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ORIGINAL ARTICLE

Evaluation of grapevines for resistance to downy mildew (*Plasmopara viticola*) under greenhouse conditions

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Abstract

Grapevine downy mildew, caused by the fungus *Plasmopara viticola* is a disease with a great economic impact on grapevine in the Brazil. The objective of this study was to evaluate resistance to downy mildew in seedless grapevine hybrids and one susceptible cultivar 'Thompson Seedles' using natural infection (cross-contamination) and artificial inoculation method under greenhouse conditions. The experiments were performed at Embrapa Semiarid, Petrolina, Pernambuco, Brazil. The first used natural infection from plants with diseased vines, and the second artificial infection in which plants were sprayed with a spore suspension with a concentration of 10^5 conidia mL^{-1}. The evaluated variables were final incidence, final severity, area under the curve of disease incidence progression and area under the curve of disease severity progression. All evaluated genotypes showed symptoms of downy mildew. The CPATSA 28.14 genotype presented the lowest values of final severity, final incidence, area under the disease severity progress curve and area under the disease incidence progress curve in both experiments. Therefore, the CPATSA 28.14 genotype was identified as having greater resistance to grapevine downy mildew. Clustering by the unweighted pair grouping method using arithmetic averages (UPGMA) resulted in the separation of the genotypes into three and two similarity groups in Experiments I and II, respectively, indicating low diversity among the grapevine hybrids evaluated.

Keywords Epidemiological components · Incidence · Multivariate analysis · *Plasmopara viticola* L. · Severity · *Vitis* spp

Introduction

Viticulture has great socioeconomic relevance for Brazil. Grapes are the third most produced fruit in the country and occupy an area of approximately 7,500 hectares, with an average productivity of 22.9 t/ha and annual production of approximately 1.7 million tons, which corresponds to an estimated value of 3.6 billion reais (IBGE 2022). Brazil is unique in that it is the only country where all three types of viticulture are found (temperate, subtropical and tropical), which are associated with different soil and climate conditions and grapevine management practices (Pereira 2020; Silva et al. 2019).

Some climatic conditions favor the emergence of diseases, which, if not properly controlled, leads to reduced productivity in vineyards. Downy mildew is considered one of the main diseases of Brazilian viticulture, occurring in all states (Amaral et al. 2020; Buonassisi et al. 2017), in addition to being the most economically significant disease worldwide due to its high power of devastation (Atak et al. 2017).

The causal agent of grapevine downy mildew is the obligate biotrophic oomycete *Plasmopora viticola* (Berk. & Curt) Berl. & de Toni. This phytopathogen has been reported to be a complex species (Rouxel et al. 2013) able to damage stems, leaves, herbaceous branches, inflorescences and fruits, affecting quality and yield (Amaral et al. 2020), and may even cause 100% production losses and impact production in following years (Angelotti et al. 2017). High air humidity, temperatures between 20° and 25 °C and free

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water in the leaves are optimal conditions for the development of the disease (Atak et al. 2017).

The initial symptoms of downy mildew are characterized by irregular yellowish and oily spots on the adaxial surface of the leaf, which evolve into brown or necrotic spots, causing death of infected tissues (Yin et al. 2017). Under favorable climatic conditions, the reproductive structures of the pathogen, which have a cottony white appearance, cover most of the abaxial surface of the leaf and may also appear on buds and shoots, which become curved and thick when infected (Buffara et al. 2014). Symptoms in clusters are grayish berries with fungal growth on their surface (Buffara et al. 2014; Yin et al. 2017).

The evaluation of the incidence and severity of diseases is an important component in the adoption of control measures. The current strategy to control grapevine downy mildew is based on chemical treatments. Repeated applications of fungicides are performed to control the disease, leading to environmental pollution, the development of resistant strains, residual toxicity and pathogen pressure (Buonassisi et al. 2017). Control measures based on cultural practices are also employed, such as handling the shoot system to reduce leaf wetness and allow greater ventilation of the vine, use of plastic cover, balanced nutrition avoiding excess nitrogen fertilization, as well as the use of less vigorous rootstocks. However, without the effectiveness of chemical control.

Due to the losses caused by *P. viticola*, it is desirable that new cultivars developed by breeding programs carry some degree of resistance to the pathogen. Thus, the presence of downy mildew resistance is essential in the selection of candidates for new cultivars, even before the selection of agronomic traits.

Efforts made to improving the resistance of susceptible vines, and wild-resistant vine species are important for the germplasm improvement of commercial cultivars (Ma et al. 2018). Several studies show that *Vitis labrusca* genotypes exhibit a generally higher degree of resistance to downy mildew than *Vitis vinifera* cultivars. However, the level of resistance may vary from cultivar to cultivar (Atak et al. 2017).

The use of resistant grapevine genotypes obtained through breeding programs represents an alternative for the control of grapevine downy mildew; this approach carries a low cost to the producer and can be widely used, although it depends on the limits of natural resistance (Buonassisi et al. 2017).

The development and cultivation of mildew resistant grape cultivars are promising strategies to reduce the impact of disease management. In grapevine, up to now 31 quantitative trait loci (QTL) conferring resistance to downy mildew at different levels ranging from weak to total have been identified within *Vitis* species. The *P. viticola* resistance loci (Rpv) discovered in *V. vinifera* were Rpv29, Rpv30, Rpv31 (Sargolzaei et al. 2021), while in unspecified American species were found Rpv4, Rpv7, Rpv11, Rpv17, Rpv18, Rpv20, and Rpv21 (Fischer et al. 2004; Welter et al. 2007; Bellin et al. 2009; Divilov et al. 2018).

Thus, the objective of this study was to evaluate resistance to downy mildew in seedless grapevine hybrids and one susceptible cultivar 'Thompson Seedles' using natural infection (cross-contamination) and artificial inoculation method, under greenhouse conditions, aiming to direct strategies for the genetic breeding program of the grapevine.

Materials and methods

In this study, 15 seedless hybrides resulting from crosses performed in Embrapa Semiárido (Table 1) were evaluated with the *Vitis vinifera* cultivar 'Thompson Seedless' as the susceptible standard.

Seedlings of each hybrid the cultivar Thompson Seedless were grown in polyethylene bags containing a mixture of natural soil and sand (3:1 v:v) under greenhouse conditions (27.5 ± 8 °C, photoperiod of 12 h and mean relative humidity of 65%, at Embrapa Semiarid, Petrolina, Pernambuco, Brazil).

For natural infection, vine seedlings with symptoms of downy mildew were randomly placed among the materials to be evaluated in a humid chamber for 48 h. The plants were inoculated by direct contact between healthy and infected plants (cross-contamination), at the ratio of five healthy plants to one infected plant at 90 days of age.

Evaluations of disease severity and incidence were performed every three days after samples were removed from the humid chamber for up to seventeen days, totaling five evaluations. Disease incidence was calculated as the percentage of leaves with symptoms in relation to the total number of evaluated leaves per plant. Disease severity was quantified as the percentage of diseased leaf area using a diagrammatic scale (Buffara et al. 2014). The area under the curve of disease incidence progress (AUCDIP) and area under the curve of disease severity progress (AUCDSP) were calculated by the expression $AUCDP = \sum d_{ti}$, where y_i and y_{i+1} represent the values of incidence or severity observed in two consecutive evaluations and d_{ti} is the interval between evaluations (Shaner and Finney 1977).

Table 1 Seedless grape hybrides evaluated for resistance to *Plasmopora viticola* under greenhouse conditions

Seedless grape hybrides			
CPATSA 79.23	CPATSA 28.14	CPATSA 51.01	CPATSA 49.114
CPATSA 79.49	CPATSA 67.24	CPATSA 49.05	CPATSA 49.86
CPATSA 42.49	CPATSA 49.32	CPATSA 49.25	CPATSA 63.114
CPATSA 65.04	CPATSA 64.83	CPATSA 63.108	'Thompson seedless'

The experimental design was completely randomized, with 16 treatments (genotypes) and five replicates (plants/genotype), and four leaves per plant were evaluated.

For artificial infection, plants with active infection of downy mildew on the leaves were grown in a greenhouse to prepare the inoculum. Leaves containing spores (sporangiospores) of the pathogen were taken to the laboratory, and with the aid of a brush, the spores were removed and placed in a beaker with sterile distilled water. Spore counting was performed in a Neubauer chamber; the suspension was adjusted to a concentration of 10^5 conidia mL^{-1}, and Tween 80 (0.05%) was added.

Healthy seedlings were inoculated by spraying the leaves to the run-off point using a DeVilbiss sprayer. After inoculation, the plants were kept in a humid chamber for 48 h. Three days after removal from the humid chamber, the evaluations began as described above.

The experimental design was completely randomized, with 16 treatments (genotypes) and five replicates (plants/genotype), and four leaves per plant were analyzed.

The experiments were analyzed separately. Univariate and multivariate analyses were performed. In the univariate analysis, the data were transformed into $(x)^{0.5}$ and subjected to analysis of variance, and the means were compared by the Scott−Knott cluster test ($p < 0.05$) using SISVAR software. In the multivariate analysis, which considered all the evaluated epidemiological parameters, the Euclidean distance was adopted as a measure of dissimilarity, and groups were clustered based on the average distance between genotypes using the hierarchical unweighted pair grouping method using arithmetic averages (UPGMA) in PAST software. The cutoff line was established at the location of the abrupt change in the branches in the dendrogram (Cruz et al. 2014).

Results

The occurrence of grapevine downy mildew was observed in all evaluated seedless grape hybrids, but at different levels of incidence and severity. There were significant differences ($p < 0.05$) between genotypes for the four epidemiological components, final severity (FS), final incidence (FI), AUCDSP and AUCDIP, in both experiments.

Among all epidemiological components, the genotypes were distributed into two groups with high similarity, except for AUCDIP in the first experiment, in which the genotypes were separated into three groups by the Scott−Knott cluster test (Tables 2 and 3).

Under natural infection, the lowest values for all the analyzed variables (FS, FI, AUCDSP and AACPID) were presented by CPATSA 28.14. Regarding FS, which indicates the percentage of injured leaf area, the genotype CPATSA 28.14 (0.3%) did not show a significant difference from CPATSA 42.49, CPATSA 49.32, CPATSA 49.86, CPATSA 63,108, CPATSA 63,114, CPATSA 64.83, CPATSA 65.04, CPATSA 79.23 or CPATSA 79.49. These same genotypes also did not differ from one another for AUCDSP.

Table 2 Reaction of seedless grapevine genotypes to natural infection by *Plasmopara viticola* based on the epidemiological components of downy mildew under greenhouse conditions

Hybrid	FS (%)	FI (%)	AUCDSP	AUCDIP
CPATSA 28.14	0.33a	20a	0.50a	30a
CPATSA 42.49	12.73a	80b	58.30a	440b
CPATSA 49.05	13.47b	60a	133.10b	630c
CPATSA 49.114	24.73b	100b	226.90b	1140c
CPATSA 49.25	15.67b	93b	103.50b	960c
CPATSA 49.32	5.27a	47a	49.70a	490b
CPATSA 49.86	8.33a	93b	27.70a	320b
CPATSA 51.01	19.60b	100b	133.70b	1070c
CPATSA 63.108	7.80a	80b	77.10a	680c
CPATSA 63.114	4.47a	60a	22.10a	310b
CPATSA 64.83	9.73a	87b	61.00a	610c
CPATSA 65.04	9.40a	87b	83.50a	720c
CPATSA 67.24	16.67b	100b	116.30b	1000c
CPATSA 79.23	7.60a	93b	37.20a	510b
CPATSA 79.49	11.33a	87b	83.60a	630c
'Thompson seedless'	35.30b	100b	218.21b	1080c

FS final disease severity at 17 days after inoculation of the pathogen, *FI* final incidence of leaves with disease symptoms 17 days after inoculation of the pathogen, *AUCDSP* area under curve of disease severity progression, *AUCDIP* area under the curve of disease incidence progression. Means followed by the same letter in the column do not differ from each other ($p \geq 0.05$) by the Scott−Knott test. Original data. For analysis purposes, all data were transformed into $(x)^{0.5}$

Regarding FI, that is, the number of leaves with symptoms, the genotypes CPATSA 28.14, CPATSA 49.32, CPATSA 49.05 and CPATSA 63,114 presented lower values than the seedless Thompson cultivar, indicating lower susceptibility to downy mildew (Table 2). For AUCDIP, the lowest value was observed for the genotype CPATSA 28.14. This genotype, together with the genotypes CPATSA 42.49, CPATSA 49.32, CPATSA 49.86, CPATSA 63.114 and CPATSA 79.23, exhibited higher resistance to downy mildew than the 'Thompson Seedless' cultivar (Table 2).

In the artificial infection treatment, the genotypes CPATSA 28.14 and CPATSA 67.24 presented similar results for all the analyzed variables, in contrast to the results for natural infection of *P. viticola.* Although resistance levels were confirmed for the other genotypes, CPATSA 67.24 exhibited distinct behavior in both experiments. This may have occurred due to the pathogen concentration being lower under artificial infection than natural infection conditions or due to a failure to inoculate some plants. Regarding FS, eleven genotypes were more resistant to downy mildew because their FS values differed significantly from that of

Table 3 Reaction of seedless grapevine genotypes to artificial infection of *Plasmopara viticola* based on the epidemiological components of downy mildew under greenhouse conditions

Hybrid	FS (%)	FI (%)	AUCDSP	AUCDIP
CPATSA 28.14	8.30a	80a	52.73a	765a
CPATSA 42.49	24.35b	100b	131.10b	795a
CPATSA 49.05	4.60a	100b	28.35a	593a
CPATSA 49.114	20.35b	100b	140.85b	1095b
CPATSA 49.25	10.00a	100b	57.45a	900b
CPATSA 49.32	5.80a	100b	31.50a	690a
CPATSA 49.86	12.15a	100b	83.85a	720a
CPATSA 51.01	10.25a	100b	64.95a	743a
CPATSA 63.108	14.55a	100b	104.10b	998b
CPATSA 63.114	13.75a	100b	76.95a	900b
CPATSA 64.83	18.80b	100b	120.68b	1028b
CPATSA 65.04	12.25a	100b	74.40a	945b
CPATSA 67.24	12.15a	75a	67.95a	758a
CPATSA 79.23	15.45a	100b	82.35a	773a
CPATSA 79.49	26.40b	100b	179.55b	923b
'Thompson seedless'	32.70b	100b	180.53b	990b

FS final disease severity at 17 days after inoculation of the pathogen, *FI* final incidence of leaves with disease symptoms 17 days after inoculation of the pathogen, *AUCDSP* area under curve of disease severity progression, *AUCDIP* area under the curve of disease incidence progression. Means followed by the same letter in the column did not differ from each other ($p \geq 0.05$) according to the Scott−Knott test. Original data. For analysis purposes, all data were transformed into (x) $^{0.5}$

the 'Thompson Seedless' cultivar. The genotypes CPATSA 67.24 and CPATSA 28.14 showed low FI values, thus demonstrating greater resistance than 'Thompson Seedless' (Table 3). Regarding AUCDSP, ten genotypes were more resistant than 'Thompson Seedless'. Considering AUCDIP, eight genotypes were more resistant than the cultivar used as the susceptible standard.

Under natural infection, only the CPATSA 28.14 genotype showed the lowest values for all the epidemiological components evaluated, whereas under artificial infection, two genotypes (CPATSA 28.14 and CPATSA 67.24) showed lower levels of disease for all variables.

UPGMA cluster analysis based on the set of epidemiological components of grapevine downy mildew resulted in the distribution of genotypes into three similarity groups in the natural infection experiment and two groups in the artificial infection experiment (Figs. 1 and 2).

Among the seedless grape hybrids naturally infected with downy mildew, one group contained only the genotype CPATSA 28.14, which presented FS, FI, AUCDSP and AUCDIP values of 0.33%, 20%, 0.50 and 30, respectively. A second group was formed by CPATSA 49.32, CPATSA 79.23, CPATSA 42.49, CPATSA 63.114, CPATSA 49.86, CPATSA 64.83, CPATSA 79.49, CPATSA 49.05, CPATSA

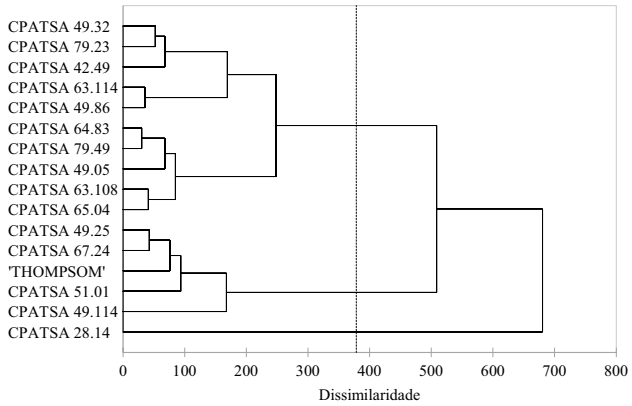

Fig. 1 Dendrogram of the cluster analysis of 16 grapevine genotypes based on the mean values of the epidemiological components of grapevine downy mildew under natural infection conditions

63.108, and CPATSA 65.04. These two groups, in descending order, include the genotypes that presented the best resistance to grapevine downy mildew. The remaining genotypes constituted the third similarity group and were the most susceptible to downy mildew (Fig. 1).

For the seedless grape hybrids subjected to artificial downy mildew infection, a group was formed by the genotypes CPATSA 51.01, CPATSA 28.14, CPATSA 67.24, CPATSA 79.23, CPATSA 49.86, CPATSA 49.32, CPATSA 42.49 and CPATSA 49.05, which presented values ranging from 4.6% to 24.35% for FS, 75% to 100% for FI, 31.5 to 131.1 for AUCDSP and 593 to 795 for AUCDIP. The genotypes most resistant to downy mildew are found in this group. The second group corresponds to the other genotypes, which had greater susceptibility to grapevine downy mildew (Fig. 2).

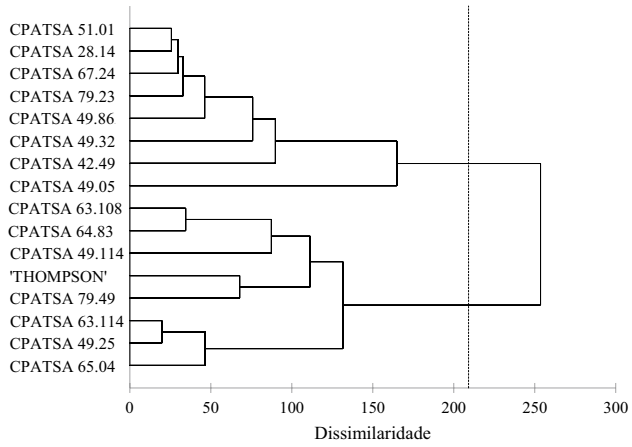

Fig. 2 Dendrogram of the cluster analysis of 16 grapevine genotypes subjected to artificial infection

The CPATSA 28.14 genotype exhibited the greatest resistance to grapevine downy mildew because it presented the lowest values of severity, incidence, AUCDSP and AUCDIP under both natural and artificial infection, differing from the 'Thompson Seedless' susceptibility pattern and indicating great potential for use in genetic breeding programs.

Discussion

The studies were carried out in a greenhouse due to the possibility of having optimal conditions for infection and development of downy mildew under controlled conditions, like what happens with the leaf disk assay. Even though the evaluation in a greenhouse is more laborious and takes longer to obtain results, it was the method chosen because the disease occurred naturally in some of the plants used in the work, and because the results obtained in a greenhouse were significantly related to the strength verified in the field. The test with leaves of seedlings *in vitro* tends to be more resistant than leaves of plants grown in a greenhouse (Deglène-Benbrahim et al. 2010).

In this study, varying degrees of resistance were observed, and none of the cultivars showed complete resistance to downy mildew. The degree of susceptibility or resistance of the host to the pathogen is a trait inherited by progeny, and the host may be susceptible or resistant or have varying degrees of resistance (Merdinoglu et al. 2018).

Sargolzaei et al. (2021) stated that V. labrusca and hybrid grape cultivars are more resistant than V. vinifera. Similarly, Toffolatti et al. (2016) stated that cultivars of V. vinifera are generally more susceptible to downy mildew. However, Atak et al. (2017) observed that in addition to cultivars of V. vinifera, some interspecific crosses are susceptible to downy mildew.

The symptomal pattern of the disease in the most susceptible cultivars corresponds to rapidly expanding oil-spot lesions, and under conditions of high humidity, the abaxial surface of the leaf presents sporulation with a cottony appearance, which may affect the entire leaf blade.

The CPATSA 28.14 genotype, exhibited the greatest resistance to grapevine downy mildew, is a table grape hybrid without seeds or with small traces of seeds resulting from the cross between the cultivars 'A 1581' and 'Marroo'; its berries have a red color, natural size of 20 mm × 17 mm, fleshy consistency, and neutral flavor. These traits endow this genotype with important qualitative traits for selection and for advancing to the next stages of genetic improvement or selection.

Beyond to CPATSA 28.14, other hybrids stood out such as 51.01, 67.24, 79.23, 49.86, 49.32, 42.49 and 49.05. Of these hybrids, both 28.14, 79.23, 49.86, 49.32 and 49.05 are crosses with the Australian cultivar 'Marroo Seedless', an interspecific hybrid characterized by being resistant to diseases, resulting from the crossing of resistant 'Carolina Blackrose' and 'Ruby Seedless' (Antcliff and Antcliff 1990).

Several genes of Vitis species closely related to cultivated grapes that confer protection against downy mildew have been identified. However, many of the major resistance genes were outperformed by virulent strains of pathogens in various phytopathogenic interactions. Resistance is horizontal, resistance genes are a limited resource, and their introduction into a new cultivar is an expensive and long-term process. That is why the evaluation and improvement of resistance are crucial, especially in the case of perennial species (Merdinoglu et al. 2018).

The infection process of grapevine downy mildew i.e., the speed and extent of infection, is determined by climatic factors, including temperature and humidity. Angelotti et al. (2017) in an evaluation of the effects of climate change on grapevine downy mildew, observed downy mildew in plants subjected to temperatures of up to 31.8 °C and concluded that temperature interfered with grapevine downy mildew infection, reduced disease severity and increased the latency period, delaying the onset of the disease. Schmidt et al. (2012) when studying the pathogenicity mechanisms of P. viticola, observed that the sporangia tolerate dehydration at 37 °C without loss of pathogenicity. During the evaluation of this experiment, the average temperature was 27.5 ± 8 °C, i.e., favorable for mildew infection and disease development.

Through marker-assisted selection, Sánchez et al. (2017) sought to develop homozygous grapevine lines at the Rpv1 and Rpv3 loci for resistance against downy mildew. Among 637 genotyped plants, 300 were homozygous for at least one resistance locus, and 10 were homozygous for both loci, suggesting that these plants have great potential as resistance donors in grapevine breeding. Ma et al. (2018) developed a transgenic VpPR10.1 grapevine line that showed resistance to P. viticola and concluded that the VpPR10.1/VpVDAC3 complex is responsible for the cell death defense response of P. viticola in grapevine. In addition, the molecular marker-based identification of the locus of resistance to grapevine downy mildew allows tracking resistant hybrid vines in breeding programs (Buonassisi et al. 2017).

Currently, the most widely used method to control grapevine downy mildew is the application of fungicides, which increases production costs, favors the emergence of strains resistant to the active ingredients and negatively impacts the environment (Santos et al. 2020). Thus, the identification and use of cultivars with greater resistance to downy mildew is considered the most effective and economical disease management strategy for producers because it allows reducing the number of fungicide sprayings and, therefore, contributes to viticulture sustainability (Buonassisi et al. 2017).

Thus, the use of resistant hybrides developed through genetic breeding represents an alternative for the integrated control of grapevine downy mildew. Accordingly, the results of this study should be complemented with evaluations of the behavior of the hybrids under field conditions and serve as a foundation for the development of cultivars resistant to grapevine downy mildew in genetic improvement programs.

In conclusion, all seedless grape hybrids evaluated showed grapevine downy mildew symptoms. The CPATSA 28.14 genotype demonstrated the greatest resistance to grapevine downy mildew because it presented the lowest levels of disease in all the evaluated epidemiological components under natural and artificial infection, indicating great potential for selection and use in genetic improvement programs. According to the cluster analysis, there is low diversity among the evaluated hybrids regarding resistance to downy mildew.

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

Declarations

Competing interest The authors declare to have no conflict of interest, whether financial or non-financial, associated with this research.

References

Amaral BD, Viana AP, Santos EA, Ribeiro RM, Silva FA, Ambrósio M, Walker AM (2020) Prospecting for resistance of interspecific hybrids of Vitis spp. to *Plasmopara viticola*. Euphytica 216:1–14

Angelotti F, Hamada E, Magalhães EE, Ghini R, Garrido L, Da R, Pedro Júnior MJ (2017) Climate change and the occurrence of downy mildew in Brazilian grapevines. Pesquisa Agropecuária Brasileira 52:426–434

Antcliff AJ, Antcliff FG (1990) Grape variety named Marroo Seedless. Assignee: Commonweath Scientific and Industrial Research Ornagization. Plant 7,377. Filed: jan.25,1989. Date of patent: nov.6,1990

Atak A, Akkurt M, Polat Z, Çelik H, Kahraman KA, Akgül DS, Özer N, Söylemezoğlu G, Şire GG, Eibach R (2017) Susceptibility to downy mildew (*Plasmopara viticola*) and powdery mildew (*Erysiphe necator*) of different Vitis cultivars and genotypes. Ciência e Técnica Vitivinícola 32:23–32

Bellin D, Peressotti E, Merdinogl D, Wiedemann-Merdinoglu S, Adam-Blondon AF, Cipriani G et al (2009) Resistance to *Plasmopara viticola* in grapevine 'Bianca' is controlled by a major dominant gene causing localised necrosis at the infection site. Theoretial and Applied Genetics 120:163–176

Buffara CRS, Angelotti F, Vieira RA, Bogo A, Tesmann DJ, De Bem BP (2014) Elaboration and validation of a diagrammatic scale to assess downy mildew severity in grapevine. Ciência Rural 44:1384–1391

Buonassisi D, Colombo M, Migliaro D, Dolzani C, Peressotti E, Mizzotti C, Velasco R, Masiero S, Perazzolli M, Vezzulli S (2017) Breeding for grapevine downy mildew resistance: a review of "'omics"' approaches. Euphytica 213:1–21

Cruz CD, Carneiro PCS, Regazzi AJ (2014) Modelos biométricos aplicados ao melhoramento genético. 3th Ed. UFV, Viçosa

Deglène-Benbrahim L, Wiedemann-Merdinoglu S, Merdinoglu D, Walter B (2010) Evaluation of downy mildew resistance in grapevine by leaf disc bioassay with *in vitro*- and greenhouse-grown plants. The American Journal of Enology and Viticulture 61:521–528

Divilov K, Barba P, Cadle L, Bruce D (2018) Single and multiple phenotype QTL analyses of downy mildew resistance in interspecific grapevines. Theoretical and Applied Genetics 131:1133–1143

Fischer B, Salakhutdinov I, Akkurt M, Eibach R, Edwards K, Toepfer R et al (2004) Quantitative trait locus analysis of fungal disease resistance factors on a molecular map of grapevine. Theoretical and Applied Genetics 108:501–515

IBGE (2022) Instituto Brasileiro de Geografia e Estatística. Levantamento Sistemático da Produção Agrícola. Available at: https://sidra.ibge.gov. br/home/lspa/brasil. Accessed on: January 12, 2022

Ma H, Xiang G, Li Z, Wang Y, Dou M, Su L, Liu R, Wang Y, Xu Y (2018) Grapevine VpPR10.1 functions in resistance to *Plasmopara viticola* through triggering a cell death-like defence response by interacting with VpVDAC3. Plant Biotechnology Journal 16:1488–1501

Merdinoglu D, Schneider D, Prado E, Wiedemann-Merdinoglu S, Mestre P (2018) Breeding for durable resistance to downy and powdery mildew in grapevine. OENO One 52:189–195

Pereira GE (2020) The three different wine-growing zones in Brazil according to climate conditions and vine managements. In: Jordão AM, Botelho RV (eds) Vitis: biology and species. Nova Science Publishers, New York. Avaiable at http://magarach-institut.ru/wp-content/uploads/2021/09/ebook-vitis.pdf. Acessed 22 May 2022

Rouxel M, Mestre P, Comont G, Lehman BL, Schilder A, Delmotte F (2013) Phylogenetic and experimental evidence for host-specialized cryptic species in a biotrophic oomycete. New Phytologist 197:251–263

Sánchez FDM, Saifert L, Zanghelini J, Assumpção WT, Guginski-Piva CA, Giacometti R, Novak EI, Klabunde GH, Eibach R, Dal Vesco L, Nodari RO, Welter LJ (2017) Behavior of grape breeding lines with distinct resistance alleles to downy mildew (*Plasmopara viticola*). Crop Breeding and Applied Biotechnology 17:141–149

Santos RF, Fraaije BA, Garrido LR, Monteiro-VItorello C, Amorim L (2020) Multiple resistance of *Plasmopara viticola* to QoI and CAA fungicides in Brazil. Plant Pathology 69:1708–1720

Sargolzaei M, Rustioni L, Cola G, Ricciardi V, Bianco PA, Maghradze D, Failla O, Quaglino F, Toffolatti SL, De Lorenzis G (2021) Georgian Grapevine Cultivars: Ancient Biodiversity for Future Viticulture. Frontiers in Plant Science 12:1–13

Schmidt C, Görtz A, Bieler E, Thomann R, Kassemeyer HH (2012) Downy mildew (*Plasmopara viticola*) - mechanisms of pathogenicity. Phytomedizin 42:1–3

Silva JN, Ponciano NJ, Souza CLM, Souza PM, Viana LH (2019) Caracterização da viticultura tropical nas regiões norte e noroeste fluminense. Revista Brasileira de Fruticultura 41:1–8

Toffolatti SL et al (2016) Evidence of resistance to the downy mildew agent *Plasmopara viticola* in the Georgian Vitis vinifera germplasm. Vitis 55:121–128

Welter LJ, Göktürk-Baydar N, Akkurt M, Maul E, Eibach R, Töpfer R et al (2007) Genetic mapping and localization of quantitative trait loci affecting fungal disease resistance and leaf morphology in grapevine (*Vitis vinifera* L). Molecular Breeding 20:359–374

Yin L, An Y, Qu J, Li X, Zhang Y, Dry I, Wu H, Lu J (2017) Genome sequence of *Plasmopara viticola* and insight into the pathogenic mechanism. Scientific Reports 7:1–12