selecionados, Rosa Intenso e MAR20#41 foram os mais promissores por apresentarem os menores valores de severidade para a VEF e para a bacteriose.

PALAVRAS-CHAVE: *Cowpea aphid-borne mosaic vírus. Xanthomonas axonopodis* pv. *Passiflorae.* Melhoramento genético

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