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ESCALA DIAGRAMÁTICA, FENOTIPAGEM, GENOTIPAGEM E MAPEAMENTO DE RESISTÊNCIA DA VIDEIRA À ANTRACNOSE

Florianópolis
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Tese submetida ao Programa de Pós-graduação em Recursos Genéticos Vegetais da Universidade Federal de Santa Catarina para a obtenção do título de Doutor em Ciências.
Orientador: Prof. Aparecido Lima da Silva

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RESUMO

A antracnose é uma doença fúngica que causa danos econômicos para viticultura brasileira. O objetivo desse trabalho foi avaliar as modificações bioquímicas e enzimáticas causadas pelo ataque do *Elsinoë ampelina* nos frutos, mapear QTLs ligados a resistência a antracnose e desenvolver escala diagramática para a fenotipagem da doença. Para a avaliação das modificações bioquímicas e enzimáticas causadas pelo *Elsinoë ampelina*, utilizou-se frutos com e sem sintomas de cinco variedades de videira que apresentam diferentes níveis de suscetibilidade a antracnose. O mapeamento de QTL ligados a resistência antracnose foi realizado a partir da fenotipagem e genotipagem de duas populações segregantes com background genético de *Vitis amurensis*. Para o desenvolvimento de escala diagramática utilizou-se fotos de ramos e cachos com sintomas da doença e 12 avaliadores classificaram o nível de severidade da doença sem e com ajuda das escalas diagramáticas desenvolvidas. Variedades menos suscetíveis ao desenvolvimento de sintomas de antracnose no fruto, apresentam mecanismo de defesa ligados a atividade enzimática. A produção de açúcar na casca e concentração de sólidos solúveis no mostro da uva foram o mesmo em bagas com e sem sintomas de antracnose, porém a acidez do mosto foi maior quando os frutos apresentaram sintomas da doença. O mapeamento de QTLs revelou dois loci ligados a resistência da videira a antracnose, *Rea1* e *Rea2*. Os loci mapeados estão ligados a síntese de terpenos, efeitos de recepção NB-ARC e transportadores de espécies reativas de oxigênio. As escalas diagramáticas elaboradas para a avaliação da antracnose nos ramos e frutos aumentaram a precisão e reprodutibilidade entre os avaliadores sendo recomendadas para a fenotipagem da antracnose. Conclui-se que existe fonte de resistência a antracnose da videira e que utilizando escala diagramática com alta reprodutibilidade será possível selecionar variedades resistentes para o melhoramento genético da espécie.

Palavras-chave: *Elsinoë ampelina*, *Vitis*, fenotipagem, QTL.

ABSTRACT

Anthracnose is a fungal disease that causes economic damage to Brazilian viticulture. This study aimed to evaluate the biochemical and enzymatic modifications caused by *Elsinoë ampelina* attack on fruits, to map QTLs linked to anthracnose resistance and to develop a diagrammatic scale for the disease phenotyping. Evaluating the biochemical and enzymatic changes caused by *Elsinoë ampelina*, fruits with and without symptoms of five grapevine varieties that present different levels of susceptibility to anthracnose were used. QTLs linked with anthracnose resistance were found using phenotypic and genotypic data from two segregating populations with genetic background of *Vitis amurensis*. For the development of the diagrammatic scale, photos of branches and bunches with symptoms of the disease were used and 12 evaluators classified the level of severity of the disease without and with the help of the diagrammatic scales developed. Varieties less susceptible to the development of anthracnose symptoms in the fruit have a defense mechanism linked to enzymatic activity. The production of sugar in the skin and the concentration of soluble solids in the grape must were the same in berries with and without symptoms of anthracnose, but the acidity of the must was higher when the fruits showed symptoms of the disease. QTL mapping revealed two loci linked to anthracnose resistance of the grapevine, *Rea1* and *Rea2*. The mapped loci are linked to terpene synthesis, NB-ARC reception effects and reactive oxygen species transporters. The diagrammatic scales elaborated for the evaluation of anthracnose in the branches and fruits increased the precision and reproducibility among the evaluators, being recommended for anthracnose phenotyping. It is concluded that there is a source of grapevine resistance against anthracnose and with the use of a diagrammatic scale with high reproducibility it will be possible to select resistant varieties for the genetic improvement of the species.

Keywords: *Elsinoë ampelina*, *Vitis*, QTL.

SUMÁRIO

1 INTRODUÇÃO

A família *Vitis* apresenta aproximadamente 70 espécies, porém a produção mundial concentra-se principalmente em *Vitis vinifera*, por apresentar maior qualidade dos frutos comparado as outras espécies *Vitis* (Töpfer et al., 2011; Dal Santo et al., 2016). No Brasil, a região Sul, maior produtora de uva para elaboração de vinhos e espumantes, caracteriza-se por verões quentes (25 a 35°C) e úmidos (Malohlava et al., 2017; Santos et al., 2018a). Esta condição ambiental favorece o ataque de doenças fúngicas, como a antracnose causada pelo ascomiceto *Elsinoë ampelina* (de Bary) (Carisse et al., 2020). A antracnose ataca principalmente tecidos jovens da videira, como folhas, ramos e frutos (Santos et al. 2018a), dificultando o cultivo de variedades de *V. vinifera*, suscetível a doença, sendo necessárias aplicações preventivas de fungicidas para o controle da doença (Li et al. 2021).

O ataque de doenças fúngicas nos cachos da videira, causam danos visuais no produto final, o fruto (Sonker et al., 2016). Para a produção de vinho, é importante entender se a injúria causada pelos sintomas da doença resulta em modificações bioquímicas na casca e no mosto da uva (Ky et al., 2012). Além disso, estudar a atividade enzimática de variedades com diferentes níveis de suscetibilidade ao ataque do fitopatogêno, permite que se compreenda mecanismos de defesa ligados a resistência a doença (Murria et al., 2018a). As espécies reativas de oxigênios são o principal mecanismo de defesa ligado a resistência a infeção de fungos fitopatogênicos no cacho da videira (Bézier et al., 2002; Rotter et al., 2009). Para antracnose sabe-se que variedades tolerantes ao ataque de *E. ampelina* apresentam maior atividade enzimática na folha (Murria et al., 2018b). Porém, estudos para avaliar mecanismos de defesa ao ataque de *E. ampelina* nos frutos ainda não foram desenvolvidos.

A resistência à antracnose ainda não foi estudada em nível genético, necessitando de estudos que comprovem o tipo de herança genética dessa característica (Mortensen, 1981; Gao et al., 2012). Entretanto, as variedades de origem Euroasiáticas, *V. vinifera*, são classificadas como suscetíveis ao ataque da doença, enquanto as espécies originárias da América e Ásia, *V. labrusca*, *V. betulifolia*, *V. caribea* e *V. amurensis*, apresentam diferentes níveis de tolerância ao ataque de *E. ampelina* (Mortensen, 1981; Jang et al., 2011). A Alemanha apresenta longa tradição em desenvolvimento de variedades de videira para a resistência às doenças fúngicas como o míldio (*Plasmopara vitícola*) e oídio (*Uncinula necator*). O principal progresso alcançado pelo país foi em 1995 com as primeiras variedades introduzidas no mercado com resistências a essas duas doenças, um gene para míldio e um para o oídio, além de apresentar boa qualidade para produção de vinho. Essas variedades foram chamadas

PIlzWIderstandsfähige Rebsorten (PIWI), que traduzindo para português significa variedades de videira resistentes a doenças fúngicas (Eibach et al., 2007). Nos anos seguintes, diversas variedades com gene de resistência para oídio, como *Ren3*, e míldio (ex: *Rpv3* ou *Rpv10*) foram registradas e recomendadas (Ruehl et al. 2015). Porém, o espectro muito estreito de genes de resistências precisava ser ampliado. Na última década um progresso significativo no melhoramento da videira levou à identificação de vários locos de resistência para essas doenças (Töpfer et al., 2011). Entretanto, as alterações climáticas como chuva frequente, vem provocando surtos de patógenos comumente encontrados no Brasil, sendo *E. ampelina* exemplo disso (Lipps e Harms, 2004), proporcionando risco de dano econômico imediatos para o Brasil e futuros para a Alemanha, o que resultou em acordo bilateral entre instituições dos dois países, Universidade Federal de Santa Catarina (UFSC) e Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI) no Brasil e Julius Kühn Institute (JKI) na Alemanha, para estudar a resistência à antracnose. Nesse contexto, foi aprovado o projeto "Mapping of Grapevine Genetic Loci for Resistance to Downy Mildew and to Anthracnose, (89-MGGL)", financiado pelo edital Bioeconomy International and fits the module -Basis (BI-Basis), do governo alemão. O referido projeto incluiu a realização de cruzamentos no Brasil conduzidos pelo Grupo de Estudo da Uva e do Vinho (NEUVIN/UFSC) e mapeamento de genes na Alemanha em busca de resistência às principais doenças fúngicas da videira, míldio, oídio e antracnose.

O controle químico da antracnose resulta no aumento do custo de produção do cultivo da videira, além de ser feito em todos os anos de forma preventiva podendo aumentar a pressão para selecionar isolados do patógeno com resistência aos fungicidas. Além disso fungicidas em excesso podem provocar riscos à saúde humana, animal e ambiental, comprometendo a sustentabilidade do sistema produtivo (Magarey et al., 1993; Ghini, 2000). O uso de métodos de controle químico, cultural e genéticos associados à estudos epidemiológicos podem garantir um melhor manejo das doenças. Neste cenário, os programas de melhoramento de videira do mundo vêm desenvolvendo novas variedades adaptadas às condições locais de cultivo, resistentes às doenças fúngicas e com elevado potencial enológico na busca de um sistema de cultivo mais sustentável (Töpfer et al., 2011). A fenotipagem dessas progênies quanto à suscetibilidade a antracnose deve ser feita de maneira precisa para permitir reprodutibilidade dos resultados (Modesto et al., 2020), assim como estudos de progresso da doença no tempo (Barros et al 2015). Para estes tipos de estudos é importante padronizar o sistema de avaliação. O uso de escala diagrama para a avaliação da antracnose na folhas de videira aumenta a precisão

e reprodutibilidade da avaliação entre os avaliadores (Santos e Spósito, 2018). Porém, além das folhas, os ramos e frutos devem ser avaliados já que não existem trabalhos que comprovem a correlação entre a resistência ou suscetibilidade de diferentes partes da videira atacadas pelo *E. ampelina*. Os objetivos desse tese foram avaliar as alterações enzimáticas e bioquímicas em bagas com sintomas da antracnose (primeiro capítulo), mapear QTLs ligados a resistência a antracnose (segundo capítulo) e desenvolver escala diagramática para a fenotipagem da antracnose no cacho e ramo de maneira mais precisa (terceiro capítulo).

2 HIPOTÉSES

1. O ataque do *Elsinoë ampelina* em bagas da videira com diferente níveis de suscetibilidade pode ajudar a compreender alterações bioquímicas e enzimáticas que estariam relacionadas a infeção do patógeno e desenvolvimento da doença;
2. *V. amurensis* apresenta loci ligados à resistência contra a infecção de *Elsinoë ampelina* em ramos e folhas;
3. A construção de escala diagramática para avaliar a severidade da antracnose da videira nos frutos e ramos possibilitará maior reprodutibilidade e precisão nas avaliações da doença para uso na fenotipagem e demais estudos epidemiológicos.

3 REFERÊNCIAS

Barros, L. B., Biasi, L. A., Carisse, O., & De Mio, L. L. M. (2015). Incidence of grape anthracnose on different VITIS labrusca and hibrid cultivars and rootstocks combination under humid subtropical climate. Australasian Plant Pathology, 44(4), 397-403.

Bézier, A., Lambert, B., Baillieul, F. (2002). Study of defense-related gene expression in grapevine leaves and berries infected with *Botrytis cinerea*. European Journal of Plant Pathology, 108, 111-120.

Carisse, O., Levasseur, A., Provost, C. (2020). Influence of leaf wetness duration and temperature on infection of grape leaves by *Elsinoe ampelina* under controlled and vineyard conditions. Plant Disease, 104(11), 2817-2822.

Dal Santo, S., Fasoli, M., Negri, S., D'Incà, E., Vicenzi, N., Guzzo, F., Zenoni, S. (2016). Plasticity of the berry ripening program in a white grape variety. Frontiers in plant science, 7, 970.

Eibach, R., Zyprian, E., Welter, L., Topfer, R. (2007). The use of molecular markers for pyramiding resistance genes in grapevine breeding. Vitis*, 46*(3), 120.

Gao, M., Wang, Q., Wan, R., Fei, Z., Wang, X. (2012). Identification of genes differentially expressed in grapevine associated with resistance to *Elsinoe ampelina* through suppressive subtraction hybridization. Plant Physiology and Biochemistry, 58, 253-268.

Ghini, R.; Kimati, H. Resistência de fungos a fungicidas Jaguariuna: Embrapa Meio Ambiente, 2000. 78p.

Hulbert SH, Ilott TW, Legg EJ et al (1988) Genetic analysis of the fungus *Bremia lactucae*, using restriction fragment length polymorphisms. Genetics 120:947–958

Jang, M.H., Ahn, S.Y., Kim, S.H., Noh, J.H., Yun, H.K. (2011) Evaluation of grapevine varietal resistance to anthracnose through treating culture filtrates from *Elsinoe ampelina.* Horticulture, Environment, and Biotechnology, 52(2):152.

Ky, I., Lorrain, B., Jourdes, M., Pasquier, G., Fermaud, M., Gény, L., Rey, P., Doneche, B. Teissedre, P. L. (2012). Assessment of grey mould (*Botrytis cinerea*) impact on phenolic and sensory quality of Bordeaux grapes, musts and wines for two consecutive vintages. Australian Journal of Grape and Wine Research, 18(2), 215-226.

Li, Z., Dos Santos, R. F., Gao, L., Chang, P., Wang, X. (2021). Current status and future prospects of grapevine anthracnose caused by *Elsinoe ampelina*: An important disease in humid grape-growing regions. Molecular Plant Pathology.

Magarey, R. D., Emmett, R. W., Magarey, P. A., Franz, P. R. (1993). Evaluation of control of grapevine anthracnose caused by *Elsinoe ampelina* by pre-infection fungicides. Australasian Plant Pathology, 22(2), 48-52.

Malohlava, I. T. C., Simon, S., Vanderlinde, G., Brighenti, A. F., Malinovski, L. I., Munhoz, B., Silva, A. L. (2016, June). Ecophysiology of 'Cabernet Sauvignon' and 'Merlot 'grown at different altitudes in Santa Catarina State, Brazil. In X International Symposium on Grapevine Physiology and Biotechnology 1188, 197-204.

Modesto, L. R., Steiner, D. R. M., Menon, J. K., Nodari, R. O., Welter, L. J., da Silva, A. L. (2020). Standard area diagram set for anthracnose severity on grapevine bunches and shoots. Australasian Plant Pathology, 49(5), 561-569.

Mortensen, J. A. (1981). Sources and inheritance of resistance to anthracnose in Vitis. Journal of Heredity, 72(6), 423-426.

Murria, S., Kaur, N., Arora, A., Arora, N. K. (2018a). Biochemical characterization of superior seedless variety of grape (*Vitis vinifera* L.) for resistance to anthracnose. Indian Phytopathology, 71, 399-405.

Murria, S., Kaur, N., Arora, N., Mahal, A. K. (2018b). Field reaction and metabolic alterations in grape (*Vitis vinifera* L.) varieties infested with anthracnose. Scientia Horticulturae, 235, 286-293.

Rotter, A., Camps, C., Lohse, M., Kappel, C., Pilati, S., Hren, M., Stitt, M., Coutos-Thévenot, P., Moser, C., Usadel, B., Delrot, S., Gruden, K. (2009). Gene expression profiling in

susceptible interaction of grapevine with its fungal pathogen *Eutypa lata*: extending MapMan ontology for grapevine. BMC Plant Biology, 9, 104.

Rühl, E., Schmid, J., Eibach, R., Töpfer, R. (2015): Grapevine breeding programmes in Germany. Grapevine Breeding Programs for the Wine Industry, Woodhead Publishing Series in Food Science, Technology and Nutrition: Number 268, 77-101.

Santos RF, Spósito MB, Ayres MR, Sosnowski MR. (2018a) Phylogeny, morphology and pathogenicity of *Elsinoë ampelina*, the causal agent of grapevine anthracnose in Brazil and Australia. J Phytopathol, 00:1–12.

Santos, R. F., & Spósito, M. B. (2018b). Improving assessments of anthracnose severity on grapevine leaves through the development of a standard area diagram set. Australasian Plant Pathology, 47(4), 357-364.

Sonker, N., Pandey, A. K., & Singh, P. (2016). Strategies to control post-harvest diseases of table grape: A review. Journal of wine research, 27(2), 105-122.

Töpfer, R., Hausmann, L., Harst, M., Maul, E., Zyprian, E., Eibach, R. (2011). New horizons for grapevine breeding. Methods Temperate Fruit Breed, 5, 79-100.

Töpfer, R., Hausmann, L., Harst, M., Maul, E., Zyprian, E., Eibach, R. (2011): New Horizons for Grapvine Breeding. In: Flachowsky, H.; Hanke M.-V. (Eds.) Methodes in Temperate Fruit Breeding. Fruit, Vegetable and Cereal Science and Biotechnology *5*, (Special Issue 1), 79-100, Global Science Books.

4 CAPÍTULO 1 – CARBOHYDRATE AND ROS ARE LINKED WITH ANTRACNOSE INFECTION ON BUNCH

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4.1 ABSTRACT

Anthracnose is a fungal disease that causes damage in grapevine berries decreasing fruit quality. Thus, this study aimed to quantify biochemical and enzymatic changes on grapevine berries of different genotypes and their associations with anthracnose symptoms. Must quality, carbohydrates production, and enzymatic activity were assessed in berries with and without anthracnose symptoms, from five genotypes that presented different degrees of anthracnose infestation. Results showed that berries with anthracnose symptoms showed high must acidity than must from berries without symptoms. However, berries without anthracnose symptoms produced more proteins and presented higher polyphenol oxidase activity than berries with symptoms. The genotypes that presented the highest anthracnose symptoms showed higher sugar and hydrogen peroxide concentrations on berry skins. In addition, the genotypes that presented the lowest anthracnose symptoms showed the highest catalase, polyphenol oxidase, and phenylalanine ammonia-lyases activity on berry skins. Specific carbohydrates and enzymatic triggers were produced for each genotype, depending on the degree of anthracnose infestation. In conclusion, the genotypes present different severities of anthracnose on berries which was correlated with high hydrogen peroxide production and carbohydrate concentration on grape skin.

Keywords: *Elsinoë ampelina*; *Vitis*; fungal disease; quality; ROS

4.2 INTRODUCTION

Anthracnose (*Elsinoë ampelina*) is an important disease that infects grapevines when conditions during the growing season are high humid and wind (Santos et al., 2018a; EPPO, 2020). During fruit development, symptoms on bunches result in necrose on berries (Hoover et al., 2011). Grape infected by *E. ampelina* exhibits delayed development and ripening, smaller yields due to fruit rot, and smaller shelf life of fruits (Carisse and Lefebvre 2011). *E. ampelina* during infection on grapevine leaves presented vesicular-like structures in parenchyma cells, suggesting hemibiotrophic (Braga et al., 2019). Hemibiotrophic fungi present a biotrophic phase that enables the pathogen to secure a foothold in the plant host, followed by a transition to necrotrophy that fuels rapid growth and reproduction (Oliver and Ipcho 2004). Defense mechanisms of plants against disease are driven by the life cycle of pathogens and their interactions with the host (Pandey et al., 2016). Plants possess many receptors capable of recognizing and evading pathogenic effectors through different strategies, such as hypersensitivity reaction directed to the site of infection or the production of enzymes to control infection (Cesari 2018).

The principal enzymes produced in plants against hemibiotrophic pathogens are catalase, superoxide dismutase, guaiacol peroxidase, ascorbate peroxidase, glutathione reductase, dehydroascorbate reductase, monodehydroascorbate reductase, and phytoalexin molecules like flavonoids, proline, phenols, and tocopherols (Cardot et al., 2019; Komives and Kiraly 2019; Poudel et al., 2019). To understand grapevine defense mechanisms against anthracnose, it is necessary to focus on enzymatic activity and biochemical routes associated with plant defense during pathogen infestation (Gao et al., 2012; Murria et al., 2018a; Murria et al., 2018b). The production of antioxidants and reactive oxygen species (ROS) is one of the most important routes activated during grapevine defense against anthracnose on leaves (Murria et al., 2018a). On the one hand, excessive accumulation of hydrogen peroxide (H_2O_2),

superoxide anion (O_2) or hydroxyl radical (^{-}OH) can lead to oxidative stress in grapevine under fungal attack, triggering cell death (Freitas et al. 2009). Many ROS triggers, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxide (APX), and guaiacol peroxidase (GPX), can efficiently use H_2O_2, O_2^{-} and ^{-}OH, reinforcing grapevine resistance to fungal infection (Freitas et al., 2009; Boubakri et al., 2013). In addition, other enzymes like polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) are important to activate grapevine defense routes, such as flavonoids, shikimic acids, and salicylic acids that control fungi symptoms (Archana et al., 2011; Welter et al., 2017). Moreover, pigments, phenol, and sugar play a role in many grapevine defense routes and protein production against the pathogen, which change fruit quality (Dikilitas et al., 2017; Murria et al., 2018b). The hypothesis tested was that the attack of *Elsinoë ampelina* on grapevine berries with different levels of susceptibility can help to understand biochemical and enzymatic alterations that would be related to the infection of the pathogen and the development of the disease.

4.3 MATERIAL AND METHODS

4.3.1 Sampling strategy

The vineyard was located at the experimental station of the Federal University of Santa Catarina, Campus of Curitibanos (27°16′58″ S, 50°30′11″ W, and 1000 m a.s.l.), state of Santa Catarina, southern Brazil. Bunches with anthracnose symptoms from the white grapevine varieties ‘Aromera’, ‘Bronner’, ‘Felicia’, and ‘Helios’, as well as from the breeding line Gf.2004–043-0024, were randomly collected during the 2018-2019 growing season (Table 1) to evaluate anthracnose severity and both biochemical and enzymatic production on berries in response to natural anthracnose infection. The bunches were collected at phenological stage 34 when berries begin to soften and Brix starts to increase (Eichhorn and Lorenz 1984). Eight randomized bunches displaying anthracnose symptoms on berries from natural infection were

collected from three replications, totaling 24 bunches per genotype. To compare the biochemical and enzymatic response in the same genotype, 20 berries with and 20 without symptoms were collected from the bunches from each of three replications, totaling 60 berries per treatment/genotype. To confirm the causal agent, pathogen isolation was conducted as described by Santos et al. (2018a), using tissues from berries showing characteristic symptoms of anthracnose and the Koch's postulate were followed.

Table 1. Genealogy and origin of grapevine genotypes used in the present study.

Genotypes	Crossing	Origin
Aromera	Eger 2 x MusKat Ottonel	InnoVitis
Bronner	Merzling x Geisenheim 6494	WBI
Felicia	Sirius x Vidal Blanc	JKI
Gf.2004-043-0024	Breeding line	JKI
Helios	Merzling x FR-986-60	WBI

Note: InnoVitis, Italy; Julius Kühn Institut (JKI), Germany; Staatliches Weinbauinstitut Freiburg (WBI), Germany.

4.3.2 Disease severity

The collected bunches were photographed by a digital camera (Nikon D3200) affixed 40 cm away from the bunch, which was laid over a white background. These pictures were used to estimate anthracnose severity (diseased area in percentage) on bunches and berries, using Quant® software (Vale et al., 2011).

4.3.3 Must quality

Must from each cultivar, with and without anthracnose symptoms, was obtained in triplicate and used to determine the soluble-solid content (SSC) (°Brix), pH, and titratable

acidity (TA) based on the percentage of tartaric acid. In addition, the SSC/TA ratio was taken as the maturity index (MI).

4.3.4 Biochemical and enzymatic analysis

To perform the biochemical and enzymatic analyses, skins were removed from berries, which were then weighed, snap-frozen in liquid nitrogen, and stored at -80°C until analysis.

4.3.5 Pigments and phenol

Frozen berry skins (100 mg) were homogenized with 3 mL of TRIS (2-Amino-2-hydroxymethyl-1,3-propanediol) (pH 7.8) containing acetone (8:2) (Sims and Gamon 2002). Chlorophylls a, b, anthocyanin and carotenoid were determined by quantification in absorbance at 663, 647, 537, and 470 nm, respectively, in a high-performance spectrophotometer (SpectraMax® 190 Microplate Reader). Pigment contents were expressed as mg 100 g^{-1} fresh mass. Afterward, phenol was extracted by Acetone (50%) using 100 mg of skins under an ultrasonic bath for 20 min (Singleton et al., 1999). Phenol content was quantified at the absorbance of 725 nm and expressed in µg ml^{-1}.

4.3.6 Carbohydrate

Carbohydrate extract was obtained from frozen berry skins (300 mg) homogenized with 1 mL of ethanol (80%) containing 50 mM of ascorbic acid and 10% of polyvinylpolypyrrolidone (PVPP) (Filson and Dawson-Andoh 2009). The homogenate was centrifuged at 13000 x g for 10 min; the supernatant was filtered through a 0.45 µm membrane. To determine carbohydrate concentration, the filtrate was injected into HPLC (Shimadzu, Kyoto, Japan) using a Prevail Carbohydrate ES 5 µm (7.5 x 4.6 mm) pre-column (Grace, Columbia, MD, USA), followed by a Prevail Carbohydrate ES 5 µm (250 x 4.6 mm) (Grace,

Columbia, MD, USA). Standard carbohydrate curve (R=0.99) was used to calculate the concentration of ribose, arabinose, xylose, fructose, mannose, glucose, sucrose, and maltose. Carbohydrate concentrations were expressed as mg mL^{-1}.

4.3.7 Enzymatic activity

4.3.7.1 Extraction

Enzymatic activity assays were performed from frozen berry skins (200 mg), sampled randomly, and homogenized with 0.8 mL of 50 mM potassium phosphate (pH 7.0) containing 1% polyvinylpolypyrrolidone (PVPP) in a ratio of 5 mL buffer g-1 of berry fresh mass (Bailly and Kranner 2011). The homogenate was centrifuged at 20,000g for 20 min at 4°C, and the supernatant was used to determine enzymatic activity, hydrogen peroxide and protein concentration using the Spectra-Max® 190 Microplate Reader.

4.3.7.2 Quantification of hydrogen peroxide

Hydrogen peroxide (H_2O_2) was quantified in the absorbance of H_2O_2 at 390 nm (Velikova et al., 2000). The reaction consisted of 75 µl of a 10 mM potassium phosphate buffer (pH 7.0), 75 µl of 1M potassium iodide, and 75 µl of enzyme extract. The samples were evaluated after keeping them in the dark for 15 min. To calculate the concentration of hydrogen peroxide, the standard H_2O_2 curve (R = 0.90) was used. The amount of hydrogen peroxide was expressed as µM of H_2O_2 g^{-1} of berries weight.

4.3.7.3 Protein total

Protein content was determined according to the Bradford (1976) method, using bovine serum albumin (BSA) as standard (R = 0.99). The reaction was performed using 193 µl of Bradford reagent (Brilliant Blue G in phosphoric acid and methanol) and 7 µl of enzyme extract.

Enzymatic activity was based on the conversion of 1M of substrate s−1. Protein was expressed as mg^{-1} protein g^{-1} berries weight.

4.3.7.4 Catalase (EC 1.11.1.6)

Catalase (CAT) activity was estimated at the absorbance of 240 nm for 3 min (Rao et al., 1996). The reaction was evaluated in a 200 µl mixture of 50 mM potassium phosphate buffer (pH 7.0), 13.3 mM H_2O_2 (extinction coefficient 39.4 mM^{-1} cm^{-1}), and 5 µl of enzyme extract. CAT activity was expressed as µKatal mg^{-1} protein.

4.3.7.5 Ascorbate peroxidase (EC 1.11.1.11)

Ascorbate peroxidase (APX) activity was determined at absorbance 290 nm for 3 min (Rao et al., 1996). The reaction was evaluated in a 200 µl mixture of 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.5 mM H_2O_2 (extinction coefficient 2.8 mM cm^{-1}), and 10 µl of the enzyme extract. APX activity was expressed as µKatal mg^{-1} protein.

4.3.7.6 Superoxide dismutase (EC1.15.1.1)

Superoxide dismutase (SOD) activity was estimated when the inhibition of photochemical reduction achieved 50% of NBT at 560 nm (Rao et al., 1996). The reaction consisted of 2 ml of a 50 mM potassium phosphate buffer (pH 7.0), containing 10 mM methionine and 56 mM nitroblue tetrazolium, 40 µl of the enzyme extract, and 24 µl riboflavin. Samples were placed under light for 15 min (15 W white lamp at approximately 12 cm from samples), and duplicate samples were kept in the dark for the same time. To estimate enzymatic activity, the standard SOD curve (R=0.98) was used. SOD activity was expressed as USOD mg^{-1} protein.

4.3.7.7 Guaiacol peroxidase (EC 1.11.1.7)

Guaiacol peroxidase (GPX) activity was measured at absorbance 470 nm for 3 min (Rao et al., 1996). The reaction consisted of 193 µl of a mixture containing 50 mM potassium phosphate buffer (pH 7.0), 12.6 mM hydrogen peroxide (extinction coefficient 25.2 mM^{-1} cm^{-1}), 0.31 M guaiacol, and 7 µl of enzyme extract. GPX activity was expressed as µKatal mg^{-1} protein.

4.3.7.8 Polyphenol oxidase (EC 1.10.3.1)

Polyphenol oxidase (PPO) was measured at absorbance 395 nm using the method provided by Kar and Mishra (1976). The reaction consisted of 100 µl of a mixture containing 20 mM potassium phosphate buffer (pH 6.7), 0.1 M Cathecol, and 10 µl of enzyme extract kept in dark for 30 min. The difference in absorbance between enzymatic reaction and blank (H_2O + enzymatic extract) was used to calculate the PPO. PPO activity was expressed as µmol catechol mg^{-1} protein.

4.3.7.9 Phenylalanine ammonia-lyases (EC 4.3.1.5)

Phenylalanine ammonia-lyases (PAL) were determined at absorbance 290 nm (Peixoto et al., 1999). The reaction consisted of 100 µl of 20 mM potassium phosphate buffer (pH 8.8), 100 µl of 0.1 M L-phenylalanine, and 100 µl of enzyme extract kept in dark for 60 min. The enzymatic activity was calculated using the molar extinction coefficient of 10^4 mM^{-1} cm^{-1}. PAL activity was expressed as µKatal mg^{-1} protein.

4.3.8 Statistical analysis

Data normality was evaluated using the Shapiro–Wilk test (Shapiro and Wilk 1965). All studied variables showed normal distribution and homoscedasticity. Then, analysis of variance (ANOVA) with two factors, genotypes, and presence of symptoms, was performed using 5 %

of significance ($p<0.05$) to evaluate the variance and interaction between two factors in random blocks, followed by the Scott-Knott test ($p<0.05$) that avoided ambiguity between the groups. The varieties were classify into classes of anthracnose infection using the disease severity. In addition, a heatmap was created, using variables that presented the significant difference between the genotypes in ANOVA. In the heatmap, the first hierarchical clustering was done for both rows and columns of the data matrix. The columns/rows of the data matrix were reordered according to the hierarchical clustering result, putting similar observations close to each other. The blocks of 'high' and 'low' values are adjacent in the data matrix. Finally, a color scheme was applied for the visualization, and the data matrix was displayed. Statistical analyses were performed using the agricolae (Mendiburu 2020), ggplot2 (Wickham and Chang 2016), and pheatmap (Kolde 2015) packages in 'R' software, v. 3.4.5 (R Core Team 2017).

4.4 RESULTS

4.4.1 Fungal isolated from anthracnose infection

From leaves with typical anthracnose symptoms were isolate *E. ampelina* and *Colletotrichum* (Figure 1). *E. ampelina* are isolated from all anthracnose symptoms, confirmed by colonies with a reddish color and wrinkled texture.

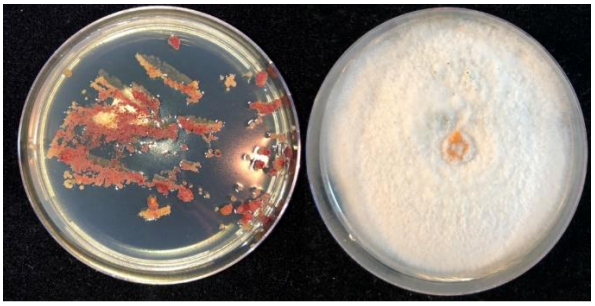

Figure 1. Colonies formed by isolates from anthracnose symptoms on grapevine bunches. Left, characteristics *Elsinoë ampelina*, and right, *Colletotrichum* spp., colonies.

4.4.2 Disease severity

Black spots typical of anthracnose were observed in all genotypes; however, the anthracnose symptoms only enlarged to characteristic necrosis, called “bird’s eye’ on Felicia berries (Figure 2 and Table 2). The ‘Felicia’ cultivar had the highest anthracnose severity on bunches (≈ 11.4% of severity), while ‘Bronner’ and ‘Aromera’ presented intermediate severity (≈3.8 and 2.7 % of severity, respectively), and GF.2004–043-0024 and Helios showed low severity (≈1.8 and 0.3% of severity, respectively) (Table 2). Felicia was the cultivar most infected by anthracnose with a major difference between the maximum and minimum severity percentage (standard deviation = 8.7%). Helios presented the lowest anthracnose severity and standard deviation (0.2%).

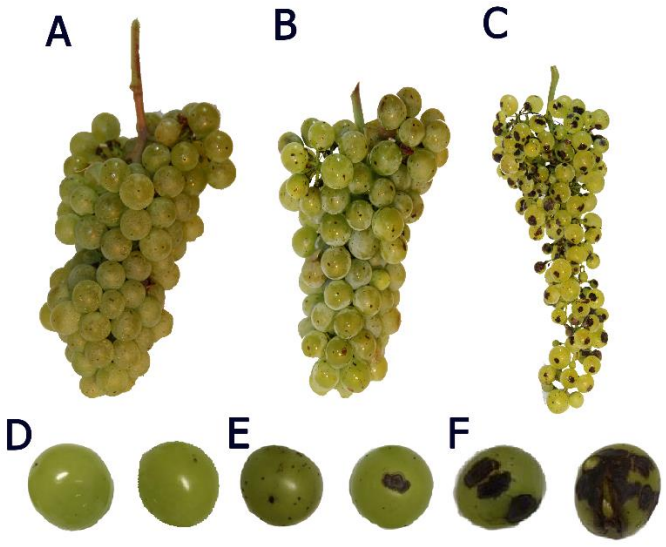

Figure 2. Grapevine anthracnose symptoms on white grapevine bunches. (A) The less infected bunch from Helios; (B) Intermediate infested, as represented by Bronner, and (C) the most infected bunch from Felicia. (D, E, F). One berry with minimum (left) and the other with maximum (right) anthracnose infected from Helios; Bronner, and Felicia bunches, respectively.

Table 2. Anthracnose severity (%) on bunches and berries of five white grapevine genotypes estimated using the software Quant.

Genotypes	Anthracnose severity on grape bunches (%)				Smax berries %	Infection level
	Mean	Minimum	Maximum	Standard deviation		
Aromera	2.7 b	1.8	3.5	0.8	3.31	Intermediary
Bronner	3.8 b	3.3	4.2	0.4	6.94	Intermediary
GF.2004–043-0024	1.8 c	1.2	2.3	0.6	0.52	Low
Felicia	11.4 a	5.2	17.6	8.7	61.09	High
Helios	0.6 c	0.5	0.9	0.2	0.28	Low

Note: Maximum anthracnose severity on berries (Smax).

4.4.3 Must quality and pigments production

The genotypes evaluated demonstrated differences in chemical components of the must (Figure 3B). However, these differences were not associated with the level of anthracnose severity (Table 2). The genotype Helios presented approximately three times more SSC than Gf.2004–043-0024, but both presented low anthracnose severity on fruits. In addition, the average value of SSC was observed on must from Felicia berries, the most susceptible genotype. Difference in pH was also verified between two genotypes with the same level of anthracnose severity, i.e., Aromera and Bronner. The genotype with the highest MI, Helios, and the genotype with lowest MI, Gf.2004–043-0024, were assigned to the low attack class (Figure 3B and Table 2). Must from berries with anthracnose symptoms presented higher acidity (TA) than without symptoms (Figure 3B). For pigments on berry skins, no difference in the content of chlorophyll a and b, carotenoid, and anthocyanin was noted in berries from different genotypes or those with and without anthracnose symptoms (Figure 3A).

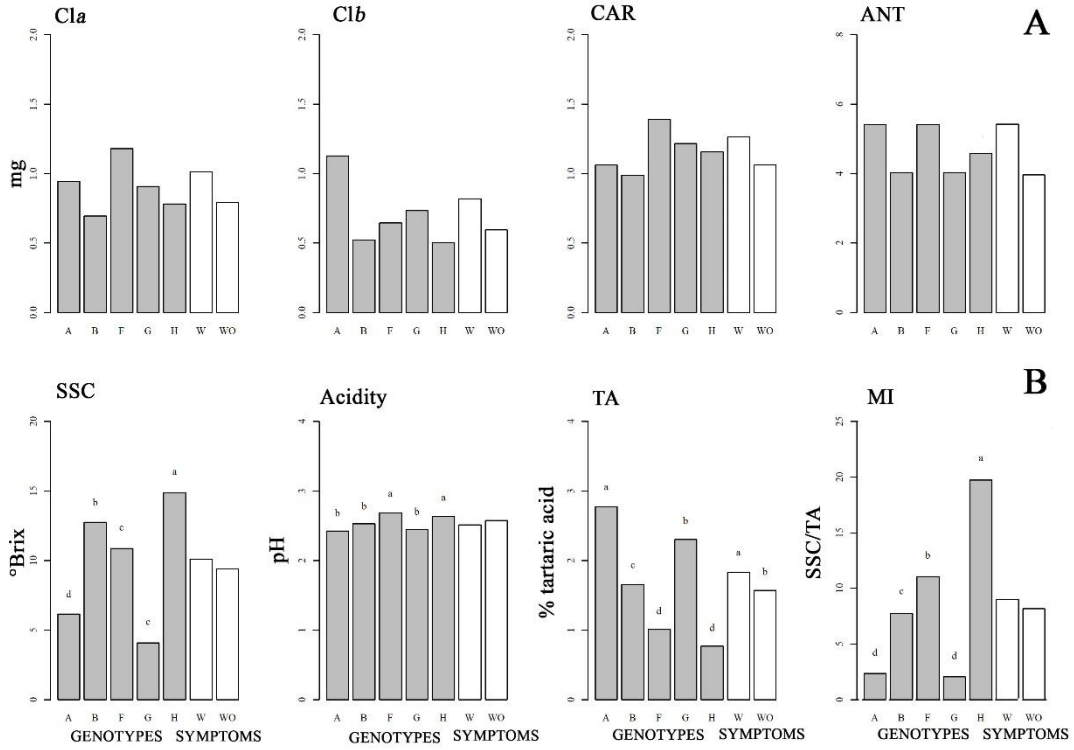

Figure 3. Chlorophyll a and b, carotenoid and anthocyanin on the skin of grape berries (A) and soluble solid contents (SSC), titratable acidity (TA) and maturation index (MI) on grape must (B) in berries, with (W) and without (WO) anthracnose symptoms, from five grapevine genotypes.
Note: Grey bars represented genotypes, and white bars represented berries with and without symptoms. Different letters at the top of bars mean difference in the Tukey test ($p>0.05$). Aromera (A), Bronner (B), Felicia (F), Gf.2004–043-0024 (G) and Helios (H).

4.4.4 Carbohydrates concentration

Genotypes with low and intermediate anthracnose severity showed lower fructose, glucose, and arabinose concentration on berry skins than the Felicia cultivar, which had a higher level of anthracnose severity (Figure 4 and Table 2). It was observed that arabinose was only detectable in Felicia. On the other hand, Aromera and Helios were the genotypes with the highest sucrose concentration, presenting low and intermediate levels of anthracnose severity, respectively. In addition, the genotypes classified as an intermediate level of anthracnose symptoms, Aromera and Bronner, produced the highest maltose and xylose concentration on berry skins, respectively. None of the evaluated genotypes produced mannose and ribose on

berry skins. Moreover, no differences in the content of glucose, fructose, sucrose, and maltose were verified in berry skins with and without anthracnose symptoms (Figure 3). However, berries with anthracnose symptoms synthesized arabinose and maltose in their skins.

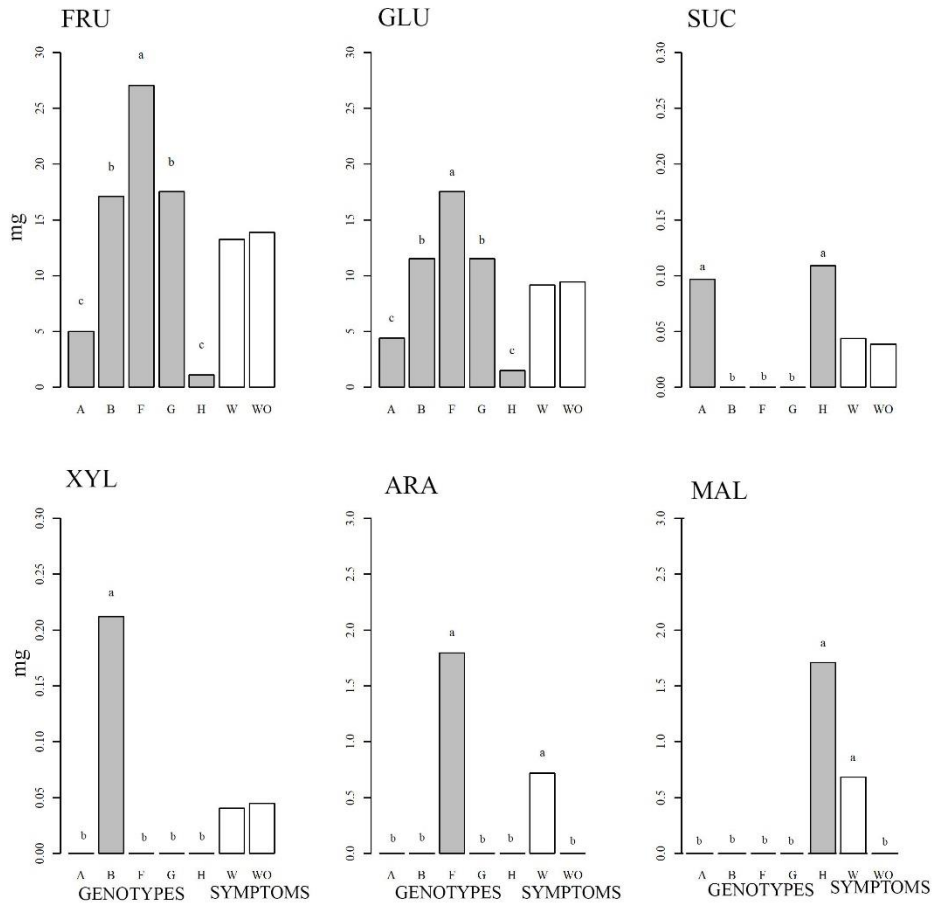

Figure 4. Concentration of carbohydrates, fructose (FRU), glucose (GLU), sucrose (SUC), arabinose (ARA), xylose (XYL), and maltose (MAL) in grape berry skins with (W) and without (WO) anthracnose symptoms from five grapevine genotypes.
Note: Grey bars represented genotypes, and white bars represented berries with and without symptoms. Different letters at the top of bars mean difference in Tukey test ($p>0.05$). Aromera (A), Bronner (B), Felicia (F), Gf.2004–043-0024 (G) and Helios (H).

4.4.5 Phenol production and enzymatic activity

The Aromera and Bronner genotypes, classified as intermediate anthracnose severity, produced less phenol on berry skins than other genotypes (Figure 5). The most susceptible genotype (cv. Felicia) showed a higher concentration of hydrogen peroxide on berry skins than

the other genotypes with fewer anthracnose symptoms (Figure 5 and Table 2). The Aromera cultivar showed higher CAT activity. However, the Bronner, Felicia, and Helios cultivars produced more GPX than the Aromera and Gf.2004–043-0024. Therefore, the genotypes with low severity, Helios and Gf.2004–043-0 024, presented higher PPO, and Gf.2004–043-0024 produced more PAL than other genotypes.

Berries with anthracnose symptoms showed less protein concentration and reduced PPO activity than those without symptoms (Figure 5). Nevertheless, the presence or absence of anthracnose symptoms on berries did not influence phenol, hydrogen peroxide, and the other enzymatic activities on the skins.

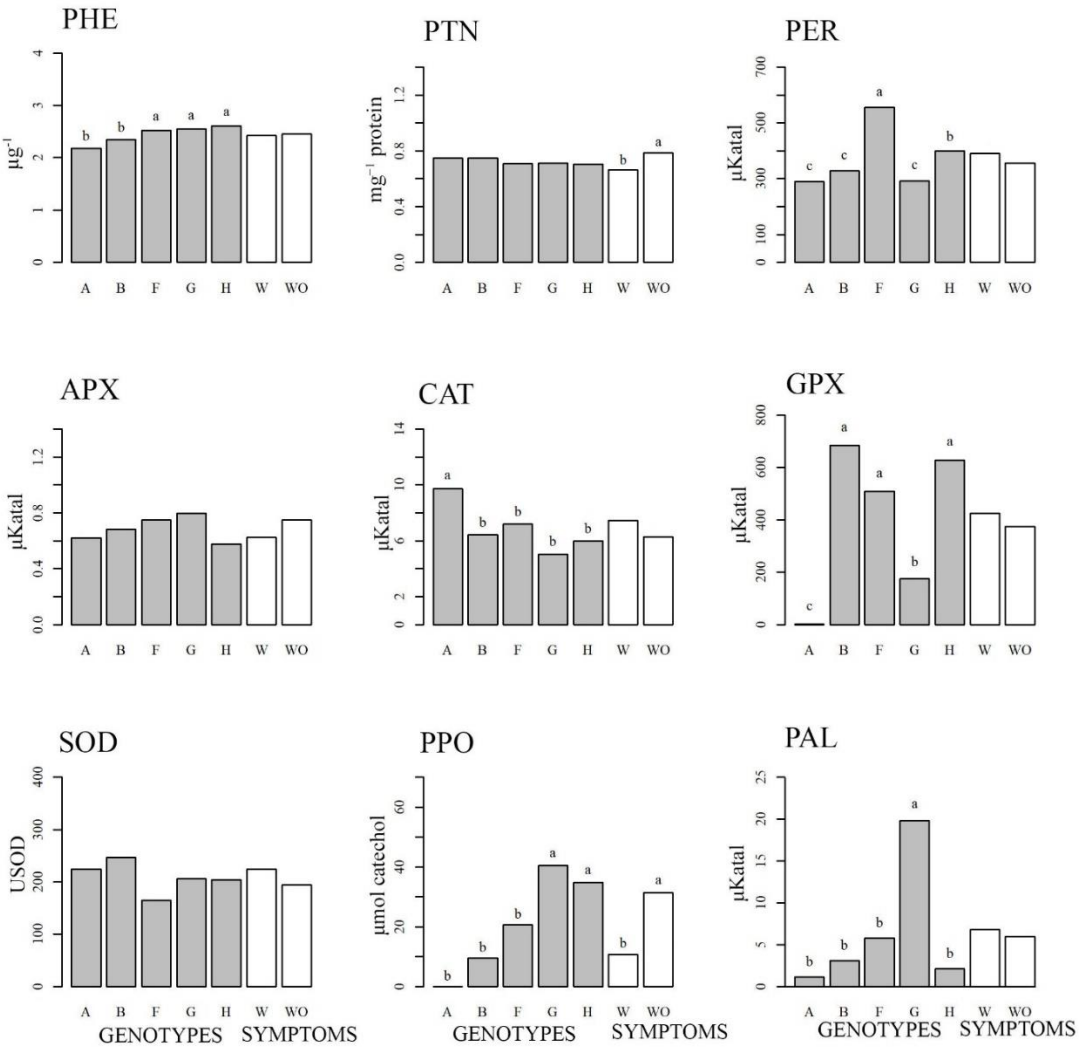

Figure 5. Concentration of phenol, protein (PTN), hydrogen peroxide (PER), ascorbate peroxidase (APX), catalase (CAT), guaiacol peroxidase (GPX), superoxide dismutase (SOD), polyphenol oxidase (PPO), and phenylalanine ammonia-lyases (PAL) in berry skins with (W) and without (WO) symptoms from five grapevine genotypes.
Note: Grey bars represented genotypes, and white bars represented berries with and without symptoms. Different letters at the top of bars mean difference in Tukey test ($p>0.05$). Unity of superoxide dismutase (USOD), Aromera (A), Bronner (B), Felicia (F), Gf.2004–043-0024 (G) and Helios (H).

4.4.6 Heatmap analysis

The heatmap portrayed three clusters that explained the linkage among the variables (Figure 5). The first cluster is comprised of TA, CAT, and sucrose. The second one grouped MI, SSC, xylose, maltose, and GPX, and in the last cluster, the severity, pH, glucose, fructose, arabinose, PER, phenol, PAL, and PPO were grouped. The genotype with the lowest anthracnose severity on berries, Helios, was grouped alone, showing the highest MI, phenol, maltose, and sucrose concentration, as well as SSC, GPX, and PAL activity. In addition, Helios exhibited the lowest severity, TA, glucose, and fructose concentration. Moreover, the genotypes classified as intermediate and low anthracnose severity, Aromera and GF.2004–043-0024, were grouped as a result of the highest TA and lowest pH and PER values (Table 2 and Figure 6). Finally, Felicia and Bronner, high and intermediate severity, respectively, were grouped by highest severity, pH, fructose, glucose, and PER, but with the lowest sucrose and maltose content.

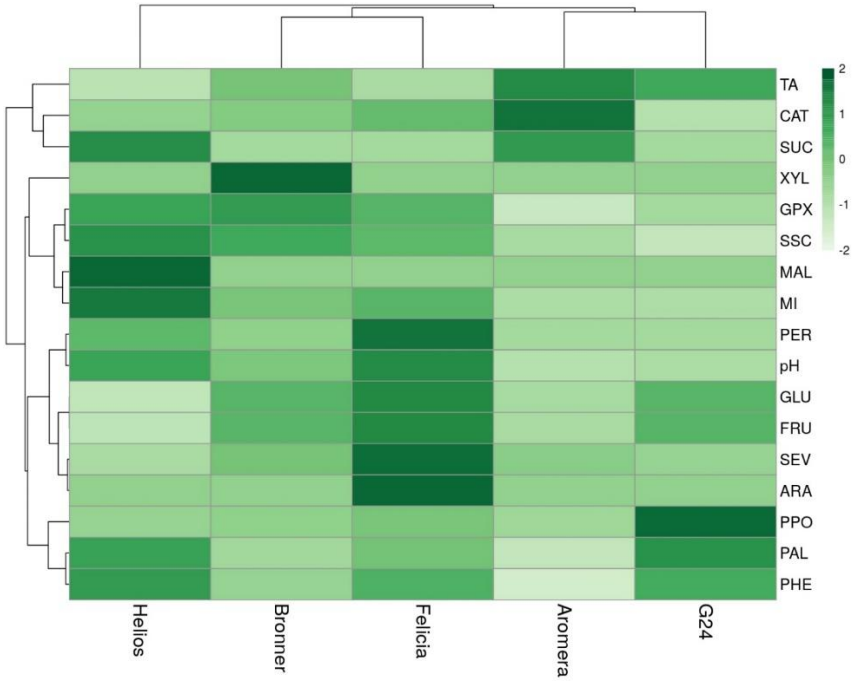

Figure 6. Heatmap using grapevine genotypes (columns) and characteristics (rows). Aromera, Bronner, Felicia, GF.2004–043-0024 (G24) and Helios are grapevine genotypes. Characteristics include anthracnose severity (SEV), soluble solid content (SSC), titratable acidity (TA), maturation index (MI), phenol content (PHE), arabinose content (ARA), xylose content (XYL), maltose content (MAL), sucrose content (SUC), fructose content (FRU), glucose content (GLU), polyphenol oxidase activity (PPO), hydrogen peroxide accumulation (PER), guaiacol peroxidase activity (GPX), catalase activity (CAT) and phenylalanine ammonia-lyases activity (PAL). A color scheme is applied for visualization, and the data matrix was displayed. The greenest cells represent the highest values of the data matrix from each characteristic per genotype, decreasing in green intensity to low values.

4.5 DISCUSSION

Anthracnose symptoms on leaves, shoots, and berries are attributed to the causal agent *E. ampelina* (Santos et al., 2018b; Li et al., 2019). *E. ampelina* and *Colletotrichum* were isolated from typical anthracnose symptoms on berries in the present study. Typical anthracnose symptoms on berries are small and circular reddish spots that initially appear in many berries, and then the lesion may extend into the pulp, causing cracks in the fruit (Ellis and Erincik 2008; Santos et al., 2020). In the present study, anthracnose symptoms were observed in all evaluated genotypes; however, the expansion of the symptoms (severity) was different among them. The

genotypes with low infection presented at least one small black spots per berry. In addition, the genotype with intermediary infection showed more than one black spot per berry. The genotype with the highest severity on bunches (Felicia) showed the greatest expansion of symptoms in berries called bid's eye.

Anthracnose attack causes loss of must quality and influences the taste of the wine (Hoover et al., 2011). However, in genotypes evaluated in the present work, anthracnose severity was not correlated with lower pH or SCC on must. Berries with anthracnose symptoms also did not show modifications in MI, but presented the highest TA on must and produced higher protein content on skins. During the necrotrophic phase, fungal diseases produce compounds that cause cell death, resulting in high acidity on the cells (Armijo et al., 2016; Braga et al., 2019). In addition, some specific proteins are produced only in leaves with anthracnose infection (Gao et al., 2012). Berries displaying disease symptoms lose quality through weight loss, color changes, and accelerated softening, and rachis browning. These deficits plus a high incidence of berry decay led to a reduction of shelf-life during postharvest storage (Alabi et al., 2016). Thus, to avoid loss of berry quality and yield, pathogens have to be controlled by using resistant varieties and disease control measures (Pedneault and Provost 2016).

Resistance to fungal diseases is linked to genetic and environmental factors (Welter et al., 2016; Merdinoglu et al., 2018). Pigment production is related to the absence of fungi on grapevine leaves (Chen et al., 2020). In the present study, no significant correlation was found between anthracnose infestation and pigment contents on grape berry skins. However, our study was carried out with white grapes that produce few pigments on the berry skins than red grapes (Niu et al., 2017; Silva et al., 2017). In addition, grapevine varieties resistant to fungal diseases showed differences in carbohydrate production on berries skin (Zyprian et al., 2016). In the present study, the presence of anthracnose symptoms was correlated with a high concentration

of two carbohydrates on berry skin, maltose and xylose. High carbohydrate concentration was also found in leaves infected by anthracnose when compared to healthy leaves (Murria et al., 2018a). In fact, the genotype with a higher level of anthracnose symptoms, Felicia, had more concentration of sucrose, fructose, and xylose than the other genotypes. The adjustment in sugar concentration and phenological phase play a determinative role in plant defense during necrotrophic interaction of *Botrytis cinerea* and *Sclerotinia sclerotiorum* in tomatoes (Lecompte et al., 2013). Supporting this finding, maturation index and sugar production are correlated with *P. viticola* (Kennelly et al., 2005) and *B. cinerea* infection (Mundy and Beresford 2007) in grapevine.

Defense mechanisms against pathogenic infections can be associated with genetic or systemic acquired resistance (SAR) induced by the pathogenic attack (Welter et al., 2016; Sawant et al., 2019; Poltronieri et al., 2020). In the present study, induction of enzymatic activity and SAR were not detected in berry skins without symptoms sampled from the same bunch with anthracnose symptomatic berries. However, differences in phenol concentration and enzymatic activity were noted on berries from the studied genotypes with different levels of anthracnose severity. Grapevine resistance to anthracnose is not a single genetic trait, but a complex and systematic network that includes physiological and biochemical processes (Gao et al., 2012). In the present study, Felicia, GF.2004–043-0024 and Helios produced more phenol than Bronner and Aromera. The early release of preformed phenolics and their later intensive production after stimulation of phenylpropanoid metabolism are part of resistance reactions to disease in many plants (Mahatma et al. 2011). Nevertheless, the antioxidant production is complex and involves the expression of more than one gene (Locato et al., 2018). ROS signaling is one activated route against fungal disease mediated by H_2O_2, O_2^- and ^-OH accumulation at specific cellular compartments (Freitas et al., 2019). High accumulation of H_2O_2 without scavenging results in dead cells and necrosis (Yang et al., 2017; Segal et al.,

2018). Compared to the other genotypes in our study, Felicia demonstrated the highest anthracnose severity on berry skins in association with the production of more H_2O_2. ROS production is concentrated in the vacuole, endoplasmic reticulum (ER), nuclei, or mitochondria and is highly regulated via calcium and different phosphorylation/dephosphorylation reactions (Mittler 2017). Each ROS presents a corresponding scavenging system involving the activity of SOD, CAT, GPX, flavonoids, and other scavenging enzymes (Cesari 2018; Yang et al., 2018). The variety with high anthracnose symptoms, Felicia, presented GPX activity similar to that observed in Bronner and Helios that presented intermediate and low anthracnose severity, respectively. GPX is the first ROS scavenging enzyme shown to act as an essential component in the signal transduction cascade(s) leading to defense reactions, such as the hypersensitive response and SAR (Pandey et al., 2016; Mauch-Mani et al., 2017). However, we found that genotypes with less anthracnose severity than Felicia showed high enzymatic activity of other ROS scavengings, such as CAT, PAL, and PPO. Tight control is needed to balance ROS production, including the activation of more than one signaling pathway by ROS-responsive regulatory genes and buffering of ROS by ROS-scavenging enzymes and antioxidant molecules (Suzuki et al., 2012). In leaves with anthracnose symptoms, additional ROS-scavenging pathways were activated, e.g., CAT and SOD (Murria et al., 2018a; Murria et al., 2018b; Li et al., 2019). CAT performs dismutation of H_2O_2 into H_2O and oxygen that is indispensable for ROS detoxification during abiotic stress (Suzuki et al., 2012). Moreover, high PAL activity was induced in grapevines infected with *Eutypa lata* and *B. cineria* (Bézier et al., 2002; Rotter et al., 2009). Studies suggested that the PAL route plays a role in early defense response in grapevine attacked by the fungus (Yun et al., 2015; Yang et al., 2018). Furthermore, PPO activity was correlated with grapevine resistance against *Eutypa lata* (El-Habbaa et al., 2016). PPO is involved in the lignification of cell walls, playing a protective role against pathogenic penetration, owing to reactive quinones produced from phenolic compound catalysis (Li et al.,

2017). The enzymes CAT, PAL and PPO are involved in phytoalexin production resulting in fungal control (Oku and Shiraishi 2017). In the present study, genotypes that presented low and intermediate anthracnose infestation produced a high activity of specific enzymes, one or two signaling molecules involved in plant defense mechanisms. Aromera produced higher CAT, reducing the production of phenol and other enzymes, such as GPX, PPO, and PAL when compared with other genotypes with the same anthracnose severity. Helios presented high GPX and PPO activities, reducing PAL activity, and Gf.2004–043-0024 presented the highest PAL and lowest CAT and GPX activities. In general, the routes activated in the genotypes with low anthracnose infestation, like in the present study, are correlated with salicylic acid, melanin, quinones, phytoalexin, flavonoids, phenolic compounds, and protein production (Mittler 2017; Cesari 2018; Murria et al., 2018a).

In conclusion, it was observed that berry skins with fewer carbohydrates, such as sucrose and fructose, resulted in lower anthracnose severity (Figure 6). However, the highest anthracnose severity on berries was correlated with high H_2O_2 accumulation because no efficient mechanisms could stop disease development, resulting in cell death and necrosis enlargement, which decreases grape quality. The genotype Felicia that presented high anthracnose severity also showed higher H_2O_2 accumulation, as well as glucose and fructose concentration, on berry skins. Nevertheless, the genotypes with less anthracnose severity than Felicia, showed more than one enzymatic activity probably linked with anthracnose resistance on berries. Anthracnose symptoms in berries can contain both, *E. ampelina* and *Colletotrichum* spp. Therefore, our study provides important information for grapevine breeding and phytopathology, highlighting the enzymatic route to defend against anthracnose symptoms on fruit and supporting future studies to map loci linked with anthracnose resistance and plant-pathogen interaction.

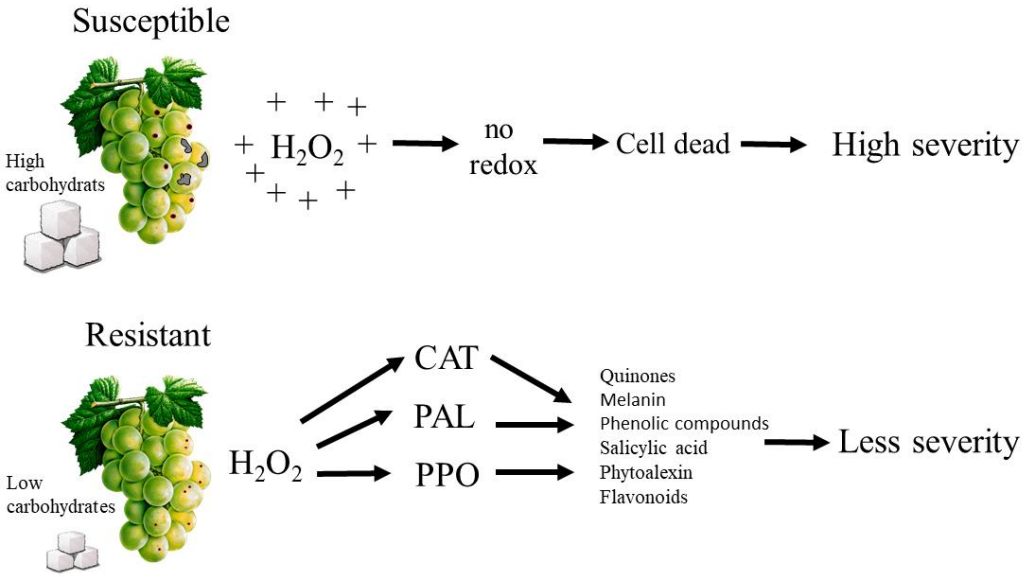

Figure 6. Biochemical and enzymatic characterization of grapevine berries infected with anthracnose. High carbohydrate concentration on berry skins, fructose, and glucose, are correlated in the presence of high anthracnose severity. Berries from genotypes without efficient defense mechanisms and less enzymatic activity present more hydrogen peroxide accumulation. Increase of pathogen on the skin then causes dead cells, leading to even higher disease severity and decay of berries. However, berries from genotypes with low and intermediate levels of anthracnose severity still possess one or more defense mechanisms able to stop the development of symptoms and prevent cell death. These mechanisms are correlated with phenylalanine ammonia-lyases (PAL), polyphenol oxidase (PPO), and catalase (CAT). According to Mittler (2017), Cesari (2018), and Murria et al. (2018a), these ROS-scavenging enzymes play a role in defense routes such as scavenging hydrogen peroxide, quinones, melanin, salicylic acid, phytoalexin, flavonoids, phenolic compounds, and protein production.

4.6 REFERENCES

Alabi, O. J., Casassa, L. F., Gutha, L. R., Larsen, R. C., Henick-Kling, T., Harbertson, J. F., & Naidu, R. A. (2016). Impacts of grapevine leafroll disease on fruit yield and grape and wine chemistry in a wine grape (*Vitis vinifera* L.) cultivar. PloS one, 11, e0149666. https://doi.org/10.1371/journal.pone.0149666

Alleweldt, G. & Possingham, J. V. (1988). Progress in grapevine breeding. Theoretical and Applied Genetics, 75(5), 669-673.

Archana, S., Prabakar, K., Raguchander, T., Hubballi, M., Valarmathi, P. & Prakasam, V. (2011). Defense responses of grapevine to *Plasmopara viticola* induced by azoxystrobin and Pseudomonas fluorescens. International Journal of Sustainable Agriculture, 3, 30-38.

Armijo, G., Schlechter, R., Agurto, M., Muñoz, D., Nuñez, C. & Arce-Johnson, P. (2016). Grapevine pathogenic microorganisms: understanding infection strategies and host response scenarios. Frontiers in plant science, 7, 382. https://doi.org/10.3389/fpls.2016.00382

Bailly, C., & Kranner, I. (2011). Analyses of reactive oxygen species and antioxidants in relation to seed longevity and germination. Seed dormancy, 773:343–367. https://doi.org/10.1007/978-1-61779-231-1_20

Baroncelli, R., Sreenivasaprasad, S., Lane, C. R., Thon, M. R., & Sukno, S. A. (2014). First report of *Colletotrichum acutatum* sensu lato (*Colletotrichum godetiae*) causing anthracnose on grapevine (*Vitis vinifera*) in the United Kingdom. New Disease Reports, 29, 26-26. http://dx.doi.org/10.5197/j.2044-0588.2014.029.026

Barros, L. B., Biasi, L. A., Carisse, O., & De Mio, L. L. M. (2015). Incidence of grape anthracnose on different *Vitis labrusca* and hibrid cultivars and rootstocks combination under humid subtropical climate. Australasian Plant Pathology, 44, 397-403. https://doi.org/10.1007/s13313-015-0353-8

Bézier, A., Lambert, B., & Baillieul, F. (2002). Study of defense-related gene expression in grapevine leaves and berries infected with Botrytis cinerea. European Journal of Plant Pathology, 108, 111-120. https://doi.org/10.1023/A:1015061108045

Boubakri, H., Wahab, M. A., Chong, J., Gertz, C., Gandoura, S., Mliki, A., Bertsch, C., & Soustre-Gacougnolle, I. (2013). Methionine elicits H2O2 generation and defense gene expression in grapevine and reduces *Plasmopara viticola* infection. Journal of plant physiology, 170, 1561-1568. https://doi.org/10.1016/j.jplph.2013.06.008

Bradford M.M., 1976 A rapid and sensitive method for the quantitationof microgram quantities of protein utilizing the principle of protein-dye binding. Analitycal Biochemistry, 72, 248-254.

Braga, Z. V., Santos, R. F., Amorim, L., & Appezzato-da-Glória, B. (2019). Histopathology of infection and colonisation of Elsinoë ampelina on grapevine leaves. Eur J Plant Path, 154, 1009-1019. https://doi.org/10.1007/s10658-019-01721-2

Brook, P. J. (1973). Epidemiology of grapevine anthracnose, caused by *Elsinoe ampelina*. New Zealand Journal of Agricultural Research, 16, 333-342. https://doi.org/10.1080/00288233.1973.10421113

Calderón, A. A., Zapata, J. M., Pedreño, M. A., Muñoz, R., & Barceló, A. R. (1992). Levels of 4-hydroxystilbene-oxidizing isoperoxidases related to constitutive disease resistance in in vitro-cultured grapevine. Plant cell, tissue and organ culture, 29, 63-70. https://doi.org/10.1007/BF00033609

Cardot, C., Mappa, G., La Camera, S., Gaillard, C., Vriet, C., Lecomte, P., Ferrari, G., & Coutos-Thévenot, P. (2019). Comparison of the molecular responses of tolerant, susceptible and highly susceptible grapevine cultivars during interaction with the pathogenic fungus *Eutypa lata*. Frontiers in plant science, 10, 991. https://doi.org/10.3389/fpls.2019.00991

Carisse, O., & Lefebvre, A. (2011). A model to estimate the amount of primary inoculum of Elsinoë ampelina. Plant disease, 95(9), 1167-1171. https://doi.org/10.1094/ PDIS-11-10-0798

Cesari, S. (2018). Multiple strategies for pathogen perception by plant immune receptors. New Phytologist, 219, 17-24. https://doi.org/10.1111/nph.14877

Chen, J. C., Yu, M., Liu, F., Qu, J. Z., Pan, X. X., Zhang, H. B., & Yang, M. Z. (2020). Diversity Distributions and the Anthocyanin Associations of Fungal Endophytes in Different Colored Grapevine Leaves. Journal of Plant Biology, 1-10. https://doi.org/10.1007/s12374-020-09233-x

Conrath, U., Pieterse, C. M., & Mauch-Mani B. (2002). Priming in plant–pathogen interactions. Trends in Plant science, 7, 210-216. https://doi.org/10.1016/S1360-1385(02)02244-6

de Souza, A. L. K., Brighenti, A. F., Brighenti, E., Caliari, V., Stefanini, M., Trapp O., Gardin, J. P.P., Dalbó, M. A., Welter, L. J., & Camargo, S. S. (2019). Performance of resistant varieties (PIWI) at two different altitudes in Southern Brazil. BIO Web Conference, 12, 01021. https://doi.org/10.1051/bioconf/20191201021

Dikilitas, M., Yucel, N., & Dervis, S. (2017). Production of Antioxidant and Oxidant Metabolites in Tomato Plants Infected with Verticillium dahliae Under Saline Conditions. In: Khan M., Khan N. (eds) Reactive Oxygen Species and Antioxidant Systems in Plants: Role and Regulation under Abiotic Stress. Springer, Singapore. https://doi.org/10.1007/978-981-10-5254-5_13

Eibach, R., Zyprian, E., Welter, L., & Topfer, R. (2007). The use of molecular markers for pyramiding resistance genes in grapevine breeding. Vitis, 46(3), 120.

El-Habbaa, G., Abdou, M., & El-Shaery, S. (2016). Biological and chemical control of grapevine die-back disease and their effect on defense related enzymes. International Journal of Scientific & Engineering Research, 7, 345-351.

Ellis, M. A., & Erincik, O. (2008). Anthracnose of grape. Agriculture and Natural Resources, 1-3.

European and Mediterranean Plant Protection Organisation (2020). https://gd.eppo.int/taxon/ELSIAM/distribution. Accessed 23 Set 2020.

Filson, P. B., & Dawson-Andoh, B. E. (2009). Sono-chemical preparation of cellulose nanocrystals from lignocellulose derived materials. Bioresource Technology, 100(7), 2259-2264. https://doi.org/10.1016/j.biortech.2008.09.062

Freitas, R., Rego, C., Oliveira, H., & Ferreira, R. B. (2009). Interactions among grapevine disease-causing fungi. The role of reactive oxygen species. Phytopathologia Mediterranea, 48(1), 117-127.

Gao, M., Wang, Q., Wan, R., Fei, Z., & Wang, X. (2012). Identification of genes differentially expressed in grapevine associated with resistance to *Elsinoe ampelina* through suppressive subtraction hybridization. Plant Physiology and Biochemistry, 58, 253-268. https://doi.org/10.1016/j.plaphy.2012.07.009

Guginski-Piva, C. A., Bogo, A., Gomes, B. R., Menon, J. K., Nodari, R. O., & Welter, L. J., (2018). Morphological and molecular characterization of *Colletotrichum nymphaeae* and *C. fructicola* associated with anthracnose symptoms of grape in Santa Catarina State, southern

Brazil. Journal of Plant Diseases and Protection, 125, 405-413. https://doi.org/10.1007/s41348-018-0176-2

Hoover, E. E., Wold-Burkness, S., Hilton, J., Mollov, D., Burkness, E., Galvan T., Hemstad, P., & Hutchison, W. D. (2011). Grape IPM guide for Minnesota producers, 1-65.

Kar, M.,& Mishra, D. (1976). Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. Plant Physiology, 57, 315-319.

Kennelly, M. M., Gadoury, D. M., Wilcox, W. F., Magarey, P. A., & Seem R. C. (2005). Seasonal development of ontogenic resistance to downy mildew in grape berries and rachises. Phytopathology, 95, 1445-1452. https://doi.org/10.1094/PHYTO-95-1445

Kolde, R. (2015). Package 'pheatmap'. R Package, 1(7), 790.

Komives, T., & Kiraly, Z. (2019). Disease resistance in plants: The road to phytoalexins and beyond. Ecocycles, 5, 7-12. https://doi.org/10.19040/ecocycles.v5i1.132

Lecompte, F., Nicot, P. C., Ripoll, J., Abro, M. A., Raimbault, A. K., Lopez-Lauri, F., & Bertin, N. (2017). Reduced susceptibility of tomato stem to the necrotrophic fungus Botrytis cinerea is associated with a specific adjustment of fructose content in the host sugar pool. Annals of botany, 119(5), 931-943. https://doi.org/10.1093/aob/mcw240

Li, C., Li, D., Li, J., Shao, F., & Lu S. (2017). Characterization of the polyphenol oxidase gene family reveals a novel microRNA involved in posttranscriptional regulation of PPOs in Salvia miltiorrhiza. Scientific reports, 7, 44622. https://doi.org/10.1038/srep44622

Li, Z., Zhang, S., Han, R., Zhang, H., Li, K., & Wang, X. (2019). Infection process and host responses to *Elsinoë ampelina*, the causal organism of grapevine anthracnose. European Journal of Plant Pathology, 155, 571-582. https://doi.org/10.1007/s10658-019-01793-0

Locato, V., Cimini, S., & De Gara, L. (2018). ROS and redox balance as multifaceted players of cross-tolerance: epigenetic and retrograde control of gene expression. Journal of experimental botany, 69, 3373-3391. https://doi.org/10.1093/jxb/ery168

Mahatma, M. K., Bhatnagar, R., Mittal, G. K., & Mahatma, L. (2011). Phenol metabolism in downy mildew resistant and susceptible genotypes of pearl millet. Archives of Phytopathology and Plant Protection, 44, 623-636. https://doi.org/10.1080/03235400903266404

Mauch-Mani, B., Baccelli, I., Luna, E., & Flors, V. (2017). Defense priming: an adaptive part of induced resistance. Annual review of plant biology, 68, 485-512. https://doi.org/10.1146/annurev-arplant-042916-041132

Mendiburu, F. (2020). Package 'agricolae'. R package version, 1-2.

Merdinoglu, D., Schneider, C., Prado, E., Wiedemann-Merdinoglu, S., & Mestre, P. (2018). Breeding for durable resistance to downy and powdery mildew in grapevine. Oeno one, 52, 203-209. https://doi.org/10.20870/oeno-one.2018.52.3.2116

Mittler, R. (2017). ROS are good. Trends in Plant Science, 22(1), 11-19. https://doi.org/10.1016/j.tplants.2016.08.002

Modesto, L. R., Steiner, D. R. M., Menon, J. K., Nodari, R. O., & Welter, L. J., da Silva, A. L. (2020). Standard area diagram set for anthracnose severity on grapevine bunches and shoots. Australian Plant Pathology, 49, 561-569. https://doi.org/10.1007/s13313-020-00728-2

Mundy, D. C., & Beresford, R. M. (2007). Susceptibility of grapes to *Botrytis cinerea* in relation to berry nitrogen and sugar concentration. New Zealand Plant Protection, 60, 123-127. https://doi.org/10.30843/nzpp.2007.60.4636

Murria, S., Kaur, N., Arora, A., & Arora, N. K. (2018a). Biochemical characterization of superior seedless variety of grape (*Vitis vinifera* L.) for resistance to anthracnose. Indian Phytopathology, 71, 399-405. https://doi.org/10.1007/s42360-018-0051-x

Murria, S., Kaur, N., Arora, N., & Mahal, A. K. (2018b). Field reaction and metabolic alterations in grape (*Vitis vinifera* L.) varieties infested with anthracnose. Scientia Horticulturae, 235, 286-293. https://doi.org/10.1016/j.scienta.2018.03.016

Niu, S., Hao, F., Mo, H., Jiang, J., Wang, H., Liu C., Fan, X., & Zhang, Y. (2017). Phenol profiles and antioxidant properties of white skinned grapes and their coloured genotypes during growth. Biotechnology & Biotechnological Equipment, 31, 58-67. https://doi.org/10.1080/13102818.2016.1258329

Oku, H., & Shiraishi T. (2017). Phytoalexins and host specificity in plant diseases. In Handbook of Phytoalexin Metabolism and Action, 41-60.

Oliver, R.P., & Ipcho, S.V. (2004). Arabidopsis pathology breathes new life into the necrotrophs-vs.-biotrophs classification of fungal pathogens. Molecular Plant Pathology, 5, 347–352. https://doi.org/10.1111/j.1364-3703.2004.00228.x

Pandey, D., Rajendran, S. R. C. K., Gaur, M., Sajeesh, P. K., & Kumar, A. (2016). Plant defense signaling and responses against necrotrophic fungal pathogens. Journal of Plant Growth Regulation, 35, 1159-1174. https://doi.org/10.1007/s00344-016-9600-7

Pedneault, K., & Provost, C. (2016). Fungus resistant grape varieties as a suitable alternative for organic wine production: Benefits, limits, and challenges. Scientia Horticulturae, 208, 57-77. https://doi.org/10.1016/j.scienta.2016.03.016

Peel, M. C., Finlayson B. L., & McMahon T. A. (2007). Updated world map of the Köppen-Geiger climate classification. https://hal.archives-ouvertes.fr/hal-00298818.

Peixoto, P. H. P., Cambraia, J., Sant'Anna, R., Mosquim, P. R., & Moreira, M. A. (1999). Aluminum effects on lipid peroxidation and on the activities of enzymes of oxidative metabolism in sorghum. Revista Brasileira de Fisiologia Vegetal, 11, 137-143.

Petit-Houdenot, Y., & Fudal, I. (2017). Complex interactions between fungal avirulence genes and their corresponding plant resistance genes and consequences for disease resistance management. Frontiers in plant science, 8, 1072. https://doi.org/10.3389/fpls.2017.01072

Poltronieri, P., Marrazzo, M. T., & Cipriani, G. (2020). Grapevine: Resistance genes, sRNAs and immunity. In Applied Plant Biotechnology for Improving Resistance to Biotic Stress, 151-179. https://doi.org/10.1016/B978-0-12-816030-5.00007-0

Poolsawat, O., Tharapreuksapong, A., Wongkaew, S., Reisch, B., & Tantasawat, P. (2010). Genetic diversity and pathogenicity analysis of *Sphaceloma ampelinum* causing grape anthracnose in Thailand. Journal of Phytopathology, 158, 837-840. https://doi.org/10.1111/j.1439-0434.2010.01696.x

Poudel, A., Navathe, S., Chand, R., Mishra, V. K., Singh, P. K., & Joshi, A. K. (2019). Hydrogen peroxide prompted lignification affects pathogenicity of hemi-biotrophic pathogen Bipolaris sorokiniana to wheat. The plant pathology journal, 35, 287. https://doi.org/10.5423/PPJ.OA.09.2018.0180

R Core Team (2017). R: A Language and environment for statistical computing. R Foundation for Statistical Computing. Vienna. Austria. http://www.r-project.org

Rao, M. V., Paliyath, G., & Ormrod, D. P. (1996). Ultraviolet-B-and ozone-induced biochemical changes in antioxidant enzymes of Arabidopsis thaliana. Plant physiology, 110(1), 125-136.

Ren, Z., & Lu, J. (2002). Muscadine rootstock increased the resistance of Florida hybrid bunch grape cv. Blanc du Bois to Pierce and Anthracnose diseases. In Proceedings of the Florida State Horticultural Society, 115, 108-110.

Rotter, A., Camps, C., Lohse, M., Kappel, C., Pilati, S., Hren, M., Stitt, M., Coutos-Thévenot, P., Moser, C., Usadel, B., Delrot, S. & Gruden, K. (2009). Gene expression profiling in susceptible interaction of grapevine with its fungal pathogen *Eutypa lata*: extending MapMan ontology for grapevine. BMC Plant Biology, 9, 104. https://doi.org/10.1186/1471-2229-9-104

Santos, R. F., Amorim, L., Zini, P. B., & Spósito, M. B. (2020). Temporal and spatial dynamics of grapevine anthracnose and its relationship to pathogen survival. Journal of Phytopathology, 168, 380–390 https://doi.org/10.1111/jph.12902

Santos, R. F., Ciampi-Guillardi, M., Amorim, L., Massola, N. S., & Spósito, M. B. (2018a). Aetiology of anthracnose on grapevine shoots in Brazil. Plant Pathology, 67, 692-706. https://doi.org/10.1111/ppa.12756

Santos, R. F., Spósito, M. B., Ayres, M. R., & Sosnowski, M. R. (2018b). Phylogeny, morphology and pathogenicity of *Elsinoë ampelina*, the causal agent of grapevine anthracnose in Brazil and Australia. Journal of Phytopathology, 166(3), 187-198. https://doi.org/10.1111/jph.12675

Sawant, I. S., Salunkhe, V. P., Ghule, S. B., Wadkar, P. N., Chavan, V., Ranade, Y. H., & Sawant S. D. (2019). Induction of resistance in grapevines against powdery mildew by Bacillus strains. Indian Phytopathology, 1-10. https://doi.org/10.1007/s42360-019-00171-4

Segal, L. M., & Wilson, R. A. (2018). Reactive oxygen species metabolism and plant-fungal interactions. Fungal Genetics and Biology, 110, 1-9. https://doi.org/10.1016/j.fgb.2017.12.003

Shapiro, S.S., & Wilk M.B. (1965). An Analysis of Variance Test for Normality (Complete Samples). Biometrika, 52, 591-611.

Silva, M. J. R., Vedoato, B. T. I. F., Lima, G. P. P., Moura, M. F., Coser, G. M. D. A. G., Watanabe, C. Y., & Tecchio, M. A. (2017). Phenolic compounds and antioxidant activity of red and white grapes on different rootstocks. African Journal of Biotechnology, 16(13), 664-671.. https://doi.org/ 10.5897/AJB2016.15837

Sims, D. A., & Gamon, J. A. (2002). Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. Remote sensing of environment, 81, 337-354. https://doi.org/10.1016/S0034-4257(02)00010-X

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M., (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In Methods in enzymology, 299, 152-178. https://doi.org/10.1016/S0076-6879(99)99017-1

Suzuki, N., Koussevitzky, S. H. A. I., Mittler, R. O. N., & Miller, G. A. D. (2012). ROS and redox signalling in the response of plants to abiotic stress. Plant, cell & environment, 35, 259-270. https://doi.org/10.1111/j.1365-3040.2011.02336.x

Töpfer, R., Hausmann, L., Harst, M., Maul, E., Zyprian, E., & Eibach, R. (2011). New horizons for grapevine breeding. Methods Temperate Fruit Breed, 5, 79-100.

Toruño, T. Y., Stergiopoulos, I., & Coaker, G. (2016). Plant-pathogen effectors: cellular probes interfering with plant defenses in spatial and temporal manners. Annual Review of Phytopathology, 54, 419-441. https://doi.org/10.1146/annurev-phyto-080615-100204

Vale, F.X.R., Filho, E.I.F., Liberato, J.R., Zambolim, L. (2001). QUANT – a software to quantify plant disease severity. In: Proceedings of the international workshop on plant disease epidemiology 2001, 1–160

Velikova, V., Yordanov, I., & Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Science, 151, 59-66. https://doi.org/10.1016/S0168-9452(99)00197-1

Vezzulli, S., Doligez, A., & Bellin, D. (2019). Molecular Mapping of Grapevine Genes. In The Grape Genome, 103-136. https://doi.org/10.1007/978-3-030-18601-2_7

Welter, L. J., Grando, M. S., & Zyprian E. (2016). Basics of grapevine genetic analysis. In Genetics, Genomics, and Breeding of Grapes, 165-187.

Wickham, H., & Chang, W. (2016). Package 'ggplot2'. Create Elegant Data Visualisations Using the Grammar of Graphics. Version, 2, 1-189.

Wiesner-Hanks, T., & Nelson, R. (2016). Multiple disease resistance in plants. Ann Rev Phyt, 54, 229-252. https://doi.org/10.1146/annurev-phyto-080615-100037

Yang, C., Li, W., Cao, J., Meng, F., Yu, Y., Huang, J., Jiang, L., Liu, M. Zhang, Z., Chen, X., Miyamoto, K., Yamane, H., Zhang, J., Chen, S., & Li, J. (2017). Activation of ethylene signaling pathways enhances disease resistance by regulating ROS and phytoalexin production in rice. The Plant Journal, 89, 338-353. https://doi.org/10.1111/tpj.13388

Yang, M. Z., Huang, L. H., Ao, X. J., Ren, A. Y., Yuan, M. Q., & Zhang, H. B. (2018). Endophytic Fungal strains Specifically Modified the Biochemical Status of Grape Cells. Journal of Plant Biology, 61, 210-216. https://doi.org/10.1007/s12374-017-0413-4

Yun, Y. H., Koo, J. S., Kim, S. H., & Kong, W. S. (2015). Cloning and expression analysis of phenylalanine ammonia-lyase gene in the mycelium and fruit body of the edible mushroom flammulina velutipes. Mycobiology, 43, 327-332. https://doi.org/10.5941/MYCO.2015.43.3.327

Zanghelini, J. A., Bogo, A., Dal Vesco, L. L., Gomes, B. R., Mecabô, C. V., Herpich, C. H., & Welter L. J. (2019). Response of PIWI grapevine cultivars to downy mildew in highland region of southern Brazil. European Journal of Plant Pathology, 154, 1051–1058. https://doi.org/10.1007/s10658-019-01725-y

Zyprian, E., Ochßner, I., Schwander, F., Šimon, S., Hausmann, L., Bonow-Rex, M., Moreno-Sanz, P., Grando, M. S., Wiedemann-Merdinoglu, S., Merdinoglu, D., Eibach, R. & Töpfer, R. (2016). Quantitative trait loci affecting pathogen resistance and ripening of grapevines. Molecular genetics and genomics, 291, 1573-1594. https://doi.org/10.1007/s00438-016-1200-5

5 CAPÍTULO 2 - *REA1 AND REA2:* TWO GENOMIC LOCI FROM *VITIS AMURENSIS* ASSOCIATED WITH THE RESISTANCE TO GRAPEVINE ANTHRACNOSE (*Elsinoë ampelina)*

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5.1 ABSTRACT

Grapevine anthracnose is a devastating fungal disease in tropical and subtropical vine-growing areas, and it is assumed that it will also become a future threat to vineyards in temperate regions, due to climate change. Until now no resistance locus against anthracnose was mapped in the grapevine genome that could be used in breeding programs to facilitate a safe and sustainable production of grapes. Thus, the present study aimed to identify loci linked with grapevine resistance to anthracnose. An F_1 progeny (n=121) with a background of *Vitis vinifera* and *Vitis amurensis* and parental was infected with a conidia suspension of *Elsinoë ampelina* in three experiments to screen phenotypically for segregation of anthracnose resistance. In addition, the same F_1 individuals and parental were genotyped with SSR markers for genetic mapping and QTL analysis. Transgressive segregation was revealed by F_1 plants. Two loci linked to anthracnose resistance, *Rea1* and *Rea2*, were identified on the chromosomes18 and 19, respectively. Candidate genes located within the two loci play putative roles in antioxidation, effector reception, transport of reactive oxygen species, as well as terpene production. This is the first report on mapping resistance genes to anthracnose in grapes.

Keywords: black spot, QTL, transgressive segregation.

5.2 INTRODUCTION

Grapevine is one of the economically most important crops and is used for the production of fresh fruit, dried fruit, juice, and wine. Nevertheless, fungal diseases are a challenge to the sustainable production of *Vitis vinifera* varieties grown all around the world (Bois et al. 2017). Breeding for genetic resistance against fungal diseases is the most sustainable strategy to reduce the spraying of pesticides in viticulture achieving high fruit quality (Van Bruggen et al. 2016). To accelerate disease-resistant varietal development through grapevine

breeding, marker-assisted selection (MAS) is essential (Dalbó et al. 2000; Eibach et al. 2007). Quantitative trait loci (QTLs) conferring resistance to a few diseases, especially powdery and downy mildew, have been mapped, allowing the use of MAS in the routine of grapevine breeding programs (Töpfer et al. 2011). There are diseases, such as anthracnose, that no resistance loci have been mapped for yet.

Anthracnose, also known as black spot, is caused by the ascomycete *Elsinoë ampelina* (Brook 1973; Santos et al. 2018a). The pathogen attacks all young green tissues, including grapevine leaves, petioles, inflorescences, and bunches (Li et al. 2019; Pirrello et al. 2019). Anthracnose is native from Europe, but it is not a major threat to viticulture there until now, most likely due to heavy plant protection treatments against other fungi that also control *E. ampelina* infection (Pirrelo et al. 2019). However, anthracnose is a major phytosanitary problem in regions that there is frequent rain during grapevine seasons such as the USA, Korea, China, India, and Brazil (Mortensen 1981, Yun et al. 2007, Zhang et al. 2014, Murria et al. 2018; Santos et al. 2018a). Considering the effects of climate change and due to the cultivation of grapevine varieties that possess resistance to the powdery and downy mildew, allowing the reduction of fungicide treatments, the outbreak of anthracnose in temperate regions, i.e. Europe, in the future is expected.

V. vinifera varieties are susceptible to anthracnose (Mortensen 1981; Poolsawat et al. 2012). American and Asian *Vitis* species, including *V. amurensis*, were described to exhibit resistance against anthracnose (Li et al. 2008; Poolsawat et al. 2012). The genetic mapping approach facilitates the analysis of important agronomic traits at the molecular level (Töpfer et al. 2011). Simple sequence repeat (SSR) and Single Nucleotide Polymorphism (SNP) markers have been used in grapevine breeding for QTL mapping because of their high level of polymorphism, simplicity, and a known map location (Schwander et al. 2012, Fu et al. 2020). The use of genetic markers linked to a resistance QTL in MAS and the search for candidate genes within QTL regions is a promising and established approach (Welter et al. 2007, Sargolzaei et al. 2020). Thus, the present study aimed was to identify QTLs and candidate genes in *V. amurensis* genome associated with grapevine resistance against anthracnose. The hypothesis tested was that the *V. amuresins* presents loci linked with the resistance against *Elsinoë ampelina* infection on leaves and cane;

5.3 MATERIAL AND METHODS

5.3.1 Plant material

The breeding line MGM4 [‘Moscato Giallo’ x ‘Sibera’ (*V. amurensis* background)], classified as resistant to anthracnose, was crossed in 2016 with two *V. vinifera* genotypes from the grapevine breeding program at UFSC, A190, and A271, generating two half-sibling populations with a total number of 121 F_1 individuals. These 121 F_1 individuals were grown in pots in the greenhouse at the Federal University of Santa Catarina, Curitibanos, Brazil (27°17'02.9"S 50°32'05.3"W). To control downy and powdery mildew it was used Dicarboximide (Captan®) and Thiophanate-methyl (Metiltiofan®) twice in 2018 and 2019.

5.3.2 Phenotyping

The parents and both half-sib populations, considered as one segregating population, were phenotyped for their response to artificial infection with *E. ampelina*. One shoot of each two-year-old potted grapevine plant with two unfolded leaves was inoculated with 6×10^{-6} conidia suspension of *E. ampelina* (isolate AVBR 118) by spraying until runoff (Santos et al. 2018b). In addition, the parentals, MGM4, A190, and A271, were inoculated with distilled water with no conidia suspension as the negative control. After inoculation, plants were maintained for 48 h at ±25°C and 90% relative humidity (± 2% of variation) in the dark. Afterward, the vines were subjected to a photoperiod of 12 h light for 12 days (Santos et al. 2018b). Koch’s postulates were confirmed with re-isolation of *E. ampelina* from sections of the plants showing characteristic symptoms. Twelve days after inoculation, the number of anthracnose spots on leaves and canes as were assessed. Phenotypes were classified into six classes according to the number of anthracnose spots using a range from 0 to 9 (0=no symptoms,1=from one to ten anthracnose spots, 3=from eleven to twenty anthracnose spots; 5 = from twenty-one to thirty anthracnose spots, 7=from thirty-one to fifty anthracnose spots, 9=more than fifty anthracnose spots) (Figure 1). The experiment was repeated two times in the growing season of 2018 and once in 2019. In addition, the maximum score of each F1 during the three evaluations was calculated. Normal distribution (skewness) of the obtained phenotypic data was tested using the “moments" package in ‘R’ software, v. 3.4.5 (R Core Team 2017).

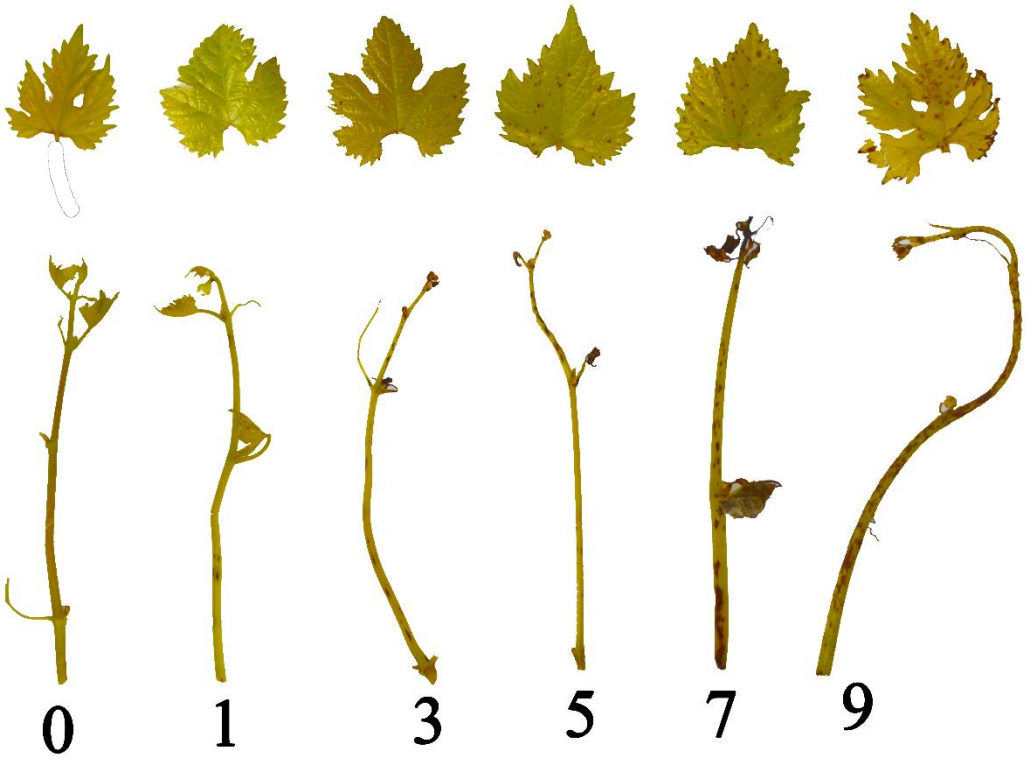

Figure 1. Classes to assess the number of anthracnose spots caused by *Elsinoë ampelina* on leaves and canes. 0= no symptoms,1=from one to ten anthracnose spots, 3=from eleven to twenty anthracnose spots; 5 = from twenty-one to thirty anthracnose spots, 7=from thirty-one to fifty anthracnose spots, 9=more than fifty anthracnose spots.

5.3.3 DNA extraction and genotyping

From each plant, it was collected 100 mg of young and healthy leaves and storaged in 2 ml microtubes until DNA extraction. The leaves were lyophilized and the DNA was isolated using the NucleoSpin® 96 Plant II Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. The parents and six of their progenies were initially used to identify polymorphic markers from a large set of simple sequence repeats (SSR) markers (Annex 1). All forward primers were labeled with a fluorescent dye (6-FAM®, HEX®, TAMRA® or ROX®) and were amplified via polymerase chain reaction (PCR) as multiplexes, combining different colors and fragment lengths. The KAPA2G Multiplex Mix Kit (KAPA Biosystems, Wilmington, USA) was used to set up 5 µl reaction mixtures, containing 0.5 pmol of each primer (forward and reverse) and 1 ng DNA template. The PCR multiplexes were amplificated with 3 min of initial denaturation at 95°C, followed by 30 cycles with denaturation at 95°C for 15 s, annealing at 60°C for 30 s and extension at 72°C for 30 s and a final extension was

performed at 72°C for 7 min using the ABI 9700 thermal cyclers (Applied Biosystems, Darmstadt, Germany). The amplified fragments were subjected to capillary electrophoresis in an ABI 3130xl Genetic Analyzer (Applied Biosystems, Darmstadt, Germany) with 16 x 36 cm capillaries and a size standard labeled with the fluorescence dye LIZ® which is identical to the GeneScan™ 500 LIZ™ (Applied Biosystems, Darmstadt, Germany). The length of the fragments was determined using the software GeneMapper® 5.0 (Applied Biosystems, Darmstadt, Germany).

5.3.4 Genetic mapping

The genetic map was generated using the informative markers for MGM4, the donor of anthracnose resistance in the examined population. Allele combinations observed in the SSR marker data were encoded using "a" for alleles inherited from the female parent of MGM4 ('Moscato Giallo') and "b" for alleles that were passed on by the male parent of MGM4 ('Sibera'). Markers with more than 20% missing data were discarded. All markers were tested for goodness-of-fit of their observed segregation about to the expected Mendelian ratio of 1:1 using a chi-square test ($p \leq 0.05$). Distorted markers were included if they did not hinder linkage map calculations and order of the markers. Markers were grouped using recombination frequency parameters by marker regression and potential problems in position were identified by looking for markers whose LOD scores were bigger than five and re-running the recombination using maximum likelihood distance (Broman et al. 2003). The linkage groups were numbered from one to nineteen, according to internationally acknowledged grapevine reference genetic maps (Doligez et al. 2006). Map units in centimorgan (cM) were generated using Kosambi's mapping function (Kosambi 1944). The collinearity between physical and genetic positions of the SSR markers was calculated using the physical position of the markers based on the reference genome PN40024 12X.v2 (Canaguier et al. 2017) and their genetic positions in the genetic map of MGM4. Statistics and charts were performed using ggplot2 (Wickham et al. 2016) and R/qtl (Broman et al. 2003) packages in 'R' software, v. 3.4.5 (R Core Team 2017).

5.3.5 QTL analysis

QTLs detection was performed using the one-dimensional scan command "scanone" LOD (logarithm of the odds). The permutation test was used to ensure the significance of QTL using 1000 permutations. The LOD score was assessed using the extended Haley-Knott model

(Feenstra et al. 2006). For interval estimation of QTL localization, it was calculated the confidence interval test as Bayesian credible intervals with a probability of coverage of 0.95. QTLs significant in all evaluation, three experiments, and maximum score, were chosen such as loci linked with Resistance to *E. ampelina* (*Rea*). The difference between the score of individuals with and without *Rea* loci were calculated. All analyses were performed using the R/qtl package (Broman et al. 2003) in R software, v. 3.4.5 (R Core Team 2007).

5.3.6 Pedigree analysis and candidate genes

Leaves from Seyanets Malengra, Severnyi, Saperavi, Saperavi Severnyi, Prachttraube, Sibera, and Moscato Giallo, genotypes that are in MGM4s pedigree, were collected from grapevine germplasm at Geilweilerhof (JKI). The DNA from these genotypes were extracted and analysis with SSR markers linked with the *Rea* loci were performed to trace back the origin of anthracnose resistance. In addition, the reference genome (PN40024 12X.v2) was used to search the QTL regions for candidate genes that can be associated with anthracnose resistance. The information of the putative proteins base on the nucleotide sequence was extracted using the annotated genome of *Vitis vinifera* in Phytozome (https://phytozome.jgi.doe.gov) and Genewise Uniprot database (http://www.uniprot.org).

5.4 RESULTS

5.4.1 Segregation and distribution of phenotypic data

The number of anthracnose spots on leaves and canes within the individuals of the evaluated population each presents quantitative segregation (Figure 2). Dataset showed normality for all evaluations and maximum datasets. Then, a test for normal distribution of the phenotypic data (skewness test) was performed to maximum values of the three phenotypic datasets (2018-1, 2018-2, 2019) for each F_1 individual. The normality ranged to a value of 0.07 for the number of anthracnose spots on leaves and 0.12 for the number of anthracnose spots on canes, with a value of 0 meaning symmetry and normal distribution of the data. The traits, number of anthracnose spot on cane and leave, showed a higher density of resistant individuals than susceptible ones, using the maximum score. The resistant donor MGM4 was assigned in class 3 according to the number of anthracnose spots on the leaves and cane. ‘Sibera’, the male parent of MGM4, revealed a lower number of anthracnose spots on the leaves and cane than MGM4, and it was classified as 0. The two *V. vinifera* parents, A190 and A271, were each assigned into classes 7 and 5 based on the number of anthracnose spots on the leaves and cane,

respectively. For both traits, F_1 individuals revealed transgressive segregation towards a higher degree of resistance than MGM4 and a higher level of susceptibility than A190 and A271.

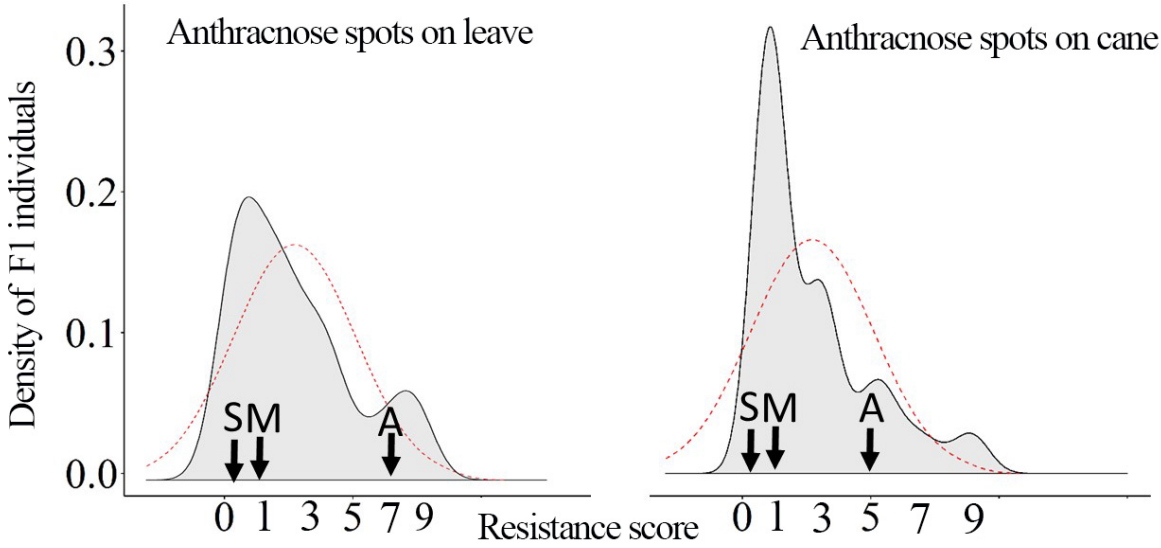

Figure 2. Phenotypic density of the 121 F_1 individuals, Sibera (S), MGM4 (M), A190, and A271 (A) for the traits: number of anthracnose spots on leaves and number of anthracnose spots on canes. Data are the maximum score of the three phenotypic evaluations (2018-1, 2018-2, 2019) for F_1 individuals. Susceptibility to anthracnose symptoms increases from a class 0 to 9. Grey curve is the frequency distribution of phenotypes of the progenies and the dotted red line indicates the normal distribution. Arrows show the scores of the parentals.

5.4.2 Genetic map

The SSRs were distributed over 19 linkage groups (LG), spanning a total genetic distance of 1530.8 cM, with linkage groups ranging from 40.1 cM (LG 15) to 112.2 cM (LG09), with an average distance between the markers of 9.3 cM (Table 1 and Figure 3). The largest number of markers was anchored on LG14 (24), covering a genetic distance of 92.2 cM, and the fewest number was anchored on LG17 (5 covering 83.3 cM; Figure 3). A high degree of collinearity was observed between genetic and physical positions of the SSR markers on the 19 LGs (Figure 4). All regressions generated for each of the 19 LGs to test for collinearity indicate that the genetic position of SSR markers on MGM4 map corresponds to a physical position on reference genome PN40024. The collinearity between physical and genetic position was the lowest on LG02 and LG19 and the highest on LG04 and LG08.

Table 1. Genetic size and coverage with SSR markers of the genetic map of *V. amurensis*-derived MGM4.

Linked group	Genetic size (cM)	Number of SSR markers	Average distance between SSR markers (cM)
LG01	105	8	13.1
LG02	104	6	17.3
LG03	48.6	10	4.9
LG04	74.4	12	6.2
LG05	73.9	8	9.2
LG06	72.6	7	10.4
LG07	81.8	9	9.1
LG08	87.2	10	8.7
LG09	113.2	9	12.6
LG10	66.9	9	7.4
LG11	86.8	8	10.9
LG12	106	9	11.8
LG13	84.7	12	7.1
LG14	94.7	23	4.1
LG15	41.2	7	5.9
LG16	64.2	9	7.1
LG17	83.3	5	16.7
LG18	72.6	14	5.2
LG19	69.7	8	8.7
Average	80.6	10	9.3

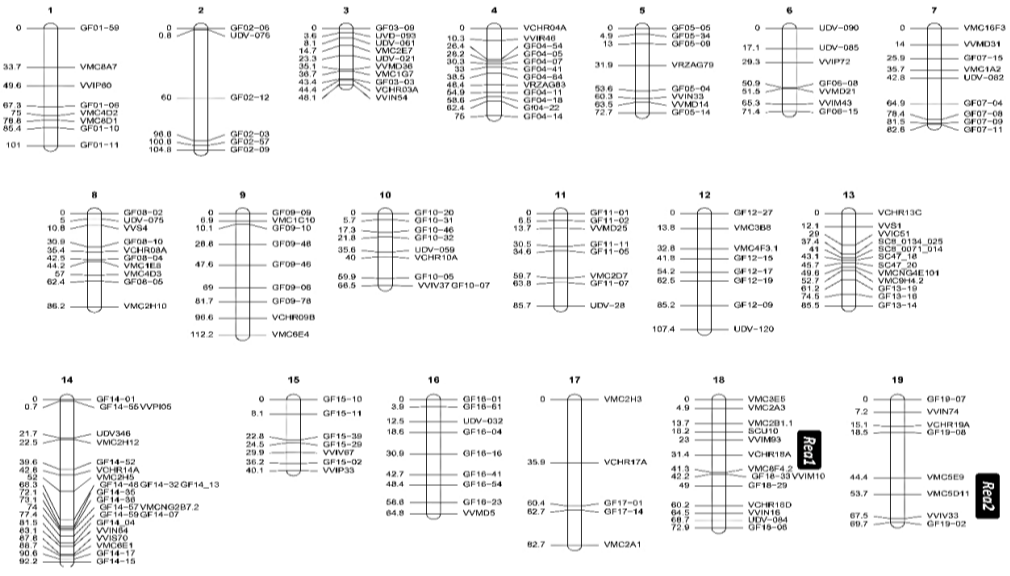

Figure 3. Genetic map of *V. amurensis*-derived MGM4 using SSR markers. Linkage groups (LG) were numbered from 1 to 19 according to the internationally acknowledged grapevine reference genetic map. Numbers on the left side of the LGs are the genetic positions (cM) of the SSR markers and labels on the right side are the names of the respective markers. Black boxes indicate the position of identified resistance loci to *Elsinoë ampelina* (*Rea*).

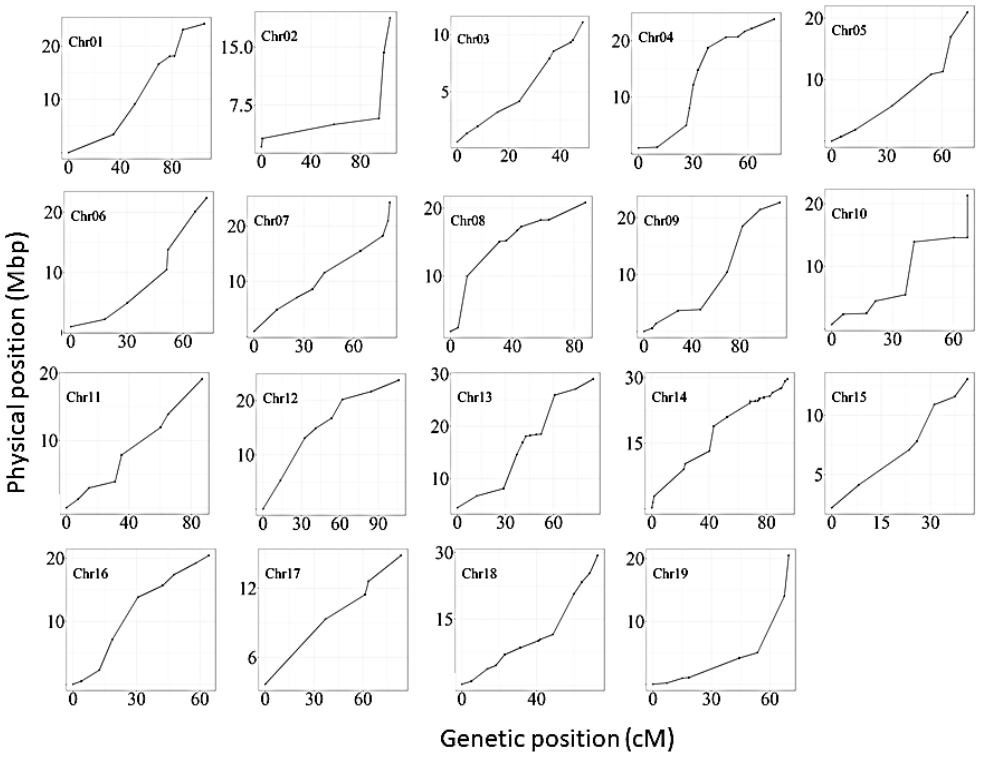

Figure 4. Collinearity between the genetic and physical position of SSR markers in the derived MGM4 genetic map. The x-axis indicates the genetic position (cM) of SSRs in the genetic map and the y-axis indicates the physical position (Mbp) of SSRs in the reference genome PN40024 12X.v2.

5.4.3 QTLs associated with grapevine anthracnose resistance

A significant QTL was identified on LG18 associated with the number of anthracnose spots on leaves (Table 2). The QTL was observed in the genetic map of MGM4 with VCHR18A (31.4 cM) as LOD_{max} marker (30-32.8 cM of confidence interval). This QTL was observed in all three phenotypic evaluations (2018-1, 2018-2, 2019) and using the maximum score, explaining from 4.50 to 15.60 % of total phenotypic variation. The maximum LOD score of this QTL was 2.98, using the maximum score from phenotypic data collected during three experiments, surpassing the permutation test significance of genome-wide threshold of 1.25 (1000 permutations; $p<0,05$) (Figure 5A). In addition, the LOD score was confirmed using standard interval mapping, Harley-Knott regression, and extended Haley-Knott method. This resistance locus was named *Rea1* (Resistance to *Elsinoë ampelina*) and decreased the susceptibility regarding the number of anthracnose spots on leaves from score 4.5 to 2.5 (Figure 5C). The F_1 with this locus presented from 10 to 15 anthracnose spots on leaves and the F_1 without this locus presented from 20 to 27 anthracnose spots on leaves. A second QTL was

detected associated with the number of anthracnose spots on the cane using all three phenotypic datasets (Table 2). This QTL is located on LG19 and is linked with VMC5D11 at 53.7 cM (52.3-55.1 cM of confidence interval) as LOD_{max} marker. The LOD value of this QTL ranged from 1.77 to 3.17, explaining 4.37 to 9.47% of the phenotypic variation. Harley-Knott regression and extended Haley-Knott method confirmed the QTLs and LOD score values found in standard interval mapping of the QTL, which exceeded the significance threshold (Figure 5B). The *Rea2* locus decreased the number of anthracnose spots on the cane from a score 4.0 to 2.5 (Figure 5D). The number of anthracnose spots on canes decreased from 20 to 25 in F_1 with this locus to from 10 to 15 in F_1 without *Rea2*.

Table 2. Genetic location of QTLs linked to grapevine anthracnose resistance using the genetic map of MGM4 and phenotypic data of number of anthracnose spots on leaves and canes, scored three times; two evaluations in 2018, one in 2019, and maximum score in the three evaluations.

Trait	Disease Scoring	LG	LOD max[1]	Variation (%)	LOD_{max} marker	Locus	Physical position [*] (Mbp)	Genetic position (cM)	Confidence interval (cM)[2]
Anthracnose on leaves	2018-1	18	2.13	12.13	**VCHR18A**	*Rea1*	8.51	31.4	30-32.8
	2018-2		1.96	4.5					
	2019		1.98	7.13					
	Maximum		2.98	15.6					
Anthracnose on canes	2018-1	19	3.17	9.47	**VMC5D11**	*Rea2*	5.03	53.7	52.3-55.1
	2018-2		1.78	4.37					
	2019		1.77	5.36					
	Maximum		1.82	7.98					

[*] Physical position of SSRs in the reference genome PN40024 12X.v2

[1]Determined significance of genome wide threshold of LOD values is 1.25 using 1000 permutations

[2]Confidence intervals calculated as Bayesian credible intervals with a probability of coverage of 0.95.

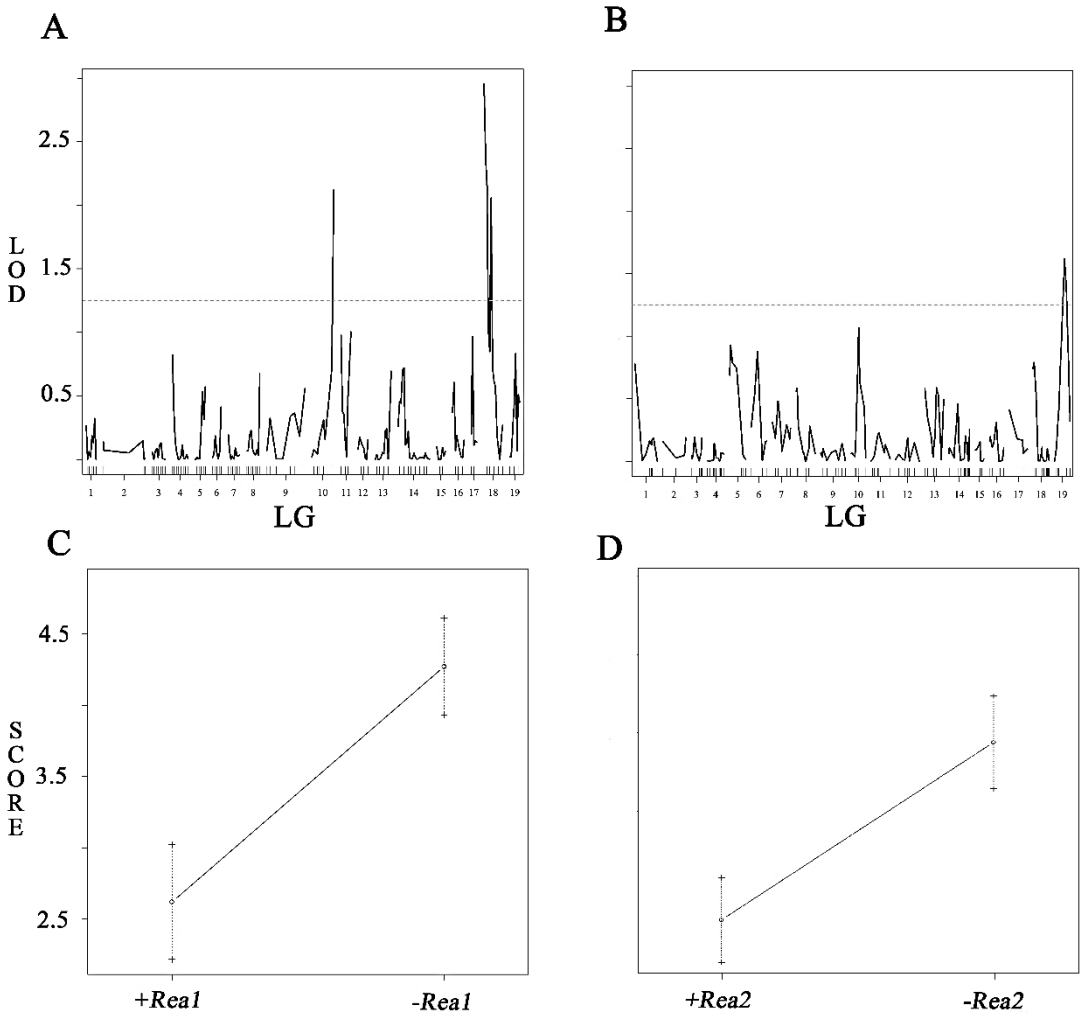

Figure 5. QTL analysis and effect of genetic anthracnose resistance on the phenotype using the maximum score. The QTL analysis with the traits: numbers of anthracnose spots on the leaves (A) and canes (B). The dotted line indicates the significance threshold calculated using 1000 permutation. Genetic effect of the QTLs on the resistance to anthracnose spot on the leaves (*Rea1*) (C) and canes (*Rea 2*) (D).

5.4.4 Pedigree analysis

The LOD_{max} marker VCHR18A of the detected QTL linked to anthracnose resistance (*Rea1*) exhibits a length of 141 bp just in the genotype MGM4, resistance donor not in the susceptible parentals, A190 and A271. The genotypes 'Sibera', 'Severnyi' and 'Saperavi Severnyi' carry the same allele (141bp) of the SSR marker confirming that *Rea1* was inherited from *V. amurensis* (Figure 6 and Annex 2). The same information was obtained for the adjacent SSR marker VVIM93, in which the resistance donor MGM4 and the genotypes with *V. amurensis* background possessed the allele with 91 bp. However, the SSR marker VMC8F4.2,

which flanks VCHR18a on the lower arm of LG18 exhibits the same fragment length in *V. amurensis*-derived and *V. vinifera* genotypes. Regarding *Rea2*, all tested genotypes derived from *V. amurensis* showed the QTL-linked allele of the LOD_{max} marker VMC5D11 (228 bp). In addition, all *V. amurensis*-derived genotypes present the same number of base pairs for the neighboring marker VVIV33 (362 bp). However, the evaluated *V. vinifera* genotypes exhibit the same fragment sizes observed for MGM4 in the SSR marker VMC5E9 flanking the other side of the LODmax marker.

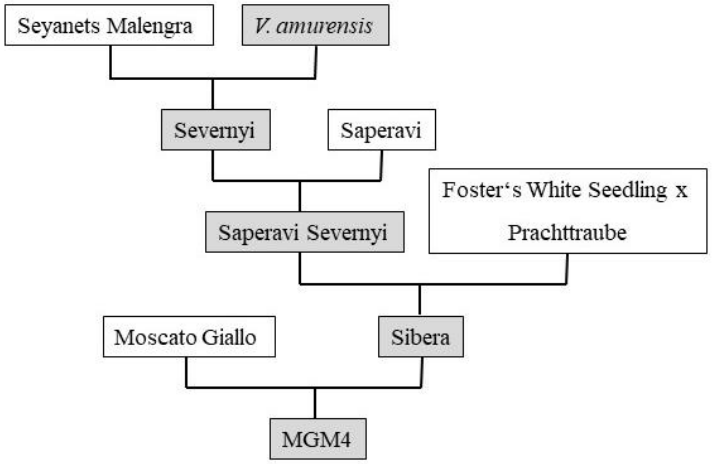

Figure 6. Pedigree of the breeding line MGM4. Grey boxes are *Vitis amurensis*-derived genotypes and white boxes are *V. vinifera* genotypes.

5.4.5 Candidate genes within the QTL regions

Several genes are located within the genomic regions of the two QTLs associated with anthracnose resistance. In the region of *Rea1* at LG18, from 3.25 to 11.53 Mbp, 506 genes are annotated in the reference genome (PN40024 12X.v2). From them, six were determined as candidate genes because their protein products have predicted functions in protein detoxification and as peroxidase, which are associated with disease resistance (Figure 7 and Annex 3). In addition, within the *Rea2* region on LG19, from 1.07 to 14.01 Mbp, there are 415 annotated genes, three of them are translate in putative proteins linked with disease resistance, NADPH dehydrogenase (quinone), NB-ARC domain-containing protein, and Terpene synthase C domain-containing protein.

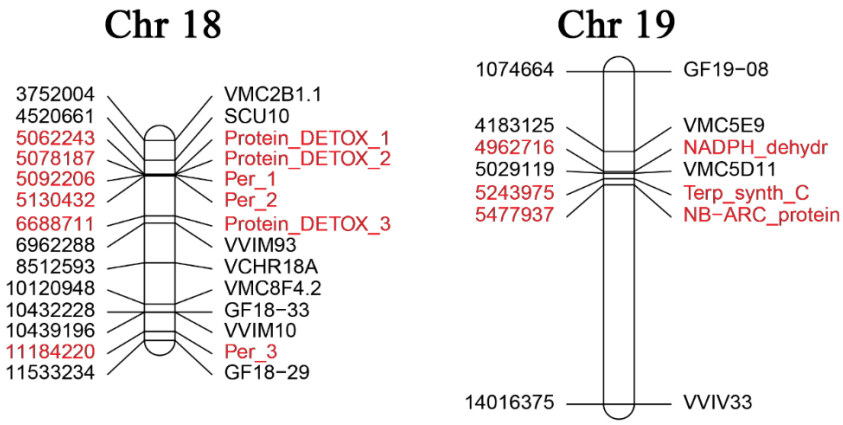

Figure 7. QTL region of *Rea1* and *Rea2*, on the chromosome 18 and 19, respectively. Labels on the left side are physical positions in base pairs and labels on the right side of the linkage group are the names of the respective SSR markers (black) and predicted proteins (red) using the reference genome sequence PN40024 12X.v2.

Note: Protein detoxification (Protein_DETOX), Peroxidase (Per), NADPH dehydrogenase (NADPH_dehydr), NB-ARC domain-containing protein (NB-ARC_protein), and Terpene synthase C domain-containing protein (Terp_synth_C).

5.5 DISCUSSION

5.5.1 Phenotype segregation

In the present study, the number of anthracnose spots was classified into six classes to phenotype the F_1 individuals and the parental lines. There is no unique protocol to score anthracnose resistance (Li et al. 2008; Poolsawat et al. 2012; Santos and Spósito 2018; Modesto et al. 2020). However, the phenotyping of grapevine anthracnose should have reproducibility and accuracy classifying the genotypes into resistant and susceptible (Modesto et al. 2020). The individuals were phenotyped as potted plants in a greenhouse because it is difficult to grow the fungus *E. ampelina* on leaf disc to evaluate in the laboratory (Poolsawat et al. 2012; Li et al. 2019). Previous studies on anthracnose resistance compared the susceptibility of *V. vinifera* varieties with other *Vitis* genotypes that could be sources of resistance (Hopkins and Harris 2000; Li et al. 2008). In the present study, the *V. vinifera* parents were more susceptible to anthracnose than the parent that is *V. amurensis*-derived. It has been shown before that other *V. amurensis* genotypes exhibit a higher level of resistance than *V. vinifera* and *V. labrusca* genotypes (Li et al. 2008; Yun et al. 2007). In grapevine crosses between pure *V. vinifera* and pure *V. amurensis* genotypes, it was observed that 100% of the F_1 individuals showed more resistance against anthracnose than the susceptible parental *V. vinifera* (Wang et al. 1998; Zhang et al. 2014). However, the present study, it was observed transgressive segregants more

tolerant than MGM 4 (*V. amuresis* background) and more susceptible than *V. vinifera*. Previously, the anthracnose resistance from *V. amurensis* was classified as dominant and from American *Vitis* as recessive (Mortensen, 1991; Wang et al. 1998; Jang et al. 2011; Kono et al. 2013). Thus, the segregation ratio expected between resistant and susceptible progenies from *V. vinifera* and *V. labrusca* crossing is 1:1 or 3:1, respectively, for heterozygous or homozygous resistant parental (Kim et al. 2008). In the present study, the number of progenies tolerant to anthracnose were bigger than susceptible progenies, fit in the segregation ratio 3:1.

5.5.2 Genetic map

The agreement between the genetic and physical position of SSR markers (collinearity) in the generated genetic map of MGM4, was also detected in a genetic map created with GBS (Fu et al. 2020). SSR segregation depends on the genetic distance between the parents (Welter et al. 2007; Schwander et al. 2012). Backcrossing using the same genetic background decreases SSR segregation due to the genetic proximity between the parents (Töpfer et al. 2011). Three backcrossings, after an interspecific cross, done with different *V. vinifera* varieties led to the mapping population used in the present study. A total of 182 markers from 806 tested SSR markers presented polymorphism locus for MGM4. In grapevine breeding, numerous backcrossing are done to obtain a high level of *V. vinifera* genome (>85%) with some resistance loci from other *Vitis* species combined in an interspecific cross (Eibach et al. 2007; Töpfer et al. 2011). The *V. vinifera* varieties showed lower genetic polymorphism between each other than comparatively the polymorphism between these varieties and genotypes from other *Vitis* species (Myles et al. 2010).

5.5.3 QTL Analysis

The LOD score observed for QTLs linked with anthracnose resistance in the present study, *Rea1* and *Rea2*, are comparable with other minor loci for grapevine resistance against downy mildew (*Plasmopara viticola*) (Welter et al. 2007; Fu et al. 2020; Sargolzaei et al. 2020), powdery mildew (*Erysiphe necator*) (Welter et al. 2007), rip rot (*Colletotrichum gloeosporioides*) (Fu et al. 2019) and black rot (*Guignardia bidwellii*) (Rex et al. 2014).

V. vinifera varieties are classified as susceptible to anthracnose, scored between 7 and 9, using the scale where genotypes classified near score 0 present no symptoms and near 9 are

very susceptible to this disease (Li et al. 2008). In the present study, the number of anthracnose spots on leaves and canes decreased in the F_1 individuals carrying the *Rea1* or *Rea2* loci (by 2.5 and 2.0 respectively) compared to those F_1 individuals without these loci. The QTLs linked with disease resistance are important to improve the resistance of susceptible varieties that are largely grown (Eibach et al. 2007). The use of MAS to pyramid major and minor loci in new breeding lines was studied to powdery and downy mildew resistance (Eibach et al. 2007; Welter et al. 2007). Therefore, the same can be done for anthracnose as well. Anthracnose is an important disease in humid regions that could become a huge problem in other temperate wine-growing regions because of the effects of climate change (Duchêne 2016; Santos et al. 2018a). The use of MAS to add resistance loci against anthracnose in *V. vinifera* genotypes is a sustainable method that decreases anthracnose infection and pesticide use as well in tropical and subtropical regions and prevents future outbreaks in temperate regions (Töpfer et al. 2011).

5.5.4 Pedigree

The Asian *Vitis* germplasm presents higher resistance to anthracnose than *V. vinifera* varieties, even in artificial or either on field conditions (Wang et al. 1998, Li et al. 2008). The two QTLs, *Rea1* and *Rea2*, detected in the MGM4 genome, evaluated in the present study, were traced back to *V. amurensis* background. The genotypes 'Severnyi', 'Saperavi Severnyi' and 'Sibera' had the same resistance alleles (fragments size) for all tested markers. 'Savernyi' and 'Saperavi Severnyi' are genotypes obtained in Russia with *V. amurensis* background (https://www.vivc.de/). 'Saperavi Severnyi' was crossed with *V. vinifera* (Foster's white seedling X Prachttraube), during the year 1964 in Germany (by researchers of Hochschule Geisenheim University), resulting in the anthracnose resistant variety 'Sibera'. 'Sibera' is the male parent of MGM4. The presence of the two resistance loci in all of the *V. amurensis*-derived genotypes that are ancestors of MGM4 supports the hypothesis that the anthracnose resistance loci *Rea1* and *Rea2* originate from *V. amurensis*. This *Vitis* specie is largely used in grapevine breeding because of its resistance against fungal diseases (Wang et al. 1998; Schwander et al. 2012; Liu and Li 2013).

5.5.5 Candidate genes and their predicted proteins

Many QTLs that provide grapevine resistance against the disease were correlated with candidate genes and predicted proteins (Schwandner et al. 2012, Fu et al. 2019; Sargolzaei et al. 2020). It is important to find genes and proteins that play a role in resistance to understand host-pathogen interactions (Sargolzaei et al. 2020). In this context, *Vitis vinifera* variety*,* Chardonnay*,* selected *in vitro* to *E. ampelina* resistance presented specific protein production (Jayasankar et al. 2000). In the present study, the predicted proteins with functions that are associated with disease resistance mechanisms were found within the genomic regions of *Rea1* and *Rea2*. Within the region of *Rea1* the candidate genes that lead to protein detoxification and peroxidase are located in the reference genome (PN40024). Genotypes resistant to anthracnose attack on leaves presented hypersensitive responses (Murria et al. 2018). The biggest number of genes expressed by tolerant variety to anthracnose (*V. quinquangularis* clone 'Shang-24') after *E. ampelina* inoculation encoded proteins and enzymes linked with energy and metabolism, such as protein detoxification (chlorophyll a/b-binding protein) and peroxidase (ribulose-1,5-bisphosphate carboxylase/oxygenase, catalase, ascorbate peroxidase, hypersensitive-induced reaction protein) (Gao et al. 2012). The expression of defense related-gene increased in susceptible variety, Red globe (*V.vinifera*), after *E. ampelina* inoculation, which was linked with ROS signaling (Li et al. 2021). Peroxidase and phenolic compounds increased during *E. ampelia* infection on *V. labrusca* leaves (Braga et al. 2021). Moreover, within the region of *Rea2*, genes coding for NADPH dehydrogenase (quinone), NB-ARC domain-containing protein, and Terpene synthase C domain-containing protein were found, and they are involved in the production of reactive oxygen species and quinone, in the detection of pathogen effector proteins and the activation of innate immune response accumulating chemical compounds (terpenoids), respectively (Wen et al. 2017; Chen et al. 2018; Barcarolo et al. 2020). Quinone outside inhibitor (QoI or strobilurin) is used as a fungicide against *E. ampelina* (Li et al. 2021). The gene encoded stilbene synthase, phenolic compound, was highly expressed in anthracnose tolerant varieties with *V. rotundifolia* background after *E. ampelina* inoculation than susceptible varieties, i.e. Cabernet Sauvignon (*V. vinifera*) (Louime et al. 2011). This gene was also expressed in *V. quinquangularis* after anthracnose inoculation (Gao et al. 2012). The tolerant hybrids to anthracnose attack, Lake Emerald and Blue Lake, expressed more stilbene synthase than the susceptible hybrids, Blanc du Bois, Suwannee (Vasanthaiah et al. 2010). The stilbene synthase gene was highly expressed 12 hours after *E. ampelina* inoculation in leaves of tolerant variety to anthracnose (*V. quinquangularis*) than in leaves of susceptible one

(*V. davidii* and *V. vinifera*) (Han et al. 2021). During the infection, *E. ampelina* triggered the Red globe defense linked with Terpene synthase (Li et al. 2021). In addition, R-genes linked with resistance to anthracnose, nucleotide-binding site (NBS)-leucine rich repeat (LRR), were isolated from grapevine hybrid (Seehalak et al. 2011), which shared high homology to putative RGA/P-loop NTPase genes presented in the study resistance donor (*V. amurensis*) (Seehalak et al. 2011). Furthermore, during *E. ampelina* infection on susceptible variety, 21 differentially expressed genes linked with LRR were up-regulated and 56 LRR were down-regulated (Li et al. 2021).

5.5.6 The use of Rea loci in grapevine breeding

Grapevine breeding is challenged to develop novel varieties well suited for sustainable viticulture with disease resistance and high fruit quality (Töpfer et al. 2011; Hajdu 2015)., Therefore introgression of genetic resistances in elite varieties using MAS is the method of choice in pyramidization strategies (Eibach and Töpfer 2002; Eibach et al. 2007; Di Gaspero and Foria 2015). Grapevine anthracnose is a major disease in tropical and subtropical viticulture but also infects vineyards located in temperate regions when the climate conditions are favorable (Santos et al., 2018a, Pirrello et al. 2019). In our study, the two QTLs detected associated with anthracnose resistance, *Rea1* and *Rea2*, are the first highlight to select markers to use in MAS and to breed new resistance varieties. These loci can be introgressed in *V. vinifera* varieties for adaptation to climate change in regions where anthracnose is a problem and it is not a primary disease yet. The cultivation of the resulting varieties will need less amount of pesticide, mainly in regions with a high incidence of anthracnose, which will reduce the production costs and adverse impact on the environment and human health. In addition, *Rea1* and *Rea2* can be used to study resistance mechanisms linked with anthracnose resistance.

5.6 REFERENCES

Alabi OJ, Casassa LF, Gutha LR, Larsen RC, Henick-Kling T, Harbertson JF, Naidu RA (2016) Impacts of grapevine leafroll disease on fruit yield and grape and wine chemistry in a wine grape (*Vitis vinifera* L.) cultivar. PLOS one, 11(2):e0149666. https://doi.org/10.1371/journal.pone.0149666

Barcarolo MV, Garavaglia BS, Gottig N, Ceccarelli EA, Catalano-Dupuy DL, Ottado J (2020) A novel Xanthomonas citri subsp. citri NADPH quinone reductase involved in salt stress

response and virulence. Bioch Biophy Acta (BBA)-Gen Sub, 1864(3):129514. https://doi.org/10.1016/j.bbagen.2020.129514

Bavaresco L (2018) Impact of grapevine breeding for disease resistance on the global wine industry. In XII International Conference on Grapevine Breeding and Genetics 1248, 7-14.

Bois B, Zito S, Calonnec A (2017) Climate vs grapevine pests and diseases worldwide: the first results of a global survey. OENO one, 51(2-3), 133-139. https://doi.org/ 10.20870/oeno-one.2016.0.0.1780

Broman KW, Wu H, Sen Ś, Churchill GA (2003) R/qtl: QTL mapping in experimental crosses. Bioinformatics 19:889–890

Brook PJ (1973) Epidemiology of grapevine anthracnose, caused by *Elsinoë ampelina*. New Zealand Journal of Agricultural Research, 16(3):333-342.

Canaguier A, Grimplet J, Di Gaspero G, Scalabrin S, Duchêne E, Choisne N et al (2017) A new version of the grapevine reference genome assembly (12X. v2) and of its annotation (VCost. v3). Genomics data, 14, 56. https://doi.org/10.1016/j.gdata.2017.09.002

Chen X, Chen H, Yuan JS, Köllner TG, Chen Y, Guo Y et al (2018). The rice terpene synthase gene Os TPS 19 functions as an (S)-limonene synthase in planta, and its overexpression leads to enhanced resistance to the blast fungus *Magnaporthe oryzae*. Plant biot jour, 16(10):1778-1787. https://doi.org/10.1111/pbi.12914

Core Team R (2017) R: A Language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria http://www.r-project.org

Dalbo MA, Ye GN, Weeden NF, Steinkellner H, Sefc KM, Reisch BI (2000) A gene controlling sex in grapevines placed on a molecular marker-based genetic map. Genome, 43(2):333-340. https://doi.org/10.1139/g99-136

Di Gaspero G, Foria S (2015) Molecular grapevine breeding techniques. In Grapevine breeding programs for the wine industry, 23-37. https://doi.org/10.1016/B978-1-78242-075-0.00002-8

Doligez A, Adam-Blondon A-F, Cipriani G, Di Gaspero G, Laucou V,Merdinoglu D, Meredith C, Riaz S, Roux C, This P (2006) An integrated SSR map of grapevine based on five mapping populations. Theor Appl Genet 113:369–382. https://doi.org/10.1007/s00122-006-0295-1

Duchêne E (2016) How can grapevine genetics contribute to the adaptation to climate change?. Oeno One, 50(3). https://doi.org/10.20870/oeno-one.2016.50.3.98

Eibach R, Zyprian E, Welter L, Topfer R (2007) The use of molecular markers for pyramiding resistance genes in grapevine breeding. Vitis, 46(3):120-124.

Eibach, Töpher R (2002) Success in resistance breeding:" Regent" and its steps into the market. In VIII International Conference on Grape Genetics and Breeding 603, 687-691.

Feenstra B, Skovgaard I M, Broman K W (2006). Mapping quantitative trait loci by an extension of the Haley–Knott regression method using estimating equations. Genetics, 173(4), 2269-2282. https://doi.org/10.1534/genetics.106.058537

Fu P, Tian Q, Lai G, Li R, Song S, Lu J (2019) Cgr1, a ripe rot resistance QTL in Vitis amurensis 'Shuang Hong' grapevine. Hort resear, 6(1):1-9. https://doi.org/10.1038/s41438-019-0148-0

Fu P, Wu W, Lai G, Li R, Peng Y, Yang B et al (2020) Identifying *Plasmopara viticola* resistance Loci in grapevine (*Vitis amurensis*) via genotyping-by-sequencing-based QTL mapping. Plant Phys Bioch, 154:75-84. https://doi.org/10.1016/j.plaphy.2020.05.016

Gao M, Wang Q, Wan R, Fei Z, Wang X (2012) Identification of genes differentially expressed in grapevine associated with resistance to *Elsinoë ampelina* through suppressive subtraction hybridization. Plant Phys Bioch, 58, 253-268. https://doi.org/10.1016/j.plaphy.2012.07.009

Hajdu E (2015) Grapevine breeding in Hungary. In Grapevine breeding programs for the wine industry, 103-134. https://doi.org/10.1016/B978-1-78242-075-0.00006-5

Han R, Yin W, Ahmad B, Gao P, Li Z, Wang X (2021) Pathogenesis and immune response in resistant and susceptible cultivars of grapevine (Vitis spp.) against *Elsinoe ampelina* infection. Phytopath, PHYTO-03. https://doi.org/10.1094/PHYTO-03-20-0079-R

Hopkins DL, Harris JW (2000) A greenhouse method for screening grapevine seedlings for resistance to anthracnose. HortScience, 35(1):89-91. https://doi.org/10.21273/HORTSCI.35.1.89

Jang MH, Ahn SY, Kim SH, Noh JH, Yun HK (2011) Evaluation of grapevine varietal resistance to anthracnose through treating culture filtrates from *Elsinoë ampelina*. Hort Envir Biotec, 52(2):152. https://doi.org/10.1007/s13580-011-0107-7

Jayasankar S, Li Z, Gray DJ (2000) In-vitro selection of *Vitis vinifera* Chardonnay with *Elsinoë ampelina* culture filtrate is accompanied by fungal resistance and enhanced secretion of chitinase. Planta, 211(2):200-208. https://doi.org/10.1007/s004250000285

Kim GH, Yun HK, Choi CS, Park JH, Jung YJ, Park KS, Dane K, Kang KK (2008) Identification of AFLP and RAPD markers linked to anthracnose resistance in grapes and their conversion to SCAR markers. Plant breed, 127(4), 418-423. https://doi.org/10.1111/j.1439-0523.2008.01488.x

Kono, A., Sato, A., Ban, Y., & Mitani, N. (2013). Resistance of *Vitis* germplasm to *Elsinoë ampelina* (de Bary) Shear evaluated by lesion number and diameter. HortScience, 48(12): 1433-1439. https://doi.org/10.21273/HORTSCI.48.12.1433

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugenics 12:172–175

Li D, Wan Y, Wang Y, He P (2008) Relatedness of resistance to anthracnose and to white rot in Chinese wild grapes. Vitis, 47(4):213-215.

Li Z, Dos Santos RF, Gao L, Chang P, Wang X (2021) Current status and future prospects of grapevine anthracnose caused by *Elsinoë ampelina*: An important disease in humid grape-growing regions. Mol Plant Pathol. https://doi.org/10.1111/mpp.13076

Li Z, Wang Y, Fan Y, Ahmad B, Wang X, Zhang S, Zhu Y, Gao L, Chang P, Wang X (2021) Transcriptome Analysis of the Grape-*Elsinoë ampelina* Pathosystem Reveals Novel Effectors and a Robust Defense Response. Molecular Plant-Microbe Inter, 34(1), 110-121. https://doi.org/10.1094/MPMI-08-20-0227-R

Li Z, Zhang S, Han R, Zhang H, Li K, Wang X (2019) Infection process and host responses to *Elsinoë ampelina*, the causal organism of grapevine anthracnose. Eur Jour Plant Path, 155(2):571-582. https://doi.org/10.1007/s10658-019-01793-0

Liu L, Li H (2013) Research progress in amur grape, *Vitis amurensis* Rupr. Canadian Journal of Plant Science, 93(4):565-575. https://doi.org/10.4141/CJPS2012-202

Louime C, Lu J, Onokpise O, Vasanthaiah HK, Kambiranda D, Basha SM, Yun HK (2011) Resistance to *Elsinoë ampelina* and expression of related resistant genes in Vitis rotundifolia Michx. grapes. Inter jour mol sci, 12(6), 3473-3488. https://doi.org/10.3390/ijms12063473

Modesto LR, Steiner DRM, Menon JK, Nodari RO, Welter LJ, da Silva AL (2020) Standard area diagram set for anthracnose severity on grapevine bunches and shoots. Austr Plant Path, 1-9. https://doi.org/10.1007/s13313-020-00728-2

Mortensen JA (1981) Sources and inheritance of resistance to anthracnose in *Vitis*. Jour Hered, 72(6):423-426.

Murria S, Kaur N, Arora A, Arora NK (2018) Biochemical characterization of superior seedless variety of grape (*Vitis vinifera* L.) for resistance to anthracnose. Indian Phyt, 71:399-405. https://doi.org/10.1007/s42360-018-0051-x

Myles S, Chia JM, Hurwitz B, Simon C, Zhong GY, Buckler E, Ware D (2010) Rapid genomic characterization of the genus *Vitis*. PloS one, *5*(1):e8219. https://doi.org/10.1371/journal.pone.0008219

Pirrello C, Mizzotti C, Tomazetti TC, Colombo M, Bettinelli P, Prodorutti D et al (2019). Emergent Ascomycetes in viticulture: an interdisciplinary overview. Frontiers in Plant Science, 10, 1394. https://doi.org/10.3389/fpls.2019.01394

Poolsawat O, Tharapreuksapong A, Wongkaew S, Chaowiset W, Tantasawat P (2012) Laboratory and field evaluations of resistance to *Sphaceloma ampelinum* causing anthracnose in grapevine. Austr Plant Path, 41(3):263-269. https://doi.org/10.1007/s13313-012-0127-5

R Core Team (2017) R: A Language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.r-project.org

Rex F, Fechter I, Hausmann L, Töpfer R (2014) QTL mapping of black rot (Guignardia bidwellii) resistance in the grapevine rootstock 'Börner'(*V. riparia* Gm183× *V. cinerea* Arnold). Theor Appl Genet, 127(7):1667-1677. https://doi.org/10.1007/s00122-014-2329-4

Santos RF, Ciampi-Guillardi M, Amorim L, Massola NS, Spósito MB (2018b) Aetiology of anthracnose on grapevine shoots in Brazil. Plant Path, 67(3):692-706. https://doi.org/10.1111/ppa.12756

Santos RF, Spósito MB (2018) Improving assessments of anthracnose severity on grapevine leaves through the development of a standard area diagram set. Australasian Plant Pathology, 47(4):357-364. https://doi.org/10.1007/s13313-018-0566-8

Santos RFD, Spósito MB, Ayres MR, Sosnowski MR (2018a) Phylogeny, morphology and pathogenicity of *Elsinoë ampelina*, the causal agent of grapevine anthracnose in Brazil and Australia. Jour Phyt, 166(3):187-198. https://doi.org/10.1111/jph.12675

Sargolzaei M, Maddalena G, Bitsadze N, Maghradze D, Bianco PA, Failla O, Toffolatti SL and De Lorenzis G (2020) *Rpv29*, *Rpv30* and *Rpv31*: Three Novel Genomic Loci Associated With Resistance to Plasmopara viticola in *Vitis vinifera*. Front Plant Sci 11:562432. https://doi.org/10.3389/fpls.2020.562432

Schwander F, Eibach R, Fechter I, Hausmann L, Zyprian E, Töpfer R (2012) *Rpv10*: a new locus from the Asian *Vitis* gene pool for pyramiding downy mildew resistance loci in grapevine. Theor Appl Genet, 124(1):163-176. https://doi.org/10.1007/s00122-011-1695-4

Seehalak W, Moonsom S, Metheenukul P, Tantasawat P (2011) Isolation of resistance gene analogs from grapevine resistant and susceptible to downy mildew and anthracnose. *Sci Hort*, *128*(3):357-363. https://doi.org/10.1016/j.scienta.2011.01.003

Töpfer R, Hausmann L, Harst M, Maul E, Zyprian E, Eibach R (2011) New horizons for grapevine breeding. Methods Temperate Fruit Breed, 5:79-100.

Van Bruggen AH, Gamliel A, Finckh MR (2016) Plant disease management in organic farming systems. Pest manag sci, 72(1):30-44. https://doi.org/10.1002/ps.4145

Vasanthaiah HK, Basha SM, Katam R (2010) Differential expression of chitinase and stilbene synthase genes in Florida hybrid bunch grapes to *Elsinoë ampelina* infection. Plant growth regulation, 61(2), 127-134. https://doi.org/10.1007/s10725-010-9458-9

Wang YJ, Liu YL, He PC, Lamikanra O, Lu J (1998) Resistance of Chinese *Vitis* species to *Elsinoë ampelina* (de Bary) shear. HortScience, 33(1):123-126.

Welter LJ, Göktürk-Baydar N, Akkurt M, Maul E, Eibach R, Töpfer R, Zyprian EM (2007) Genetic mapping and localization of quantitative trait loci affecting fungal disease resistance and leaf morphology in grapevine (*Vitis vinifera* L). Mol Breed, 20(4):359-374. https://doi.org/10.1007/s11032-007-9097-7

Wen Z, Yao L, Singer SD, Muhammad H, Li Z, Wang X (2017) Constitutive heterologous overexpression of a TIR-NB-ARC-LRR gene encoding a putative disease resistance protein from wild Chinese *Vitis* pseudoreticulata in Arabidopsis and tobacco enhances resistance to phytopathogenic fungi and bacteria. Plant Phys Biochem, 112:346-361. https://doi.org/10.1016/j.plaphy.2017.01.017

Wickham H, Chang W, Wickham M H (2016). Package 'ggplot2'. Create Elegant Data Visualisations Using the Grammar of Graphics. Version, 2(1), 1-189.

Yun HK, Park KS, Roh JH, Choi YJ, Jeong SB (2007) Developing a screening system for resistance to anthracnose in grapevines using culture filtrates from *Elsinoë ampelina*. The Jour Hort Sci Biotech, 82(3):360-364. https://doi.org/10.1080/14620316.2007.11512243

Zhang QT, Fan ST, Lu WP, Song RG, Yang YM, Ai J (2014) Breeding progress of amur grape (*Vitis Amurensis* RUPR.) in China. In XI International Conference on Grapevine Breeding and Genetics 1082, 33-36. https://doi.org/10.17660/ActaHortic.2015.1082.1

Braga, Z. V., Muniz, L. F., Manarim, G. R., de Aguiar, C. L., & Appezzato-da-Gloria, B. (2021). Anatomical and biochemical changes in leaves of Vitis labrusca L. cv. Niagara Rosada in response to infection by Elsinoë ampelina Shear. Brazilian Journal of Botany, 44(1), 187-196. https://doi.org/10.1007/s40415-020-00677-6

5.7 SUPPLEMENTARY MATERIAL

Annex 1. SSR markers tested for polymorphism and segregation pattern on *V. amurensis*-derived MGM4 map.

Marker series	Tested markers	Informative markers	Reference
APT3	1	0	Fechter 2012 unpublished
GF	425	95	Schwander et al. 2012, Fechter et al. 2014, Zyprian et al. 2016, Zendler et al. 2017 and Hausmann 2017 unpublished
SC	23	4	Coleman et al. 2009
SCU	4	1	Scott et al. 2000
STS	1	0	Dalbo et al. 2001
UDV	79	14	Di Gaspero et al. 2005 and 2007
VCHR	36	11	Cipriani et al. 2008
VMC	113	27	Vitis Microsatellite Consortium
VMCNG	18	2	Grando 2000
VrZAG	9	2	Sefc et al. 1999
VVI	75	18	Merdinoglu et al. 2005
VVMD	14	6	Bowers et al. 1996 and 1999
VVNT	1	0	Fournier-Level et al. 2009
VVS	5	2	Thomas & Scott 1993
VChr15_Cen Gen05	1	0	Van Heerden et al. 2014
Total	806	182	

Annex 2. Number of base pairs of each SSR marker linked with the identified anthracnose resistance QTLs in the genetic map of *V. amurensis*-derived MGM4.

Genotype	VVIM93 LG18		VCHR18A LG18		VMC8F4.2 LG18		VMC5E9 LG19		VMC5D11 LG19		VVIV33 LG19	
Seyanets Malengra[1]	106	X	158	X	86	92	210	X	X	X	345	X
Severnyi[2]	**91**	106	**141**	162	86	92	210	226	**228**	X	345	**362**
Saperavi[1]	122	X	158	X	86	92	216	208	202	X	342	345
Saperavi Severnyi[2]	**91**	122	**141**	158	86	92	208	226	202	**228**	345	**362**
Prachttraube[1]	106	X	158	162	86	92	220	X	X	X	X	X
Sibera[2]	**91**	106	**141**	158	86	92	210	226	**228**	X	341	**362**
Moscato Giallo[1]	106	122	158	X	92	X	220	226	202	X	345	X
MGM4[3]	**91**	122	**141**	X	86	92	220	226	202	**228**	345	**362**

[1]*V. vinifera* genotypes that belong to MGM4s pedigree.
[2]*V. amurensis*-derived genotypes that belong to MGM4s pedigree.
[3]*V. amurensis*-derived genotype used for genetic mapping of QTLs linked to grapevine anthracnose resistance.
(x) Homozygous loci or null allele.

Annex 3. Putative proteins deduced from candidate genes in the *Rea1* (Chr18) and *Rea2* (Chr19) regions using the reference genome PN40024 12X.v2 .

Chr	Protein	Start	Stop	Strand	Annotation
18	Protein_DETOX_1	5062243	5066242	-	ID=VIT_218s0001g06790.v2.1;Name=VIT_218s0001g06790
18	Protein_DETOX_2	5078187	5081480	-	ID=VIT_218s0001g06820.v2.1;Name=VIT_218s0001g06820
18	Per_1	5092206	5123595	+	ID=VIT_218s0001g06850.v2.1;Name=VIT_218s0001g06850
18	Per_2	5130432	5131472	+	ID=VIT_218s0001g06881.v2.1;Name=VIT_218s0001g06881
18	Protein_DETOX_3	6688711	6690565	-	ID=VIT_218s0001g08200.v2.1;Name=VIT_218s0001g08200
18	Per_3	11184220	11185662	-	ID=VIT_218s0001g13110.v2.1;Name=VIT_218s0001g13110
19	NADPH_dehydr	4962716	4967446	+	ID=VIT_219s0014g04660.v2.1;Name=VIT_219s0014g04660
19	Terp_synth_C	5243975	5244703	-	ID=VIT_219s0014g04920.v2.1;Name=VIT_219s0014g04920
19	NB-ARC_protein	5477937	5480948	+	ID=VIT_219s0014g05180.v2.1;Name=VIT_219s0014g05180

Note: Protein detoxification (Protein_DETOX), Peroxidase (Per), NADPH dehydrogenase (NADPH_dehydr), NB-ARC domain-containing protein (NB-ARC_protein) and Terpene synthase C domain-containing protein (Terp_synth_C).

6 CAPÍTULO 3 - STANDARD AREA DIAGRAM SET TO ANTHRACNOSE SEVERITY ON GRAPEVINE BUNCH AND SHOOT

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6.1 ABSTRACT

Anthracnose is one of the most destructive grapevine diseases in warm and humid regions, but no efficient tools are available to quantify disease symptoms in different parts of the plant. Therefore, this study aimed to develop and validate a standard area diagram (SAD) to evaluate grapevine anthracnose on the fruit and shoot. For fruit SAD development, fruit clusters showing signs of anthracnose attack were sampled from five different white grape genotypes: Aromera, Bronner, GF24, Felicia, and Helios. To develop shoot SAD, plants from a population that segregated for anthracnose resistance were artificially inoculated. Fruits and shoots with characteristic symptoms were photographed, and a total of 30 and 31 images, respectively, were selected to develop each SAD. The SAD from fruit and shoot included severity ranging from 0.5 to 17.6 % and from 0.8 to 45.9 %, respectively. In order to verify the applicability of both SADs, random raters evaluated the images with and without the use of SAD. Rater data were utilized for validation of both SADs through the use of linear regression, absolute error, and Lin's statistic, and repeatability was tested by inter-rate analysis. The developed SADs improved the accuracy and repeatability among the raters. In addition, diagrammatic scales for anthracnose severity on the fruit and shoot decreased absolute error and disease overestimation. In conclusion, the use of a set of both SADs improved grapevine anthracnose evaluation on the fruit and shoot.

Key words*:* *Elsinoe ampelina*; *Vitis vinifera*; disease resistance; grapevine breeding

6.2 INTRODUCTION

Grapevine (*Vitis sp*) is largely cultivated in many parts of the world for different purposes, such as wine production, table grape, and dry fruit production (OIV 2017). However, one crucial barrier to the production of grapes is disease caused by fungi (Waite et al. 2019). Grapevine anthracnose (*Elsinoë ampelina*) is one such disease that occurs mainly in tropical and subtropical regions under warm and humid conditions (Santos et al., 2018). Nevertheless, climate change may favor anthracnose development in other regions as well (Ponti et al., 2018). *Elsinoë ampelina*, the causal agent of grapevine anthracnose, attacks leaves, shoots, tendrils, and fruits, affecting the productivity of vines and fruit quality (Poolsawat et al. 2012; Braga et al. 2019). Therefore, the development of resistant cultivars and adequate field management of the disease are two alternatives for controlling this pathogen (Ricketts et al. 2017). The most widely grown cultivars are susceptible to anthracnose. This demonstrates the need for new methods to evaluate the disease, including epidemiological models and disease progress curves, as well as grapevine breeding programs and integrated disease management (Bock et al. 2016; Fantin et al. 2018).

Epidemiological models and disease progress curves are fundamental approaches toward understanding pathogenic infection and development (Sompong et al. 2012; Santos and Spósito 2018; Braga et al. 2019). In addition, no useful methodology is currently available to screen anthracnose resistance in grapevine breeding programs (Wang et al. 1998; Hopkins and Harris 2000; Poolsawat et al. 2012). Moreover, viticulturists use preventive applications of fungicides because they don't have the correct management and tools to control the anthracnose on grapevines (Ricketts et al. 2017). The development of a standard area diagram (SAD) would be a useful aid in quantifying disease attacks with accuracy and repeatability (Sachet et al. 2017). This can be used following disease development toward the goal of efficient

management before anthracnose is able to do substantial economic damage (Barros et al. 2015; Ricketts et al. 2017).

Diagrammatic scale, SAD, involves the development of a set of illustrations of plant parts with different levels of disease damage (Domiciano et al. 2014). It sets a standard for evaluation to enable fair comparison and reduce subjectivity among raters (Del Ponte et al. 2017). Accordingly, SAD was used in many species, such as cotton (*Gossypium hirsutum*) (Fantin et al. 2018), passion fruit (*Passiflora edulis*) (Costa et al. 2018), and coconut (*Cocos nucifera*) (Santos et al. 2017). Few SADs have been developed for grapevine diseases, such as grapevine rust, downy and powdery mildew (IPGRI 1997; Angelotti et al. 2008; Buffara et al. 2014). For grapevine anthracnose, SAD is only available for leaves (Santos and Spósito 2018). However, under field conditions and in artificial *E. ampelina* inoculations, the first symptoms are observed on shoots, but symptoms on fruits are also seen (Ellis and Erincik, 2008; Santos et al., 2018).

Normally, diagrammatic scales to evaluate plant diseases were based solely on the severity of disease progression on leaves (Angelotti et al. 2008; Buffara et al. 2014; Domiciano et al. 2014). However, *E. ampelina* also attacks the fruits; therefore, to evaluate anthracnose by only looking at leaves ignores the impact of the disease on fruit productivity and quality (Martinelli et al. 2015). In addition, an *E. ampelina* pathogenicity test should be done on shoots, instead of leaves (Santos et al. 2018), because young shoots provide a larger area to evaluate than young leaves. Then this perspective, grapevine anthracnose should be evaluated at two specific phenological phases that show the results of disease: they include sprouting to control seedling necrosis and during fruit development to prevent fungal growth (Barros et al. 2015). However, no scales are currently available for anthracnose evaluation during these two phases, resulting in a lack of tools with which to monitor the progress of the disease at those important grapevine developmental phases (Fan et al. 2017). The development of such scales would allow

for better screening between different isolates and cultivars (Hopkins and Harris 2000; Poolsawat et al. 2012). Additionally, the genetic mapping of quantitative traits (QTLs) conferring disease resistance requires accurate phenotyping of segregating populations on the different plant organs (Welter et al. 2007), and SADs are very useful for this purpose as well. Therefore, the present study aimed to develop and validate SAD for assessing anthracnose severity on grapevine fruit and shoots. The hypothesis tested was that the construction of SAD to assess the severity of grapevine anthracnose in fruits and shoots will increase the reproducibility and precision in disease assessments for use in phenotyping and other epidemiological studies.

6.3 MATERIAL AND METHODS

6.3.1 Sampling collection

The present research was carried out in an experimental vineyard at the Federal University of Santa Catarina, Campus of Curitibanos, State of Santa Catarina, Brazil, during the 2018–2019 season. The vineyard is located at coordinates 27°16′58″ S by 50°30′11″ W at an altitude of 1000 m. The climate in this region is Cfb - humid mesothermic climate, according to the Köppen-Geiger climate classification (Peel et al. 2007).

Fruit clusters were sampled from four grapevine cultivars that included Aromera, Bronner, Felicia, and Helios, and the breeding selection GF24. All genotypes were grafted onto the rootstock Paulsen 1103, planted at a spacing of 3.00 × 1.20 m, and trained on a vertical shoot positioning trellis. At the phenological stage of berry formation at the beginning of bunch closure (E-L 33) (Eichhorn and Lorenz 1984), four bunches per cultivar per block with different anthracnose severity (naturally infection) were sampled in triplicate from each cultivar, totaling 12 bunches collected in three blocks. Bunches were photographed with a digital camera (Nikon D3200, Brazil) affixed 40 cm away from each bunch which had been laid over a white

background (Figure 1a). Based on the characteristic symptoms of anthracnose on berries, was isolated the pathogenic agent *E. ampelina*. Other fungi from the species *Colletotrichum* were isolated from the berries as well.

In addition, two-year-old potted vines from a crossed population that segregated for anthracnose resistance were pruned and grown in a greenhouse at ±25ºC. At 20 days after pruning, 103 plants were inoculated with a $6x10^{-6}$ conidia suspension of *E. ampelina* (isolate AVBR 118) until run off (Santos, et al. 2018). After inoculation, plants were maintained for 48 h at ±25ºC and 90% relative humidity (± 2% of variation) in the dark. Afterward, the vines were subjected to a photoperiod of 12 h for 12 days. Characteristic symptoms were reisolated to confirm the pathogen (*Elsinoë ampelina*). The first apical shoot from each plant was photographed at 12 days past inoculation using a digital camera (Nikon D3200, Brazil) mounted 30 cm from the shoot on a white background (Figure 1b).

6.3.2 Standard area diagram development

The bunch and shoot photos were evaluated for anthracnose severity (disease area in percentage), using Quant® (Vale et al. 2001). Subsequently, 30 and 31 photos at the fruit bunches and shoots were selected for scale construction, respectively (Figure 1). Both SADs were developed using Quant® by marking the anthracnose symptoms in black and grapevine parts, bunches or shoots, in green. The maximum and minimum severity range for the fruit and bunch disease areas were chosen to establish the extreme ranges for sets of SADs.

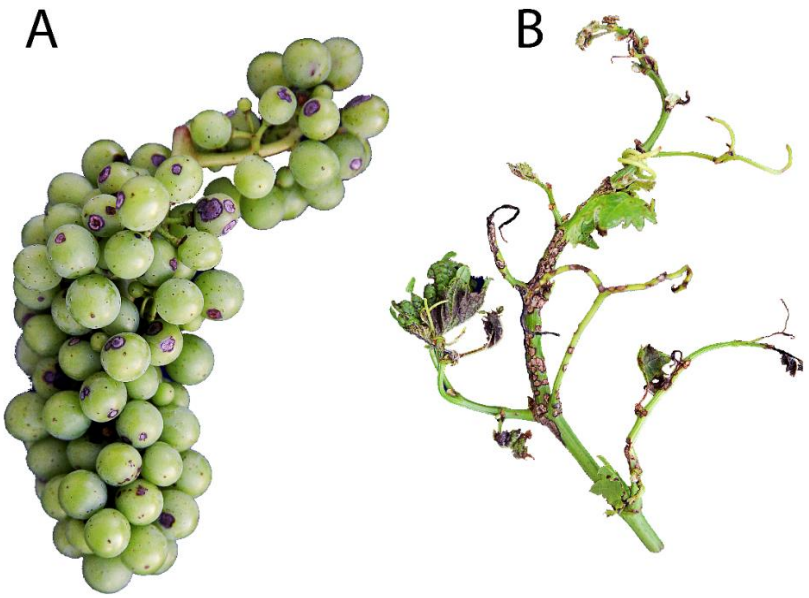

Figure 1. Characteristic symptoms of anthracnose on a grapevine bunch (a) and shoot (b). Photographer: Augusto Marques.

6.3.3 Validation of SAD

Instructions about characteristic anthracnose symptoms on bunch and shoot were given to twelve randomly chosen raters with and without experience in disease evaluation. Afterward, raters were asked to evaluate disease severity based on a sequence of pictures (31 images of bunches and 30 of shoots) with different levels of anthracnose organized at random, first on the bunch and then on shoot without the use of SAD. One day later, raters were asked to re-evaluate disease severity of the same pictures, now, however, using SAD for bunch and shoot, respectively.

6.3.4 Statistical analysis

The accuracy and precision for bunch and shoot evaluations were determined by the distance from the linear regression analysis with and without SAD. In addition, the difference among raters' evaluations was estimated by absolute error adherence, as calculated by the severity real minus estimated severity, comparing separately the use of SAD for bunch and

shoot. Lin's concordance correlation (LCC) was calculated for bunch and shoot with and without SAD (Lin 1989). For Lin's concordance, the scale (*v*), localization (*u*) and coefficient (*cb*) of bias, Pearson correlation (*r*), and Lin's concordance correlation (*pc*) were all estimated. When LCC statistics of systematic bias are $v = 1$, constant bias $u = 0$, generalized bias, $cb = 1$, precision $r = 1$, and accuracy $pc = 1$, then perfect accuracy of the estimates can be attained. Any deviation from those values indicates bias, imprecision, and loss of accuracy (Lin 1989). The assessment of inter-rater reliability (IRR) was calculated using the coefficient of determination (R^2) to provide the degree of agreement among raters with and without SAD for bunch and shoot evaluation. Moreover, the intra-class correlation coefficient (ICC) was calculated for bunch and shoot evaluation using the analysis of variance (Nita et al., 2003). For each statistic described above, i.e., *v*, *u*, *cb*, *r*, *pc,* and R^2, confidence intervals for 95% (p=0.05) were calculated by 10,000 bootstrapping samples using the percentile method (Yadav et al. 2013). Statistical analyses were performed using the epiR package (Stevenson et al. 2017) and irr (Gamer et al. 2012) was performed with 'R' software, v. 3.4.5 (R Core Team 2017).

6.4 RESULTS

SAD developed in this study for grapevine bunch was comprised of five values of anthracnose severity, ranging from 0.5 to 17.5 % (Figure 2). The anthracnose symptoms on the berries occurred up to the development of pea-size berries and were characterized initially by the development of small reddish circular spots (Figure 1a), which enlarged and became, in some cases, slightly sunken, turning into whitish-gray spots surrounded by narrow reddish-brown to black margins. The berries were more susceptible up to pea-size when compared to near onset of ripening (veraison) when they began to soften and take on color.

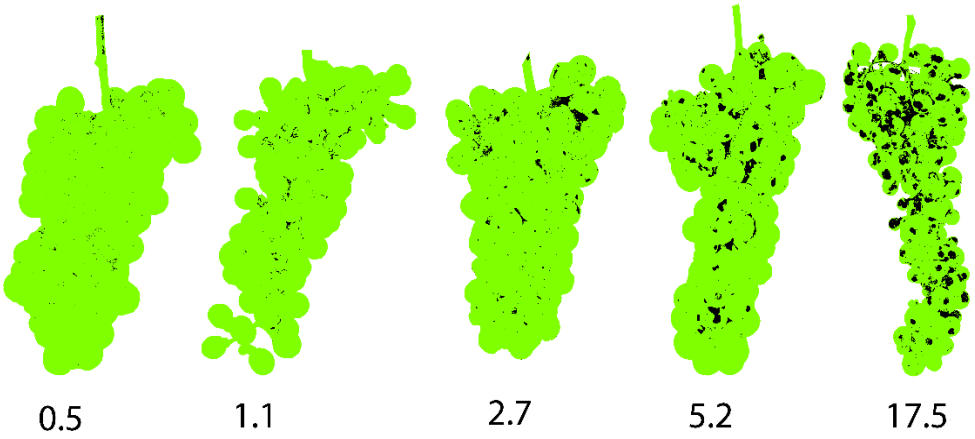

Figure 2. Standard area diagrams developed for grapevine anthracnose severity on grapevine bunches. The value below each image corresponds to the severity, according to the percentage area of the bunch covered by disease symptoms.

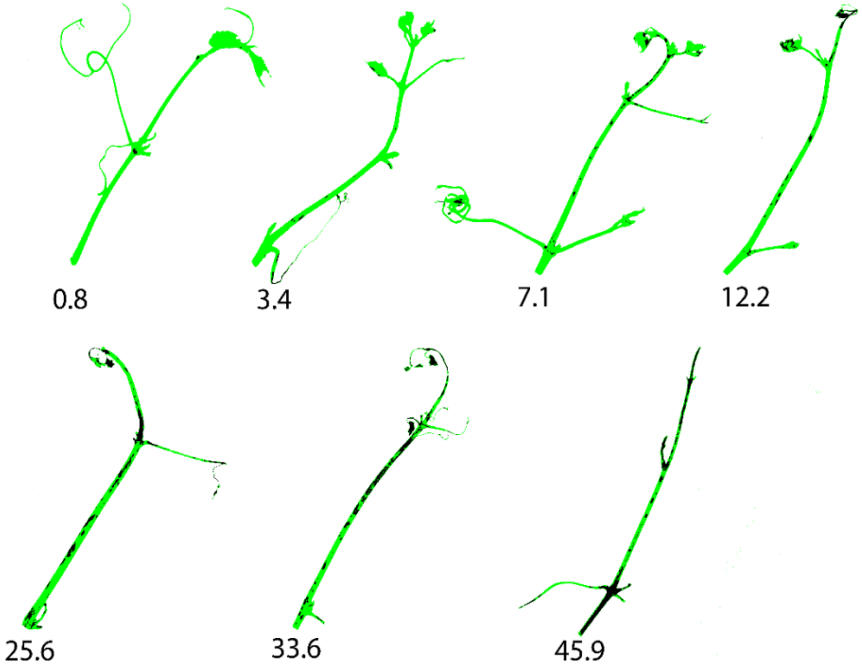

Figure 3. Standard area diagrams developed for grapevine anthracnose severity on the grapevine shoots. The value below each image corresponds to severity, according to the percentage area of the shoot covered by disease symptoms.

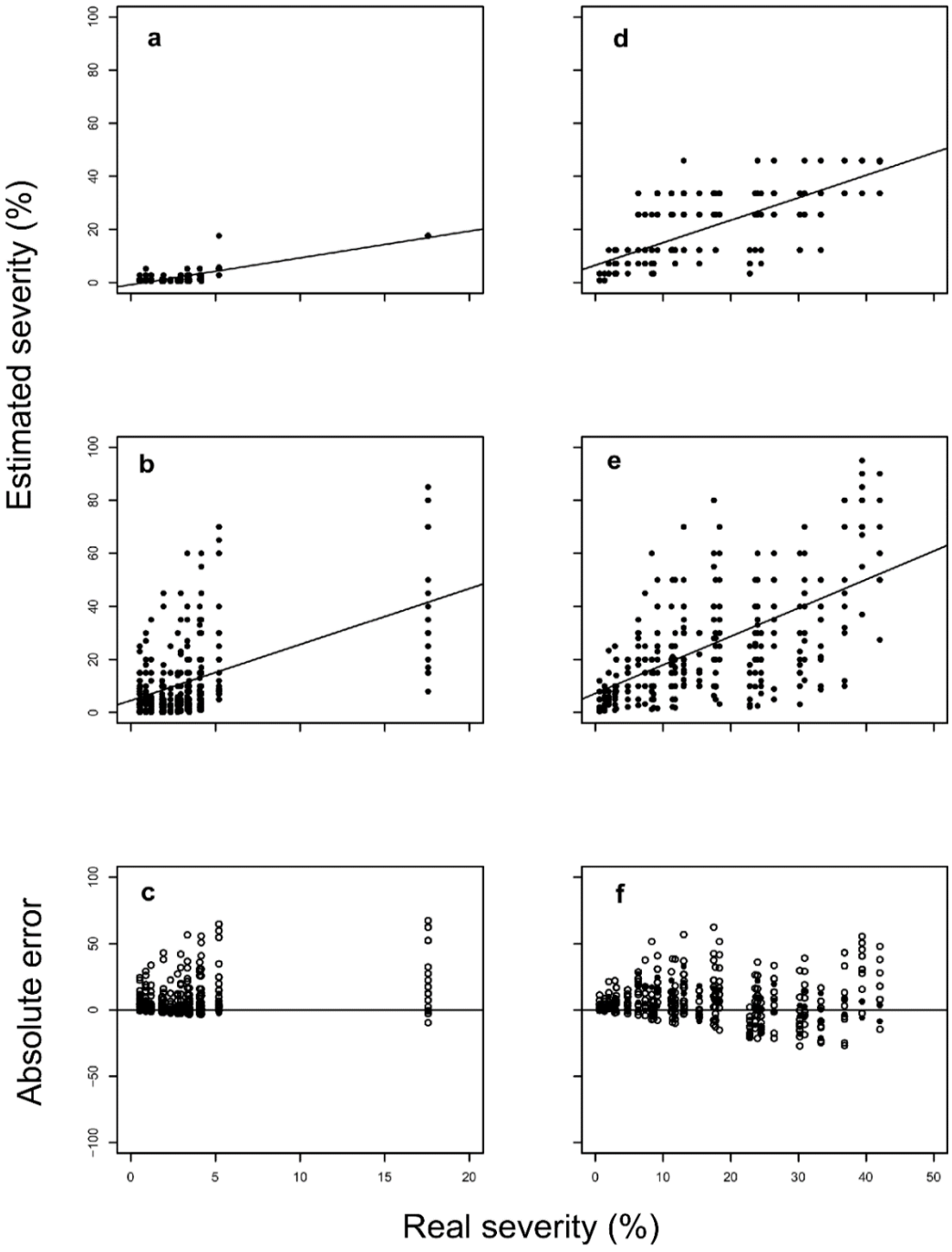

Figure 4. Linear relationship between real and estimated severity of grapevine anthracnose estimated by 12 raters on the bunch with (a) and without the use of the standard area diagram (SAD) (b) (*n*=360) and on the shoot with (d) and without the use of the SAD (e) (*n*=372). The solid line is the concordance line. Absolute error represents adherence by the use of SAD to evaluate anthracnose severity on a bunch (c) and shoot (f). Filled circles (black) are absolute errors with SAD and unfilled cycles (white) without SAD. The solid line represents the no-error line.

Shoot SAD started at 0.8% and ended at 45.9% severity (Figure 3) and was divided into seven values. On the shoots, the characteristic symptoms appeared as numerous small, circular, and reddish spots (Figure 1b) that enlarged, became sunken and produced lesions with gray centers and round or angular edges. Under severe attack, multiple lesions coalesced, often causing necrosis and breakdown of the infected shoots.

The use of SADs to estimate anthracnose severity on bunches increased the concordance of the estimative (Figure 4a). Without the use of SAD, raters overestimated, by 77.0%, the real severity on bunches (Figure 4b). In addition, the absolute error was minimized by 79.2% when SAD was used (absolute error = error without SAD - error with SAD) (Figure 4c). Corroborating this result, linear regression also demonstrated that SAD helped to estimate the severity of the shoot (Figure 4 d and e). The major concordance between estimated severity with SAD and real severity in the grape bunches occurred at lower severity (0.8 to 3.4%) were evaluated (Figure 4d). In addition, the use of SAD to evaluate anthracnose severity on shoot diminished by 35.6% the overestimate of real severity and the absolute error by 39.8% (Figure 4d, e, and f).

Accuracy was defined as variation associated with estimating disease severity using the same image with and without SAD. The use of SAD to evaluate anthracnose severity on bunches improved accuracy by 45% (LCC=*pc*) (Table 1). Scale (*v*) and coefficient of bias (*cb*), confirmed the increase in accuracy and repeatability when SAD was used to evaluate grapevine anthracnose severity on bunches, resulting in values of 2.69 and 0.55 without the use of SAD compared to a value near the perfect correlation (1), 1.06, and 0.97 with the use of SAD, respectively. Corroborating, the localization (*u*) showed values near the perfect correlation (0) when bunches were evaluated with SAD (<0.01). In addition, severity evaluation without SAD resulted in a reduction of precision when evaluating the same images since *r* decreased by 0.14 compared to using the scale.

Table 1. Scale (*v*), localization (*u*) and coefficient (*cb*) of bias, Pearson correlation (*r*), Lin's concordance correlation (*pc*), and confidence intervals (CIs) based on 10,000 bootstrap samples to grapevine anthracnose severity evaluated on bunches and shoots with and without standard area diagrams.

	Bunch			Shoot		
	Means[sd] No SADS	With SAD	CI 95%[*]	Means[sd] No SADS	With SAD	CI 95%*
LCC (*pc*)[a]	0.47 (0.29)	0.92 (0.03)	**0.29, 0.61**	0.52 (0.13)	0.69 (0.06)	**0.10, 0.26**
Scale (*v*)[b]	2.69 (1.59)	1.06 (0.05)	**-2.55, -0.88**	1.50 (0.45)	1.13 (0.10)	**-0.61, -0.11**
Localization (*u*)[c]	1.07 (1.04)	<0.01 (0.10)	**-1.85, -0.71**	0.54 (0.56)	0.31(0.17)	-0.52, 0.11
Coefficient of bias (*cb*)[d]	0.55 (0.28)	0.97 (0.01)	**0.26, 0.57**	0.72 (0.13)	0.93 (0.05)	**0.12, 0.28**
Correlation coefficient (*r*)[e]	0.81 (0.12)	0.95 (0.03)	**0.06, 0.20**	0.71 (0.10)	0.74 (0.04)	-0,02, 0,10

[Sd] standard deviation in parenthesis

[a] Scale of bias or slope shift measures accuracy using real severity (x) and estimated severity (y), ranges from -1 to 1, with perfect ratio at 1

[b] Location of bias or height shift, measures accuracy using real severity (x) and estimated severity (y), with perfect ratio at 0

[c] Coefficient of Bias measures accuracy by calculating how far the best-fit line deviates from concordance line, using v and u. No deviation from the concordance line occurs when Cb = 1

[d] Pearson correlation measures precision using real severity (x) and estimated severity (y). Ranges from -1 to 1, with perfect ratio at 1;

[e] Lin's concordance correlation combined both measures of precision (r) and accuracy (cb) resulting in the agreement to evaluate anthracnose. Ranges
from -1 to 1, with perfect agreement at 1

[*] Confidence intervals (CI) were calculated by 10,000 bootstrapping samples using the percentile method. If CIs not include zero, there were significant differences between the means ($p > 0.05$), represented by bold values

Severity on shoot estimated with SAD enhanced by 0.17 the LCC (*pc*), which demonstrates the usefulness of SAD in rating grapevine anthracnose on shoots (Table 1). In addition, the use of SAD increased the accuracy of estimating severity on shoots (*v*=1.50 without and 1.13 with SAD; *cb*=0.72 without and 0.93 with SAD). However, SAD provided less improvement in the accuracy when estimating anthracnose severity on the shoot when compared to the use of SAD for bunch evaluation. Nevertheless, the standard deviation for the interrater coefficient of determination decreased when the SADs were employed to evaluate anthracnose severity on both bunches and shoots (Table 1).

Inter-rate reliability, as measured by R^2, for both bunches and shoots showed significant improvement in repeatability among the raters when SADs were employed (Table 2). The use of SADs increased by 60% the correlation among the raters on bunch evaluations and 24% on

shoots (Table 2). Bunch evaluation with SAD increased all correlation coefficients among the raters (minimum correlation with=0.73 and without=0.17; maximum correlation with=0.99 and without=0.88). However, when severity was estimated on shoots, 71.2 % of total correlation among the raters increased when compared with and without SADs (minimum correlation with=0.46 and without=0.10; maximum correlation with=0.87 and without=0.82) (Table 2). In addition, the average correlation was higher when SADs were used for bunch and shoot evaluations (with=0.95 and 0.81; without=0.35 and 0.57; for bunch and shoot, respectively), and the standard deviation decreased (Table 2).

Table 2. Assessment of inter-rater reliability (IRR), as measured by coefficient of determination (R^2) and confidence intervals (CIs) based on degree of agreement among 12 raters to evaluate grapevine anthracnose severity on bunches and shoots with and without standard area diagram.

	Rater		1	2	3	4	5	6	7	8	9	10	11	12
								No SADs						
Bunch evaluation	1	With SADs	-	0.81	0.53	0.68	0.43	0.47	0.73	0.57	0.73	0.84	0.69	0.87
	2		0.99	-	0.37	0.64	0.56	0.34	0.86	0.46	0.72	0.94	0.54	0.96
	3		0.98	0.97	-	0.22	0.35	0.84	0.35	0.56	0.31	0.42	0.63	0.46
	4		0.94	0.93	0.94	-	0.57	0.17	0.74	0.54	0.68	0.69	0.56	0.63
	5		0.96	0.95	0.96	0.94	-	0.31	0.64	0.49	0.65	0.58	0.61	0.54
	6		0.79	0.78	0.76	0.75	0.75	-	0.30	0.39	0.26	0.35	0.44	0.41
	7		0.98	0.97	0.98	0.95	0.95	0.76	-	0.55	0.79	0.88	0.66	0.87
	8		0.97	0.96	0.98	0.92	0.93	0.76	0.96	-	0.56	0.54	0.82	0.55
	9		0.93	0.92	0.92	0.92	0.93	0.73	0.91	0.86	-	0.76	0.71	0.73
	10		0.97	0.96	0.95	0.93	0.95	0.77	0.96	0.94	0.95	-	0.67	0.93
	11		0.96	0.95	0.97	0.96	0.93	0.76	0.96	0.95	0.93	0.96	-	0.62
	12		0.97	0.98	0.97	0.94	0.94	0.78	0.97	0.96	0.90	0.94	0.96	-
								No SADs						
Shoot evaluation	1	With SADs	-	0.46	0.36	0.44	0.15	0.38	0.58	0.31	0.40	0.28	0.23	0.21
	2		0.66	-	0.69	0.64	0.23	0.81	0.76	0.75	0.80	0.69	0.59	0.59
	3		0.56	0.80	-	0.81	0.19	0.80	0.56	0.63	0.66	0.71	0.56	0.66
	4		0.61	0.67	0.65	-	0.18	0.81	0.65	0.70	0.69	0.82	0.47	0.63
	5		0.74	0.71	0.65	0.69	-	0.18	0.31	0.13	0.06	0.17	0.10	0.11
	6		0.47	0.71	0.74	0.57	0.71	-	0.69	0.81	0.81	0.74	0.61	0.74
	7		0.53	0.56	0.46	0.63	0.68	0.54	-	0.68	0.64	0.61	0.48	0.48
	8		0.65	0.69	0.59	0.79	0.76	0.60	0.75	-	0.67	0.81	0.69	0.69
	9		0.67	0.86	0.78	0.67	0.76	0.65	0.58	0.67	-	0.63	0.53	0.58

10	0.70	0.70	0.63	0.75	0.79	0.72	0.67	0.87	0.72	-	0.66	0.69
11	0.69	0.76	0.60	0.75	0.79	0.61	0.69	0.71	0.81	0.73	-	0.62
12	0.75	0.76	0.70	0.66	0.81	0.62	0.70	0.71	0.80	0.74	0.82	-

	Bunch		Shoot	
	Without SAD	With SAD	Without SAD	With SAD
Mean inter-rater coefficient of determination (R^2)	0.35 (0.19[a])	0.95 (0.07 [a])	0.57 (0.22 [a])	0.81 (0.09 [a])
Confidence interval (CI) (95%)*	**0.28 - 0.37**		**0.09 - 0.21**	

[a]Standard deviation in parenthesis; (CI) Confidence interval based on 10,000 bootstrap samples; [(*)]when CI does not include zero, there were significant differences between the means (p>0.05) (bold represents significant difference).

6.5 DISCUSSION

Anthracnose symptoms in vineyards are very distinctive, but the disease has been attributed to different causal fungi, such as *Elsinoë ampelina* and *Colletotrichum* species (Yan et al. 2015; Liu et al. 2016; Santos et al. 2017; Guginski-Piva et al. 2018). *Elsinoe ampelina* causes the formation of many black spots and sunken lesions with grey centers and dark reddish-brown to violet-black margins, infecting young shoots, leaves, tendrils, berries, petioles, and fruit stems, but lesions on shoots and berries are the most common and distinctive as previous studies suggested (Ellis and Erincik 2008; Braga et al. 2019).

SAD does not replace the experience and knowledge about characteristic symptoms of a pathogen, but it can improve accuracy in evaluating severity by providing a reference point for comparison between attacked plants (Costa et al. 2018). On fruits, research has shown that SAD estimates have improved the accuracy and reproducibility of the disease evaluation process (Pedroso et al. 2011; González-Domínguez et al. 2014; Costa et al. 2018;). Anthracnose lesions on the berries diminish the grape quality and productivity if the disease is not controlled when the first symptoms appear (Wang et al. 1998; Silva et al. 2019). The SADs proposed in the present study encompassed a range of disease anthracnose severity values in this initial phase sufficient of disease development that was to diminish economic damage (Sompong et al. 2012). In addition, it is used to help breeders select grapevine varieties with more resistance to this disease (Hopkins and Harris 2000; Murria et al. 2018).

The use of SADs to estimate anthracnose severity on shoots is less common than for other parts of the plant, such as leaves and fruit (Bock et al. 2010; Clive 1971). However, in grapevine, the appearance of anthracnose on shoots is very distinctive because the first symptoms appear on that tissue (Ellis and Erincik 2008; Silva et al. 2019). Therefore, SAD for anthracnose on a shoot can be used in grapevine breeding programs to classify seedlings or adult plants for resistance to the disease, in integrated management systems of the disease in field conditions, to map genetic marks linked with anthracnose resistance, and to evaluate the pathogenicity of isolates (Hopkins and Harris 2000; Poolsawat et al. 2012). In addition, SAD used for most diseases had maximum values of less than 40% of severity on shoots (Bock et al. 2016), near the maximum range proposed in the present study. As corroboration, under field conditions, the maximum severity that can be evaluated for anthracnose on the leaves is 46 % because, after this severity level, the leaves become brittle and can die (Santos et al. 2018). Values near 46% were observed in the present study to indicate maximum severity on the shoot. Then, the maximum disease severity of SAD was verified in both field and artificial inoculation and was used to efficiently evaluate the disease in both cases with repeatability (Bock et al. 2010).

Phenotyping is the foundation of any breeding selection process that searches for high-throughput measurements to screen many genotypes under similar conditions (Fantin et al. 2018). Increasing the accuracy and repeatability of the phenotyping process is fundamental for grapevine breeding programs to compare plants and to find resistant genes against diseases (Ricketts et al. 2017). For anthracnose, phenotyping needs to be developed further, and tools are needed to evaluate grapevine germplasm concerning resistance against this disease for which no identified QLTs have been identified (Welter et al. 2016). SAD is one tool that improves the accuracy and repeatability of phenotyping evaluation in a variety of crops (Pedroso et al. 2011; Buffara et al. 2014; Dolinski et al. 2017; Costa et al. 2018).

Accuracy assesses the closeness of a measured value to a standard, or known value, as indicated by the minimum deviation between estimated and real severity values. On the other hand, the repeatability of disease assessments refers to the precision of values with the lowest variation possible among the raters (Bock et al., 2016). Diagrammatic scales developed for anthracnose on the bunch and shoot in the present study help to estimate severity close the real value, thus increasing accuracy and repeatability (precision). A similar achievement was also obtained when SAD was employed to evaluate grapevine anthracnose severity on the leaves (Santos and Spósito 2018), brown eye spot on coffee (Azevedo de Paula et al. 2016), and black rot of coconut (Santos et al., 2017). In addition, raters, as well as experts in plant pathology, tend to overestimate the severity of disease (Costa et al. 2018). In the present study, overestimated values mainly occurred when SAD was used to evaluate severity on the shoot. Therefore, training people in the use of SAD should minimize absolute errors and overestimation to evaluate disease symptoms in the target part of the plant (Sachet et al. 2017).

The use of SAD provides a tool with which raters can estimate disease more precisely (Domiciano et al., 2014). In the present study, the scale developed with five diagrams for bunch resulted in more agreement among raters than with the seven diagram panels used for shoot evaluation. This result differs from that of Bock et al. (2016) who reported that SAD with less than seven diagrams increased the discrepancy among raters. In the current study, this may have occurred because the precision of estimates of disease severity depends on the size and shape of the lesions, coloration, and the number of lesions per unit of area (Azevedo de Paula et al. 2016). Generally, less accuracy occurs when the scale presents high severity and large-sized lesions (Bock et al. 2016). This scenario was observed for severity on the shoots, but regardless of the number of diagrams, SAD increased repeatability among the raters during disease evaluation when compared to estimates without the benefit of SAD for the fruit and shoot.

In conclusion, the use of the SADs developed in the present study increased the accuracy and repeatability of the estimation of anthracnose severity on grape bunches and shoots, and it can be applied to phenotyping, genetic mapping to find QTLs linked to resistance genes, grapevine breeding programs and evaluating field management practices.

6.6 REFERENCES

Angelotti, F., Scapin, C. R., Tessmann, D. J., Vida, J. B., Oliveira, R. R. and Canteri. M. G. (2008). Diagrammatic scale for assessment of grapevine rust. Tropical Plant Pathology, https://doi.org/10.1590/S1982-56762008000600006

Azevedo de Paula, P. V., Pozza, E. A., Santos, L. A., Chaves, E., Maciel, M. P. and Paula, J. C. A. (2016). Diagrammatic scales for assessing brown eye spot (*Cercospora coffeicola*) in red and yellow coffee cherries. Journal of Phytopathology, https://doi.org/791-800. 10.1111/jph.12499

Barros, L. B., Biasi, L. A., Carisse, O., & De Mio, L. L. M. (2015). Incidence of grape anthracnose on different VITIS labrusca and hibrid cultivars and rootstocks combination under humid subtropical climate. Australasian Plant Pathology, https://doi.org/10.1007/s13313-015-0353-8

Bock, C. H., Hotchkiss, M. W. and Wood, B. W. (2016). Assessing disease severity: accuracy and reliability of rater estimates in relation to number of diagrams in a standard area diagram set. Plant Pathology, https://doi.org/10.1111/ppa.12403

Bock, C. H., Poole, G. H., Parker, P. E. and Gottwald, T. R. (2010). Plant disease severity estimated visually. by digital photography and image analysis. and by hyperspectral imaging. Critical Reviews in Plant Sciences, https://doi.org/10.1080/07352681003617285

Braga, Z. V., dos Santos, R. F., Amorim, L. and Appezzato-da-Glória. B. (2019). Histopathology of infection and colonisation of *Elsinoë ampelina* on grapevine leaves. European Journal of Plant Pathology, https://doi.org/10.1007/s10658-019-01721-2

Buffara, C. R. S., Angelotti. F., Vieira. R. A., Bogo. A., Tessmann. D. J. and Bem. B. P. D. (2014). Elaboration and validation of a diagrammatic scale to assess downy mildew severity in grapevine. Ciência Rural, https://doi.org/10.1590/0103-8478cr20131548

Clive. J. W. (1971). An illustrated series of assessment keys for plant diseases, their preparation and usage. Edit. WL Seaman. Disease Survey, Canadian, pp 1-39

Costa, A. P., Pires, M. D. C., Peixoto, J. R., Blum, L. E. B. and Faleiro, F. G. (2018). Standard area diagram set for bacterial spot assessment in fruits of yellow passion fruit. Revista Brasileira de Fruticultura, https://doi.org/10.1590/0100-29452018039

Del Ponte, E. M., Pethybridge, S. J., Bock. C. H., Michereff. S. J., Machado. F. J. and Spolti. P. (2017). Standard area diagrams for aiding severity estimation: scientometrics. pathosystems. and methodological trends in the last 25 years. Phytopathology, https://doi.org/10.1094/PHYTO-02-17-0069-FI

Dolinski, M. A., Duarte, H. D. S. S., da Silva, J. B. and De Mio. L. L. M. (2017). Development and validation of a standard area diagram set for assessment of peach rust. European Journal of Plant Pathology, https://doi.org/10.1007/s10658-016-1138-9

Domiciano, G. P., Duarte, H. S. S., Moreira, E. N. and Rodrigues, F. A. (2014). Development and validation of a set of standard area diagrams to aid in estimation of spot blotch severity on wheat leaves. Plant Pathology, https://doi.org/10.1111/ppa.12150

Eichhorn, K. W. and Lorenz, D. H. (1984) Phaenologische Entwicklungsstadien der Rebe. European Med Plant Protection Org, 14,295–298.

Ellis, M. A. and Erincik. O. (2008). Anthracnose of grape. Agriculture and Natural Resources. The Ohio State University. https://ohiograpeweb.cfaes.ohio-state.edu/sites/grapeweb/files/imce/pdf_factsheets/Anthracnose.pdf. Accessed 10 February 2020.

Fan, X. L., Barreto, R. W., Groenewald, J. Z., Bezerra, J. D. P., Pereira, O. L., Cheewangkoon, R., Mostert, L., Tian, C. M. and Crous. P. W. (2017). Phylogeny and taxonomy of the scab and spot anthracnose fungus *Elsinoë* (*Myriangiales. Dothideomycetes*). Studies in Mycology, https://doi.org/10.1016/j.simyco.2017.02.001

Fantin, L. H., Braga, K., Canteri, M. G., Dias, A. R. and Borges. E. P. (2018). Development and validation of diagrammatic scale to assess target spot severity in cotton. Australasian Plant Pathology, https://doi.org/10.1007/s13313-018-0576-6

Gamer, M., Lemon, J., Fellows, I. and Singh P (2012) irr: Various coefficients of interrater reliability and agreement. R package version 0.84. Accessed 10 March 2019

González-Domínguez, E., Martins, R. B., Del Ponte, E. M., Michereff, S. J., García-Jiménez, J. and Armengol, J. (2014). Development and validation of a standard area diagram set to aid assessment of severity of loquat scab on fruit. European Journal of Plant Pathology, https://doi.org/10.1007/s10658-014-0400-2

Guginski-Piva, C. A., Bogo, A., Gomes, B. R., Menon, J. K., Nodari, R. O. and Welter. L. J. (2018). Morphological and molecular characterization of *Colletotrichum nymphaeae* and *C. fructicola* associated with anthracnose symptoms of grape in Santa Catarina State. southern Brazil. Journal of Plant Diseases and Protection, https://doi.org/10.1007/s41348-018-0176-2

Hopkins, D. L. and Harris, J. W. (2000). A greenhouse method for screening grapevine seedlings for resistance to anthracnose. HortScience, https://doi.org/10.21273/HORTSCI.35.1.89

IPGRI, U. (1997). OIV. 1997. Descriptors for Grapevine (*Vitis spp*.). International Union for the Protection of New Varieties of Plants, Geneva, Switzerland/Office International de la Vigne et du Vin, Paris, France/International Plant Genetic Resources Institute, Rome, Italy, 142 (34), 1-4.

Leite, I. C. H. L., Lima Filho, F. A. S., Freitas-Lopes, R. L., Michereff, S. J., Capucho. A. S. and Lopes. U. P. (2019). Standard area diagrams to aid assessments of the severity of blight leaf spot symptoms on cassava leaves. Journal of Plant Pathology, https://doi.org/10.1007/s42161-018-0132-9

Lin L. I. K. (1989) A concordance correlation coefficient to evaluate reproducibility. Biometrics 45:255–268.

Liu, M., Zhang, W., Zhou, Y., Liu, Y., Yan, J. Y., Li, X. H. and Hyde, K. D. (2016). First report of twig anthracnose on grapevine caused by *Colletotrichum nymphaeae* in China. Plant Disease, https://doi.org/10.1094/PDIS-05-16-0632-PDN

Martinelli, F., Scalenghe, R., Davino, S., Panno, S., Scuderi, G., Ruisi, P., Villa, P., Stroppiana, D., Boschetti, M., Goulart, L. R., Davis, E. C. and Davis. C. E. (2015). Advanced methods of plant disease detection. A review. Agronomy for Sustainable Development, https://doi.org/10.1007/s13593-014-0246-1

Murria, S., Kaur, N., Arora, A. and Arora, N. K. (2018). Biochemical characterization of superior seedless variety of grape (*Vitis vinifera* L.) for resistance to anthracnose. Indian Phytopathology, https://doi.org/10.1007/s42360-018-0051-x

Nita, M., Ellis, M. A. and Madden, L. V. (2003) Reliability and accuracy of visual estimation of *Phomopsis* leaf blight of strawberry. Phytopathology, https://doi.org/10.1094/PHYTO.2003.93.8.995

OIV - International Organisation of Vine and Wine (2017) World VitiViniculture Situation. OIV Statistical Report on World Vitiviniculture. www.oiv.int/public/medias/5479/oiv-en-bilan-2017

Pedroso, C., Lage, D. A. C., Henz, G. P. and Café-Filho. A. C. (2011). Development and validation of a diagrammatic scale for estimation of anthracnose on sweet pepper fruits for epidemiological studies. Journal of Plant Pathology, https://www.jstor.org/stable/41998960

Ponti, L., Gutierrez, A., Boggia, A. and Neteler. M. (2018). Analysis of grape production in the face of climate change. Climate, https://doi.org/10.3390/cli6020020

Poolsawat, O., Tharapreuksapong, A., Wongkaew, S., Chaowiset, W. and Tantasawat. P. (2012). Laboratory and field evaluations of resistance to *Sphaceloma ampelinum* causing anthracnose in grapevine. Australasian Plant Pathology, https://doi.org/10.1007/s13313-012-0127-5

R Core Team (2017) R: A Language and environment for statistical computing. R Foundation for Statistical Computing. Vienna. Austria. http://www.r-project.org

Ricketts, K. D., Gómez, M. I., Fuchs, M. F., Martinson, T. E., Smith, R. J., Cooper, M. L., Moyer, M. M. and Wise, A. (2017). Mitigating the economic impact of grapevine red blotch: Optimizing disease management strategies in US vineyards. American Journal of Enology and Viticulture, https://doi.org/10.5344/ajev.2016.16009

Sachet, M. R., Citadin, I., Danner, M. A., Guerrezi, M. T. and Pertille, R. H. (2017). DiseasePlan-a spreadsheet application for training people to assess disease severity and to assist with standard area diagram development. Ciência Rural, https://doi.org/10.1590/0103-8478cr20160924

Sachet, M. R., Danner, M. A., Citadin, I., Pertille, R. H. and Guerrezi. M. T. (2017). Standard area diagram set for olive leaf spot assessment. Ciência Rural, https://doi.org/10.1590/0103-8478cr20160923

Santos, P. H. D. D., Mussi-Dias. V., Freire, M. D. G. M., Carvalho, B. M. And Silveira, S. F. D. (2017). Diagrammatic scale of severity for postharvest black rot (*Ceratocystis paradoxa*) in coconut palm fruits. Summa Phytopathologica, https://doi.org/10.1590/0100-5405/170792

Santos, R. F., Ciampi-Guillardi, M., Amorim, L., Massola Júnior, N. S. and Spósito. M. B. (2018). Aetiology of anthracnose on grapevine shoots in Brazil. Plant Pathology, https://doi.org/10.1111/jph.12675

Santos, R. F. and Spósito, M. B. (2018). Improving assessments of anthracnose severity on grapevine leaves through the development of a standard area diagram set. Australasian Plant Pathology, https://doi.org/10.1007/s13313-018-0566-8

Silva, H. F. D., Pinto, K. M. S., Nascimento, L. C. D., Silva, E. C. D. and Souza, W. C. O. D. (2019). Evaluation of the use of biotic and abiotic resistance elicitors against anthracnose in grapevine (Vitis labrusca L.). Summa Phytopathologica, http://dx.doi.org/10.1590/0100-5405/180414

Sompong, M., Wongkaew, S., Tantasawat, P. and Buensanteai. N. (2012). Morphological. pathogenicity and virulence characterization of *Sphaceloma ampelinum* the causal agent of grape anthracnose in Thailand. African Journal of Microbiology Research, https://doi.org/2313-2320.10.5897/AJMR11.1149

Stevenson, M., Nunes, T., Heuer, C., Marshall, J., Sanchez, J., Thornton, R. et al. (2017) epiR: Tools for the analysis of epidemiological data. R package version 0.9–87. Accessed 10 March 2019

Vale, F. X. R., Filho. E. I. F., Liberato. J. R. and Zambolim. L. (2001) QUANT – a software to quantify plant disease severity. In: Proceedings of the International Workshop on Plant Disease Epidemiology 2001. Ouro Preto. Brazil. 160.

Waite, H., Armengol, J., Billones-Baaijens, R., Gramaje, D., Hallen, F., Di Marco, S. and Smart. R. (2019). A protocol for the management of grapevine rootstock mother vines to reduce latent infections by grapevine trunk pathogens in cuttings. Phytopathologia Mediterranea, https://doi.org/10.14601/Phytopathol_Mediterr-22772

Wang, Y. J., Liu, Y. L., He, P. C., Lamikanra, O. and Lu. J. (1998). Resistance of Chinese Vitis species to *Elsinoë ampelina* (de Bary) shear. HortScience, 33(1), 123-126.

Welter, L. J., Göktürk-Baydar, N., Akkurt, M., Maul, E., Eibach, R., Töpfer, R., & Zyprian, E. M. (2007). Genetic mapping and localization of quantitative trait loci affecting fungal disease resistance and leaf morphology in grapevine (*Vitis vinifera* L). Molecular Breeding, https://doi.org/10.1007/s11032-007-9097-7

Welter, L. J., Grando, M. S. and Zyprian, E. (2016). Basics of grapevine genetic analysis. In Genetics, Genomics, and Breeding of Grapes, CRC Press.

Yadav N. V. S., de Vos, S. M., Bock, C. H. and Wood, B. W. (2013) Development and validation of standard area diagrams to aid assessment of pecan scab symptoms on fruit. Plant Pathology, https://doi.org/10.1111/j.1365-3059.2012.02641.x

Yan, J. Y., Jayawardena, M. M. R. S., Goonasekara, I. D., Wang, Y., Zhang, W., Liu, M. and Bahkali. A. (2015). Diverse species of *Colletotrichum* associated with grapevine anthracnose in China. Fungal Diversity, https://doi.org/10.1007/s13225-014-0310-9

7 CONSIDERAÇÕES FINAIS

A videira é um das frutíferas mais plantadas no mundo, porém o seu cultivo de forma convencional utiliza de aplicações massivas de fungicidas para controle de doenças fúngicas, notadamente em áreas mais úmidas que o centro de origem da *Vitis vinifera*. A antracnose é uma das doenças fúngicas da videira considerada um problema fitopatológico em regiões de alta humidade e chuvas desde a brotação até a fase de frutos em meia baga (verdes). No trabalho foram devolvidas ferramentas para o manejo sustentável dessa doença, como por exemplo o uso de escala diagramática, que aumenta a precisão e reprodutibilidade da avaliação dos sintomas da antracnose. Esta ferramenta já está disponibilizada para uso e pode então melhorar o manejo da doença a campo, como também selecionar genótipos que apresentam resistência a doença, tanto na fase de brotação quando de enchimento dos frutos.

Os frutos da videira são o produto final destinado ao comércio "*in natura*" e produção de vinhos. Para a produção de vinho, a injúria por doenças pode ou não atrapalhar o uso desses frutos durante a fermentação. O estudo das alterações bioquímicas na bagas demonstrou que sintomas de *Elsinoë ampelina* não alteram a concentração de sólidos solúveis, açucares e fenóis na casca da uva. Porém, bagas com sintomas de antracnose apresentam mosto com menor acidez. Além disso, a produção enzimática nos frutos de variedades com diferentes níveis de tolerância a doença, demostrou que existem mecanismos de defesas ligados a resistência da videira ao ataque de *E. ampelina*.

Informações sobre o melhoramento genético para a resistência a antracnose ainda são incipientes. Antes do mapeamento de genes de resistência para míldio (*Plasmopara vitícola*) e oídio (*Unicula necator*) a antracnose era considerada uma doença secundária durante o manejo a campo. Porém, com o uso de variedades resistentes a essas doenças, a antracnose tornou a próxima doença a ser estudado em nível de importância econômica em regiões de alta umidade como o Brasil. Os locos mapeados durante o desenvolvimento do presente trabalho apresentam os primeiros avanços científicos sobre a localização de genes no genoma da videira ligados a resistência antracnose em folhas e ramos. Trabalhos futuros para o refinamento desse mapeamento genético, utilizando maior número de indivíduos e marcadores, resultarão em maior precisão da localização genômica dos genes ligados a resistência a essa doença, facilitando ainda mais o uso destes genes no melhoramento assistido por marcadores

moleculares. Espera-se que essa tese seja utilizada como base para o desenvolvimento de variedades de videira resistentes a antracnose, que possam ser cultivadas em diferentes condições climáticas, diminuindo o uso fungicidas para o controle de *E. ampelina*.