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PLANT PROTECTION
Article

Resistance of grapevine hybrids to bacterial canker disease

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ABSTRACT: The bacterial canker, caused by Xanthomonas citri pv. viticola, is the most important bacterial disease of grapevines cultivated in tropical areas. The objectives of this study were to select grape hybrids resistant to bacterial canker based on genotypic values and to evaluate the response of seedless grape hybrids to artificial infection with bacterial canker. Two experiments were conducted. The field experiment (Experiment I) was conducted with 569 hybrids, evaluated for symptoms of bacterial canker, in the absence of an experimental design, using a single individual of each genotype. Experiment II was conducted in a greenhouse, where ten seedless grape hybrids were evaluated in terms of incubation period, incidence, severity, and area under the progress curve of bacterial canker disease incidence, in a completely randomized design. The use of the residual maximum likelihood/best unbiased linear prediction methodology allowed the selection of 40 grape hybrids with lower genotypic values for resistance to bacterial canker. The CPATSA 49.25 and CPATSA 49.86 hybrids showed greater resistance to bacterial canker in all epidemiological parameters evaluated under greenhouse conditions.

Key words: *Vitis* spp., *Xanthomonas citri* pv. *viticola*, residual maximum likelihood/best unbiased linear prediction, genotypic values, epidemiological parameters.

INTRODUCTION

The bacterial canker of grapevine (*Xanthomonas citri* pv. *viticola*) was described by the first time in India in 1972 and later in Brazil in 1998. It is considered an A2 quarantine pest that is present in the states of Bahia, Ceará, Pernambuco and Roraima, according to Normative Instruction No. 38/2018, and is under official control.

Currently, it is the most important bacterial disease of grapevines cultivated in tropical areas (Lima et al. 2017). Bacterial canker of the vine represents a high potential risk to the development of national viticulture. It has a significant incidence, causes severe damage and significant losses in susceptible cultivars. In addition, it limits potential exports due to the effect of the disease on fruit quality and the possible imposition of phytosanitary barriers by importing countries. The 'Red Globe', as well as other cultivars originating from the 'Thompson Seedless' cultivar, was severely affected, as the incidence was up to 100%, with total production losses in some areas (Rodrigues Neto et al. 2011).

The symptoms of the disease are characterized on the leaves by small, dark, and angular spots, which can coalesce and dry, causing necrotic areas and leaf burns. Dark, elongated spots form on the veins and petioles of the leaves and on the branches and rachises of the fruits, which develop into black longitudinal cracks. The berries are uneven in size and color and may present depressed and necrotic lesions (Nayudu 1972).

Under favorable conditions for the development of the disease (high humidity and temperature), the pathogen survives on infected vine branches or is associated with latent infections in asymptomatic plants (Nascimento and Mariano

2004) and alternative hosts (Santos et al. 2014), on vine cutting tools (Naue et al. 2014), and in infected crop residue (Silva et al. 2012). Thus, dissemination can occur through propagation materials such as seedlings and cuttings, water and infected tools, colonization occurring systemically, and the bacterium has already been found in seeds, berries, and roots (Tostes et al. 2014).

For disease control, the use of healthy propagation material integrated with management practices that may limit the survival and spread of the bacterium, such as field inspection, drastic pruning of infected organs, elimination of severely infected plants, disinfestation of vehicles, equipment and materials for pruning and the use of copper protectors and windbreaks, is recommended (Nascimento et al. 2006). Due to the lack of efficient practices that act on the pathogen, genetic resistance is an important alternative for controlling the disease.

Studies to evaluate the susceptibility of different genotypes of Vitis spp. to bacterial canker have already been performed in India (Chand 1992; Kamble et al. 2017) and in Brazil, in the states of São Paulo (Malavolta Júnior et al. 2003), Pernambuco (Nascimento et al. 2006), and Bahia (Zucal et al. 2016), and the results showed that the reaction of the grapevine genotypes to this bacterium is quite variable. Although there are studies on the resistance of grape cultivars to bacterial canker, there are still no breeding studies aimed at the selection of grapevine hybrids resistant to this disease.

In this context, this is the first scientific report presenting the results of research on grapevine genetic improvement aimed at the selection of table grape hybrids for resistance to bacterial canker. The objectives of this study were to select table grape hybrids resistant to bacterial canker of the vine based on genotypic values and to evaluate the response of seedless grape hybrids to artificial infection with bacterial canker. The results obtained provide support for decision-making and future strategies in the genetic improvement of grapevines in Brazilian semiarid regions.

MATERIALS AND METHODS

Evaluation of hybrids under field conditions

Two experiments were conducted, one under field conditions (Experiment I), and the other in a greenhouse (Experiment II).

Experiment I was carried out at the Bebedouro Experimental Field of Brazilian Agricultural Research Company (Embrapa) Semiarid Region in Petrolina, Pernambuco, Brazil (9°08'03"S, 40°18'28"W, and 370 m altitude), in a field of mother plants or a field of vegetative multiplication. The soil in the experimental area was classified as Eutrophic Red-Yellow Plinth Ultisol.

The vines were grown in a 'trellis' system, with a spacing of 3 m between rows and approximately 30 cm between plants. The evaluations were performed between May and June 2019 and 2021, two months after pruning.

A total of 569 hybrids (F1) originating from 28 crosses between cultivars of *Vitis vinifera*, between interspecific hybrids, and between *V. vinifera*, and interspecific hybrids (Table 1) were evaluated. The crosses were made as part of the "Brazil's grapes" breeding program at Embrapa Semiarid in Petrolina. Each hybrid was represented by a single, freestanding vine plant without experimental design or repetition.

The evaluation of the severity of bacterial canker symptoms in naturally infected plants was performed using a rating scale, ranging from 1 to 4:

- 1 = no symptoms;
- 2 = few symptoms (a few, small lesions on branches or leaves not exceeding 8% of the leaf area);
- 3 = medium symptoms (lesions on the branches, petioles, and leaves ranging from 9 to 34% of the leaf area);
- 4 = many symptoms (large number of lesions on the branches, petioles, and leaves, with coalesced spots forming large necrotic areas greater than 35% of the leaf area).

The diagrammatic scale proposed by Nascimento et al. (2006) was used as a basis for the percentage of leaf area affected by the disease.

Table 1. Identification of crosses and number of genotypes evaluated in each cross of hybrid grapevine.

Crossing (male parent × female parent)	Code	Number of genotypes evaluated	Crossing (male parent × female parent)	Code	Number of genotypes evaluated
BRS Vitória × A1581	7	1	BRS Linda × CG351	32	1
CG351 × A Dona	10	2	BRS Isis × A1105	37	1
BRS Vitória × CG102295	11	4	Feal × Princess	47	2
CG351 × CNPUV24	12	5	Jupiter × Marroo	49	177
BRS Isis × CG102295	16	3	Thompson × CG351	61	7
BRS Isis × CNPUV24	17	4	Feal × Marroo	62	22
BRS Vitória × Jupiter	18	2	CG351 × Marroo	63	54
BRS Linda × Marroo	24	32	A1105 × Marroo	64	39
A Dona × Marroo	25	13	BRS Clara × Marroo	65	66
BRS Vitória × Marroo	26	22	CG33716 × ADona	67	8
CG102295 × CNPUV24	27	13	CNPUV8 × CG351	74	6
A1105 × CG351	29	1	A Dona × CG351	76	1
BRS Vitória × A1105	30	1	BRS Linda × Feal	78	6
CG351 × CG102295	31	11	BRS Isis × Marroo	79	65

The estimation of the variance components and the prediction of the genotypic values for the evaluated trait were performed using the procedure of maximum residual likelihood/best unbiased linear prediction (REML/BLUP), since it is used in the case of unbalanced data. The statistical model used was the additive repeatability model, with a complete parentage matrix (Model 166), which is given by Eq. 1:

$$y = Xm + Za + Wp + e \quad (1)$$

where: y: the data vector; m: the vector of the (fixed) measurement effects added to the overall average; a: the vector of individual (random) additive genetic effects; p: the vector of permanent (random) environmental effects; e: the error vector; X, Z and W: the incidence matrices for m, a and p, respectively (Resende 2016).

The choice of a linear mixed model (normally distributed residuals) over a generalized mixed model, whose residuals follow a binomial distribution, was possible because, when testing the latter, there was no improvement in the results. When there are categorical variables with more than two classes, fitting a model with a normal distribution is more appropriate than fitting a model with a binomial distribution. In addition, the REML/BLUP procedure is robust to data normality, and even if the data do not follow a normal distribution, the results are reliable. Regarding the genetic effects considered in the model, the dominance effect was tested, and it was not significant. Therefore, by the criterion of parsimony, only the additive genetic effect was considered in the analysis.

The significance of the random effects of the model (hybrids) was assessed using deviance analysis via the likelihood ratio test, as recommended by Viana and Resende (2014). The hybrids were classified according to the genotypic values (u + a) estimated using BLUP, and the 40 best hybrids (lowest values) were selected. Selegen-REML/BLUP software was used for deviance analysis and prediction of breeding values (Resende 2016).

Evaluation of hybrids under greenhouse conditions

Experiment II was conducted under greenhouse conditions (temperature 26 ± 6°C, 12-hour photoperiod and mean relative humidity of 66%, at Embrapa Semiarid Region in Petrolina, from June to July 2021.

Young vine trees were prepared from vine plants from the Bebedouro Experimental Field of Embrapa Semiárido. Among the seedless grape genotypes resulting from crosses performed at Embrapa Semiárido, 10 genotypes (CPATSA 49.05, CPATSA 49.114, CPATSA 49.25, CPATSA 49.32, CPATSA 49.86, CPATSA 63.108, CPATSA 64.83, CPATSA 67.24, CPATSA 79.23, and CPATSA 79.49) were evaluated in the previous experiment. Branches of these genotypes were collected, and, immediately after collection, the branches were reduced to stalks, 15- to 20-cm long, containing three buds and an average diameter of 6 mm. Next, the shoots were vertically immersed in a water depth of approximately 10 cm for 48 hours to facilitate rooting. After this period, the stems were taken to a greenhouse held at a controlled temperature and planted in polyethylene bags (10 × 20 cm) containing natural soil. All plants were irrigated daily.

The Xcv isolate of *Xanthomonas citri* pv. *viticola* preserved in sterilized distilled water was reactivated and tested for pathogenicity by the stem prick method using healthy young vine trees of the 'Red Globe', which were kept in a humid chamber for 48 hours in a greenhouse. The isolate used belongs to the Collection of the Laboratory of Phytopathology of Embrapa Semiárido.

The isolate was grown and maintained in a nutrient yeast dextrose agar medium (5-g meat extract, 5-g peptone, 10-g glucose, 5-g yeast extract, 18-g agar·L^{-1} distilled water) for 48 hours at 28°C. After two days of cultivation, the bacterial cells were used to prepare the suspension in sterile distilled water and adjusted in a spectrophotometer to A_{570} = 0.4, which corresponds to the concentration of 10^8 colony-forming units (CFU) mL^{-1} (Peixoto et al. 2006).

The inoculation of the genotypes was performed by using the pinning method, with the aid of a pad containing two entomological pins and deposition of the bacterial suspension (Maji and Nath 2015). The wounds were made in the middle portion of the petiole and in the upper third of the stem, in which 10 µL of the suspension was deposited using a fixed-volume micropipette. The inoculation point was covered with a moistened cotton swab and wrapped with aluminum foil (Lima et al. 1999). After inoculation, the plants were kept in a humid chamber for 48 hours to favor infection and then kept in a greenhouse.

The vines were evaluated daily, up to 30 days after inoculation, and the following epidemiological parameters were recorded: incubation period; disease incidence; area under the disease progress curve, calculated based on the incidence values; and severity of the disease.

The incubation period, which represents the number of days between inoculation and disease onset, was calculated from daily evaluations of the leaf petiole and stem. For the petiole, the mean incubation period was considered when 50% or more of the petioles of the leaves of each plant showed symptoms. For the stem, only the number of days for the onset of symptoms was considered for each plant. The incidence of petioles or stems with symptoms was calculated as the percentage of petioles or stems with symptoms in relation to the total number of petioles or stems evaluated per plant. The area under the disease incidence progress curve was calculated by using the Eq. 2:

$$AUDPC = \Sigma[(y_i + y_{(i+1)}/2)]d_{ti} \quad (2)$$

where: y_i and $y_{(i+1)}$: the incidence values observed in two consecutive evaluations; d_{ti}: the interval between evaluations (Shaner and Finney 1977).

The severity was evaluated by means of the areas of the lesions, considered a rectangle, at 30 days after inoculation.

The experimental design was completely randomized, with 10 treatments (genotypes), and five replicates. Each plot was represented by a six-month-old vines, whose four petioles and one stem were evaluated. The data were subjected to analysis of variance, and the means were compared by Tukey's test ($p < 0.05$). The data were analyzed using the Statistical Analysis System.

RESULTS AND DISCUSSION

Evaluation of hybrids under field conditions

During the field experiment, from pruning to the end of the evaluations, the climatic conditions were favorable for the emergence of bacterial canker (Fig. 1). In regard to phytopathogens, survival in infected tissue is common, and, when climatic conditions are favorable, they reproduce and provide the primary inoculum for new epidemics (Batista et al. 2015).

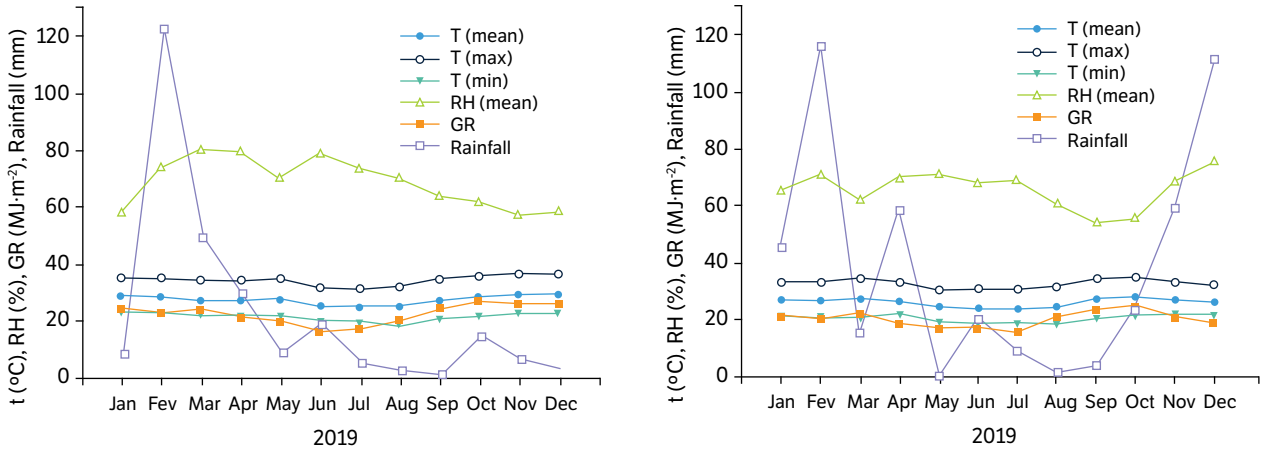

Figure 1. Meteorological data on precipitation (mm), average, minimum and maximum air temperature (T, °C), relative humidity (RH, %) and global radiation (GR, MJ m-2) for 2019 and 2021.

During the experiment, the accumulated index and the occurrence of rainfall were slightly higher in 2019 (352 mm) than in 2021 (313 mm). Consequently, there was a higher average relative humidity in 2019 (76.99%) compared to 2021 (68.26%). The average monthly temperatures ranged from 25 to 28.5°C and from 24.5 to 27°C in 2019 and 2021, respectively. Periods of high rainfall, which promote an increase in the relative humidity, and high temperature occurred after pruning, establishing an environment conducive to the emergence and increase in intensity of bacterial canker, since the bacterium colonizes the tissue more easily. In addition, rainfall favors the spread of the pathogen (Nascimento and Mariano 2004).

A significant effect ($p < 0.01$) was observed using deviance analysis for the symptoms of bacterial canker under field conditions for the hybrids (Experiment I). This indicates the existence of variability in the intensity of bacterial canker symptoms among the evaluated genotypes under naturally occurring conditions; therefore, it is possible to obtain genetic gains by selecting resistant genotypes. These symptoms ranged from asymptomatic plants to plants with large lesions (cankers) on the branches and leaves.

Estimates of genetic parameters are essential to guide the correct selection of superior individuals. When partitioning the phenotypic variance, there was a smaller contribution of genetic variance (0.07), represented by the additive variance, in the total phenotypic effects. These results agree with those of Amaral et al. (2020), who evaluated a population of interspecific hybrids of *Vitis* spp. for resistance to downy mildew and found a small contribution of additive variance. Santos et al. (2018), who evaluated the same population of hybrids for resistance to the root-knot nematode (*Pratylenchus brachyurus*), found a small contribution of additive variation to the composition of the phenotypic variance. On the other hand, Vivas et al. (2014) reported that, for the severity of phoma leaf spots (*Stagonosporopsis caricae*) on papaya, the phenotypic variance was explained almost entirely by the additive variance.

The contribution of the permanent environmental effect was also low (0.005), i.e., no environmental condition permanently affected the vines in all measurements. Consequently, the coefficient of determination of the permanent environment was also low (0.005).

On the other hand, there was a high estimate of environmental variance (0.79) influencing the phenotypic variance in the two periods evaluated. In the evaluation of progenies, high estimates of environmental variance indicate that polygenic diseases are largely influenced by the environment (Amaral et al. 2020). This demonstrates that environmental factors strongly influenced the disease. The environmental conditions were different in the two years evaluated, with a higher amount of precipitation and greater relative humidity in the months preceding 2019 (Fig. 1), which may have contributed to the high value of environmental variance. The presence of humidity associated with high temperatures is the ideal climatic condition for the development of the disease (Nascimento and Mariano 2004). In addition, the initial source of pathogen inoculum influences the occurrence of diseases in grapevines (Barbosa et al. 2016).

Results similar to these were found by Amaral et al. (2020), who obtained high estimates of environmental variance acting on the phenotypic variance for downy mildew severity in grapevines at the evaluated stations. The results obtained disagree with those of Santos (2015), who evaluated the resistance of passion fruit to the Cowpea aphid-borne mosaic virus and found low environmental variance, which resulted in high heritability values. However, these estimates vary according to the pathosystem involved and the experimental design.

The estimate of individual heritability in the narrow sense (h2a) captures the effects of additive genetic variances. Individual heritability in the narrow sense was of low magnitude, as evidenced by the low estimates of additive variances. Heritability is an important genetic parameter, because it quantifies the fraction of heritable phenotypic variation available for selection (Santos et al. 2019). It was found that 7.9% of the phenotypic values originated from genetic causes. Although the heritability estimate was considered of low magnitude, there is still a possibility of selection (Viana and Resende 2014).

The results of low individual narrow-sense heritability were observed by Santos et al. (2019) for traits of resistance to the nematode *P. brachyurus* in *Vitis* spp. Pereira et al. (2013), in selecting coffee plants for agronomic traits and disease resistance, also observed that the additive heritability within families was low, and the reaction to rust was high (7.2%). It can be inferred that the selection within the number of progenies is possible only for this variable. In studies that considered the genetic effect of dominance, heritability in the broad sense was higher than heritability in the narrow sense (Santos et al. 2018; Amaral et al. 2020).

The components of the mean (individual BLUP values), the genetic gains, and the new means of grapevine hybrids in terms of their resistance to bacterial canker are presented in Table 2. Among the 569 hybrids evaluated, the 40 best hybrids, corresponding to 7% of the genotypes, were selected according to the lowest breeding values for the symptoms of bacterial canker. The 10 most resistant genotypes under field conditions were CPATSA 18.04, CPATSA 18.05, CPATSA 49.72, CPATSA 49.34, CPATSA 49.25, CPATSA 49.264, CPATSA 49.92, CPATSA 49.90, CPATSA 49.89, and CPATSA 49.86 (Table 2).

Table 2. Ranking of the 40 interspecific hybrids of *Vitis* spp. in terms of resistance to bacterial canker (*Xanthomonas citri* pv. *viticola*), in which the additive effects (a), average or genotypic values (u + a), genetic gain and new average or genotypic values for canker symptoms are predicted.

Rk	Hybrid	a	u + a	Gain	New average
1	CPATSA 18.04	-0,4697	1,9740	-0,0228	2,4209
2	CPATSA 18.05	-0,4301	2,0136	-0,0220	2,4217
3	CPATSA 49.72	-0,3701	2,0736	-0,0213	2,4224
4	CPATSA 49.34	-0,3701	2,0736	-0,0207	2,4230
5	CPATSA 49.25	-0,3701	2,0736	-0,0201	2,4236
6	CPATSA 49.264	-0,3701	2,0736	-0,0195	2,4242
7	CPATSA 49.92	-0,3305	2,1132	-0,0189	2,4248
8	CPATSA 49.90	-0,3305	2,1132	-0,0183	2,4253
9	CPATSA 49.89	-0,3305	2,1132	-0,0178	2,4259
10	CPATSA 49.86	-0,3305	2,1132	-0,0173	2,4264
11	CPATSA 49.85	-0,3305	2,1132	-0,0167	2,4269
12	CPATSA 49.84	-0,3305	2,1132	-0,0162	2,4275
13	CPATSA 49.79	-0,3305	2,1132	-0,0156	2,4280
14	CPATSA 49.76	-0,3305	2,1132	-0,0151	2,4286
15	CPATSA 49.71	-0,3305	2,1132	-0,0145	2,4291
16	CPATSA 49.69	-0,3305	2,1132	-0,0140	2,4297
17	CPATSA 49.58	-0,3305	2,1132	-0,0134	2,4302
18	CPATSA 49.55	-0,3305	2,1132	-0,0129	2,4308
19	CPATSA 49.49	-0,3305	2,1132	-0,0123	2,4314
20	CPATSA 49.46	-0,3305	2,1132	-0,0117	2,4319

Continue...

Table 2. Continuation...

Rk	Hybrid	a	u + a	Gain	New average
21	CPATSA 49.42	-0,3305	2,1132	-0,0112	2,4325
22	CPATSA 49.40	-0,3305	2,1132	-0,0106	2,4331
23	CPATSA 49.39	-0,3305	2,1132	-0,0100	2,4336
24	CPATSA 49.38	-0,3305	2,1132	-0,0095	2,4342
25	CPATSA 49.33	-0,3305	2,1132	-0,0089	2,4348
26	CPATSA 49.20	-0,3305	2,1132	-0,0083	2,4353
27	CPATSA 49.19	-0,3305	2,1132	-0,0077	2,4359
28	CPATSA 49.14	-0,3305	2,1132	-0,0072	2,4365
29	CPATSA 49.12	-0,3305	2,1132	-0,0066	2,4371
30	CPATSA 49.253	-0,3305	2,1132	-0,0060	2,4377
31	CPATSA 49.250	-0,3305	2,1132	-0,0054	2,4382
32	CPATSA 49.242	-0,3305	2,1132	-0,0048	2,4388
33	CPATSA 49.240	-0,3305	2,1132	-0,0042	2,4394
34	CPATSA 49.235	-0,3305	2,1132	-0,0036	2,4400
35	CPATSA 49.230	-0,3305	2,1132	-0,0031	2,4406
36	CPATSA 49.221	-0,3305	2,1132	-0,0025	2,4412
37	CPATSA 49.201	-0,3305	2,1132	-0,0019	2,4418
38	CPATSA 49.200	-0,3305	2,1132	-0,0013	2,4424
39	CPATSA 49.198	-0,3305	2,1132	-0,0007	2,4430
40	CPATSA 49.197	-0,3305	2,1132	-0,0001	2,4436

Negative gains were observed for all 40 hybrids selected; in other words, it was possible to select genotypes with lower values in terms of bacterial canker symptoms. Negative values were also observed for the selected hybrids in terms of the predicted genetic effects; that is, they tended to contribute to the reduction of bacterial canker intensity in new generations. It is noteworthy that selection based on the additive genetic effect facilitates the production of superior plants (Vivas et al. 2014).

These results support those found by Vivas et al. (2014), who obtained values for desirable (negative) additive genetic effects when evaluating papaya genotypes for resistance to phoma leaf spots. Santos et al. (2019) selected grapevine individuals for nematode resistance with lower genotypic values, with predicted gains below 1%.

The progress of breeding programs is significantly influenced by the selection of the best genotypes that will constitute the next generations (Amaral et al. 2020). Therefore, it can be inferred that, within the genotype selection estimates obtained using BLUP, the genotypic values are of great relevance for genotype selection.

Thus, according to the estimated parameters and considering the lowest predicted breeding values and negative gains, it is recommended that the selected genotypes advance to the next selection stage for resistance under controlled conditions to confirm genetic resistance. In addition, we considered the evaluation of agronomic and commercial characteristics for the development of new cultivars of seedless table grapes resistant to bacterial canker and adapted to Brazilian semiarid regions.

Evaluation of hybrids under greenhouse conditions

In Experiment II, the plants of all vine hybrids showed symptoms of bacterial canker. There were significant differences ($p < 0.05$) between the genotypes for the different epidemiological components, except for the incidence of the disease at 30 days after inoculation, because both the petiole and the stem showed 100% incidence 19 days after inoculation (Table 3).

Table 3. Reaction of grapevine genotypes to Xanthomonas citri pv. viticola based on the epidemiological components of bacterial canker under greenhouse conditions*.

Hybrid	PI_s (days		SEV_s (mm^2)		$AUDPC_s$		PI_p (days)		SEV_p (mm^2)		$AUDPC_p$	
CPATSA 49.05	6.8	d	2.92	c	1,370.0	a	6.2	d	3.94	cd	1,390.0	a
CPATSA 49.25	16.8	a	2.47	c	390.0	e	12.4	ab	3.02	cd	647.5	c
CPATSA 49.32	13.2	ab	4.08	bc	740.0	de	10.0	bc	4.10	cd	882.5	bc
CPATSA 49.86	14.0	ab	2.87	c	650.0	de	14.4	a	3.87	cd	620.0	c
CPATSA 49.114	10.2	bc	3.33	bc	1,030.0	abcd	10.8	bc	2.93	cd	942.5	bc
CPATSA 63.108	11.0	bc	4.89	abc	880.0	cd	11.8	ab	5.54	bc	845.0	bc
CPATSA 64.83	8.0	cd	2.39	c	1,260.0	abc	8.8	bc	1.74	d	1,100.0	ab
CPATSA 67.24	12.0	bc	2.27	c	900.0	bcd	11.6	ab	2.05	d	872.5	bc
CPATSA 79.23	7.4	d	7.35	a	1,310.0	a	6.8	cd	11.13	a	1,310.0	a
CPATSA 79.49	6.8	d	6.50	ab	1,270.0	abc	6.8	cd	8.86	ab	1,330.0	a

s: stem; p: petiole; PI: period of incubation; SEV: severity of disease at 30 days after inoculation; AUDPC: area under the disease progress curve; *mean values followed by the same letter in the column did not differ significantly ($p \geq 0.05$) using Tukey's test.

The incubation period reflects the susceptibility of the genotype to the pathogen. The longest incubation period of the disease in the stem was observed for genotype CPATSA 49.25 (16.8 days), which did not differ statistically from genotypes CPATSA 49.86, and CPATSA 49.32; the longest incubation period of the disease in the petiole was observed for genotype CPATSA 49.86 (14.4 days), which was statistically equivalent to that of genotypes CPATSA 49.25, CPATSA 63.108, and CPATSA 67.24, indicating that these hybrids are less susceptible to bacterial canker. The CPATSA 49.25 and CPATSA 49.86 hybrids had the highest values of the disease incubation period both in the stem and in the petiole.

Kamble et al. (2019) observed canker symptoms in the 'Thompson Seedless' cultivar three days after inoculation by means of infiltration. Nayudu (1972) found an incubation period of six days in the 'Anab-e-Shahi' grape with foliar inoculation. For the 'Red Globe' grape, an incubation period of 12 days was observed based on inoculation in the leaf and petiole (Lima et al. 1999). In contrast, Zucal et al. (2016) observed an incubation period of approximately 25 days for the 'Ugni Blanc', 'Petit Verdot', 'BRS Cora', 'Moscato' and 'Paulistinha' and 'Liberty' grapes. For the 'Isabel' and 'Paulsen 1103' vines, symptoms were only observed 39 days after inoculation (Nascimento et al. 2006).

The variation in the time of onset of the disease may be related to the different genotypic constitutions of the materials, i.e., the incubation period may be reduced in susceptible cultivars, but it may be also affected by the inoculation method used, inoculation point, concentration of the bacterial suspension, aggressiveness of the inoculated isolate, temperature, air humidity, plant age, method, and point of inoculation (Kong et al. 2008). Differences in the incubation period reflect differences in the growth rate of the pathogen in the host and, consequently, in the rate of progress of the epidemic, which is an important component of resistance (Parlevliet 1979).

Regarding disease severity, at 30 days after inoculation, the genotypes with the fewest number of lesions on the stem were CPATSA 67.24, CPATSA 64.83, CPATSA 49.25, CPATSA 49.86, and CPATSA 49.05, ranging from 2.27 to 2.92 mm2; for the petiole, the genotypes were CPATSA 64.83, CPATSA 67.24, CPATSA 49.05, CPATSA 49.25, CPATSA 49.32, CPATSA 49.86, and CPATSA 49.114, ranging from 1.74 to 4.10 mm^2.

Malavolta Júnior et al. (2003), using inoculation by foliar infiltration, observed low levels of disease severity in 'White Niagara' (*Vitis labrusca* × *V. vinifera*) and 'Niagara Rosada' (natural mutation of 'White Niagara') clones, if the resistance of these hybrids came from *V. labrusca*, whose genotype must contain the genes for this trait. Nascimento et al. (2006), using the friction inoculation method with leaf gauze, observed that the 'Paulsen 1103' and 'Isabel' grapevines had lower levels of disease severity at 42 days after inoculation. Using the same method of friction with gauze, low severity was observed for the 'BRS Cora' grape (0.04%) (Zucal et al. 2016).

Inoculation into the petioles of 'Red Globe' vines led to necrosis, dark lesions and small cankers causing longitudinal cracks from the point of inoculation. At 25 days after inoculation, the lesions varied in size from 5 to 26 mm, and necrosis surrounded more than 50% of the stems (Lima et al. 1999).

Malavolta Júnior et al. (2003), who implemented inoculation by foliar infiltration, identified a high level of severity of bacterial canker in the 'Red Globe', 'Itália', 'Benitaka', and 'Rubi' clones belonging to the species *V. vinifera*. Nascimento et al. (2006), using the friction inoculation method with leaf gauze, observed that the severity at 42 days after inoculation ranged from 0.08 to 23.24%, and high levels of disease severity were observed for the 'Benitaka', 'Brasil', 'Catalunha' and 'Thompson Seedless' clones. Zucal et al. (2016), using the same method, found that the severity of leaf canker ranged from 0.04 to 10.85%, with the 'Red Globe' grape and Muscat Italy clone having the highest severity values.

It can be inferred that hybrids with smaller lesions have greater resistance to colonization, while hybrids with larger lesions have lower resistance to colonization. For the genotypes with fewer stem and petiole lesions, there were five hybrids in common (CPATSA 49.25, CPATSA 49.86, CPATSA 49.05, CPATSA 67.24, and CPATSA 64.83).

The area under the disease progress curve represents the proportion of plant organs with symptoms in relation to the total, without taking into account the amount of disease in each plant. When calculating the area under the disease progress curve for the stem, the genotypes with the lowest values were CPATSA 49.25, CPATSA 49.32, and CPATSA 49.86. The area under the disease progress curve values for the petiole were lower for the CPATSA 49.86 (620), and CPATSA 49.25 (647) genotypes. Two genotypes were common (CPATSA 49.25, and CPATSA 49.86) when comparing the genotypes that exhibited the lowest area under the disease progress curve for the stem and petiole.

In a study seeking to find sources of resistance to bacterial canker in grapevines, it was observed that, under field conditions, *V. vinifera* was highly susceptible, while *V. labrusca* showed some resistance, but, after artificial inoculation, it became 'moderately resistant'. In addition, among the *V. vinifera* cultivars, the seedless cultivars were highly susceptible compared to the seeded cultivars. It was also observed that seedless and colored cultivars showed greater susceptibility than seedless and white cultivars (Chand 1992).

Kamble et al. (2017) found that, throughout the germplasm of *Vitis* spp., 90% of the seeded and pigmented genotypes were resistant, and 86% of the seedless and white genotypes were highly susceptible. According to Chand (1992), the exact mechanism of susceptibility in seedless cultivars is not known, but it is possible that the resistance traits are linked to the presence or absence of seeds.

In all the epidemiological parameters evaluated, the CPATSA 49.25 and CPATSA 49.86 hybrids showed greater resistance to bacterial canker, while the CPATSA 79.23 and CPATSA 79.49 hybrids showed greater susceptibility to this disease. The highest resistance of the CPATSA 49.25 and CPATSA 49.86 hybrids confirms the results obtained in Experiment I. From the selection of hybrids in the field, the CPATSA 49.25 and CPATSA 49.86 hybrids were among the 40 hybrids recognized for their greater resistance to bacterial canker of grapevine under field conditions.

These two hybrids are from the cross between Jupiter and Marroo and are seedless table grapes or with grapes with small traces of seeds. The CPATSA 49.25 hybrid is a red table grape with a natural berry size of 17 × 14 mm, fleshy consistency, and neutral flavor. The CPATSA 49.86 hybrid is a green table grape with a natural berry size of 16 × 13 mm, a crunchy consistency, and neutral flavor.

CONCLUSION

The analysis using the REML/BLUP method and the model presented are suitable for the selection of grapevine genotypes with resistance to *Xanthomonas citri* pv. *viticola*. Therefore, the 40 hybrids selected in this study will be able to advance to the next stage of the grapevine breeding program.

The CPATSA 49.25 and CPATSA 49.86 seedless hybrids were resistant to bacterial canker under greenhouse and field conditions.

CONFLICT OF INTEREST

Nothing to declare.

AUTHORS' CONTRIBUTION

Data curation: Carvalho, J. N., Carvalho, P. A., Batista, D. C., Leão, P. C. S.; **Formal Analysis:** Carvalho, J. N., Carvalho, P. A., Batista, D. C.; **Investigation:** Carvalho, J. N.; **Project Administration:** Leão, P. C. S.; Resources: Leão, P. C. S.; **Methodology:** Carvalho, J. N., Leão, P. C. S.; **Software:** Carvalho, J. N.; **Supervision:** Barbosa, M. A. G., Pio, R., Leão, P. C. S.; **Validation:** Carvalho, J. N., Leão, P. C. S.; **Visualization:** Carvalho, J. N.; **Writing – Original Draft:** Carvalho, J. N.; **Writing – Review & Editing:** Carvalho, J. N., Barbosa, M. A. G., Carvalho, P. A., Pio, R., Leão, P. C. S.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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