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Agronomical and oenological characterization of grapes and wines elaborated from five red fungal resistant Italian varieties at Serra Gaúcha, Southern Brazil

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ABSTRACT

Serra Gaúcha is the main wine producing region of Brazil. Around 80% of total wines are elaborated, in the region, with *Vitis labrusca* grape varieties, while 20% from *Vitis viniefera L.* The climate condition is humid temperate, requiring new grape varieties, adapted and more resistant to pathogens. Therefore, the objective of the study was to evaluate the agronomical and oenological characteristics of grapes and wines from five red Italian varieties considered resistant to downy mildew. The experiment was performed in a randomized trial with two blocks, consisting of 12 plants each, with vine spacing of 1.15m between plants and 2.30m between rows. The red varieties tested were Merlot Kanthus, Cabernet Volos, Julius, Merlot Khorus and Cabernet Eidos, in the 2021 vintage. Disease susceptibility, cycle duration, yield, and oenological parameters of grapes and wines were evaluated. All varieties were resistant for downy mildew, and cycle duration varied between 160 to 183 days. Julius presented the highest yield, while Carbenet Eidos presented the lowest. No differences were observed for total soluble sugars, while total acidity and pH varied according to the grape variety. Phenolic compounds were strongly influenced by grape varieties, and differences were observed for skin+pulp and seeds extracts of grapes and wines. Cabernet Eidos presented the highest amounts of total anthocyanins in skin+pulp, while Merlot Khorus the lowest concentrations. In seed extracts, Cabernet Volos and Cabernet Eidos presented the highest concentrations of total flavanols, while Julius, Merlot Kanthus and Merlot Khorus presented the lowest values. In wines, Julius presented the highest concentrations of total flavanols, while Cabernet Eidos the lowest amounts. Merlot Khorus presented the highest concentrations of total anthocyanins, while Cabernet Eidos the lowest amounts. Ex-

cept Cabernet Eidos, all other varieties may have good adaptability to the region and could be commercially used by wineries in the future for winemaking.

Introduction:

Rio Grande do Sul is the main wine grape-producing state in Brazil, responsible for 62.72% of national winegrowing area, and 90% of the national production of grapes and wines (Mello and Machado, 2020). In 2019, 332.6 million liters of wine were consumed in Brazil, and Serra Gaúcha was the main wine grape-producing region. Brazilian wines are composed of 80% from *Vitis labrusca* grape varieties, and 20% from *Vitis vinifera L.* (Mello and Machado, 2020; Pereira et al., 2020; Pereira, 2020). The region is located in a humid temperate climate, with high rainfall period during grape maturation. From December to March is the period of maturation and harvesting of wine grapes in the region, and total rainfall accumulated was 606 mm. In January, there was high rainfall (226 mm) and in February, the number of rainy days decreased and was 139 mm. In March, rainfall was 147 mm. The use of varieties tolerant/resistant to pathogenic fungal diseases, in order to reduce and/or avoid risks of losses in productivity or oenological quality caused by pathogen attacks, for wineries, improving the quality of their wine. Resistant varieties could also reduce phytosanitary treatments and production costs, thinking in a sustainable viticulture at Serra Gaúcha. New varieties were developed through backcrossing to obtain resistance genes. Among them, Merlot and Cabernet Sauvignon were crossed with varieties recognized for their resistance to fungal pathogens, such as the Bianca, Regent and Konza 20-3 varieties. These new varieties have a high percentage of *Vitis vinifera L.* in their pedigree (≥85%) (Etienne et al., 2016; Casanova-Gascón et al., 2019). They are internationally known as PIWI, an abbreviation of the German word *Pilzwiderständsfhige* (resistant to fungal diseases) (Souza et al., 2019; Zanghelini et al., 2019).

In this context, the objective of the study was to evaluate the agronomical and oenological responses of five red Italian varieties considered resistant to downy mildew, recently introduced in the region.

Materials and methods:

The study was developed in an experimental vineyard located at Embrapa Grape and Wine, in Bento Gonçalves city, Rio Grande do Sul state. The experimental design was installed in 2016 in randomized blocks, with two plots, of 12 plants per replicate, totaling 24 plants per variety, spaced 1.15m between vines and 2.30m between rows. The varieties were Merlot Kanthus, Cabernet Volos, Julius, Merlot Khorus and Cabernet Eidos, grafted onto the Paulsen 1103 rootstock. Vines were trained in vertical shoot positioning-VSP, pruned in a bilateral spur-pruned cordon. Agronomical characterization was carried out by determining the cycle duration (days after pruning until the harvest-DAP), susceptibility to downy mildew disease (by checking disease symptoms in the field), and yield (kg/plant) and (kg/ha). At harvest, decided according to grape sanity and climatic conditions of the region, 100 berries were collected per plot, of which 40 berries were used for physical-chemical analyses, namely total soluble solids (°Brix), total acidity (g L-1 of tartaric acid) and pH (AOAC, 2002). The remaining 60 berries were used to characterize individual and total phenolic compounds by UPLC-MS, by extracting with ethanol skin+pulp and seeds separately, in triplicate (20 berries each) (Pereira et al., 2021). Wines were elaborated using 20 kg of grapes from each plot, following standard protocols for reds, controlling alcoholic and malolactic fermentations (25±2ºC and 18±2ºC, respectively) (Peynaud, 1997). After cold stabilization, wines were bottled and analyzed according to the following physicochemical analyzes: density, alcoholic content, total acidity, pH (AOAC) and phenolics coumpounds by UPLC-MS according to Canedo-Reis et al. (2020) methodology. Results of harvest, 2021 vintage, were subjected to analysis of variance (ANOVA) and comparison of means using Tukey test at 5% probability level, using the Action stat statistical program.

Results and discussion:

Significant differences were observed for agronomical and oenological parameters. The five varieties were resistant to downy mildew in the 2021 vintage, no spraying was applied, and no symptoms were observed. Vintage 2021 was characterized and influenced by La Niña, considered a dry season as compared to other vintages. In 2021, the rainfall was 606 mm between December to February (Ripening period), while the normal is 550 mm. The cycle duration, between pruning to harvest, varied between 160 to 183 days (Table 1).

The yield was strongly influenced by variety. Julius presented the highest yield (3.0 Kg/plant), followed by Merlot Khorus (2.7 Kg/plant), Cabernet Volos (2.3 Kg/plant), Merlot Kanthus (2.3 Kg/plant), and Cabernet Eidos (0.6 Kg/plant). The average production per plant ranged from 2,226.5 to 11,245.5 Kg ha-1. The productivity (Kg ha-1) and weight per plant obtained in this study were lower than those found by Testolin et al. (2020), with the exception for Julius variety, which showed higher productivity.

There were statistical differences were observed for total soluble sugars (ranging from 19.5 to 22.5 ºBrix), and these results were lower than those observed by Testolin et al. (2020). Total acidity and pH varied according to the grape variety (Table 1). Cabernet Volos, Merlot Khorus and Merlot Kanthus grapes presented the highest total acidity value (6.7 g L^{-1} and 5.3 g L^{-1}), while Julius and Cabernet Eidos presented the lowest values (5.2 g L^{-1}). Concerning pH, it ranged from 3.40 (Julius) to 3.79 (Cabernet Eidos). Grapes of Cabernet Eidos and Merlot Khorus presented higher values of pH, while Cabernet Volos and Julius presented lower values than those described by Testolin et al. (2020).

In skin+pulp extracts of the grapes, higher values of flavonols + stilbenes were observed for Cabernet Volos, Julius, Cabernet Eidos and Merlot Kanthus (14.47 mg kg^{-1}, 13.42, 12.69 and 9.08 mg kg^{-1}, respectively), and lower concentrations for Merlot Khorus (7.18 mg kg^{-1}) (Table 2). Individual phenolic compounds were characterized. The major compound identified was isoquercitin, varying from 5.72 mg kg^{-1} (Merlot Khorus) to 11.74 mg kg^{-1} (Cabernet Volos) (data not shown). The highest concentration of total flavanols was observed in Merlot Khorus and Cabernet Volos grapes (789.75 and 782.85 mg kg^{-1}), while Cabernet Eidos and Merlot Kanthus presented the lowest values (250.82 and 265.81 mg Kg^{-1}). The major compound was epicatechin gallate, ranging from 187.52 mg Kg^{-1} (Cabernet Eidos) to 701.08 mg Kg^{-1} (Cabernet Volos) (data not shown). The concentration of total anthocyanins varied from 57.06 mg Kg^{-1} (Merlot Khorus) to 125.76 mg Kg^{-1} (Cabernet Eidos), which concentrations were lower than those determined in the study of Susin et al (2022), in Merlot grapes at Serra Gaúcha. The major compound of total anthocyanins was malvidin-3-glucoside, whose concentrations ranged from 26.07 mg Kg^{-1} (Merlot Khorus) to 98.04 mg Kg^{-1} (Cabernet Eidos) (data not shown). Merlot Kanthus, Cabernet Volos and Julius presented the highest concentrations of caftaric acid (18.59, 16.56 and 13.26 mg Kg^{-1}, respectively), while Merlot Khorus and Cabernet Eidos presented the lowest concentration (2.71

and 5.35 mg Kg^{-1}, respectively). These variations may be due to the climate conditions in the vintage for different regions at the harvest date, and the protocols of winemaking (Peynaud, 1997).

In seed extracts, a higher total content of flavonols + stilbenes were observed for Cabernet Eidos (11.98 mg kg^{-1}) and Merlot Khorus (10.80 mg kg^{-1}), and lower for Julius, Merlot Kanthus and Cabernet Volos (5.05, 5.39 and 6.02 mg kg^{-1}, respectively). The major compound of total flavonols+stilbenes was isoquercitin, which ranged from 1.48 mg kg^{-1} (Merlot Kanthus) to 9.21 mg kg^{-1} (Cabernet Eidos) (data not shown). Different concentrations of total flavanols were found in seed extracts, ranging from 3,006.0 mg kg^{-1} in Julius to 4,777.64 mg kg^{-1} for Cabernet Volos. The major individual compound of total flavanols was epicatechin gallate, ranging from 644.47 mg kg^{-1} (Merlot Khorus) to 1,796.06 mg kg^{-1} (Cabernet Volos) (data not shown).

These results were higher than those shown by Hornedo-Ortega (2020), from Cabernet Sauvignon, Merlot and others varieties. Merlot Khorus, Cabernet Eidos and Julius presented the highest concentrations of caftaric acid in seed extracts, while Cabernet Volos and Merlot Kanthus had the lowest concentrations (Table 2). Differences between the five varieties are due to genetic factor, and adaptation to the *Terroir* of Serra Gaúcha. That could be explained by, soil type, climate conditions, vine management, harvest date, and enological protocols (Van Leeuwen et al., 2004).

All wines were considered dry, according to the density, and had different values (Table 2). Alcoholic degree ranged from 10.22 % for Cabernet Eidos, to 13.28 % for Merlot Khorus, harvested in the same DAP (183 days). Total acidity varied from 6.4 g L^{-1} (Cabernet Eidos) to 9.4 g L^{-1} (Merlot Khorus), and pH presented differences. For wine phenolic compounds determined by UPLC-MS, Julius presented the highest concentrations of total flavonols + stilbenes and total flavanols (84.96 and 566.14 mg L^{-1}, respectively), while Cabernet Eidos presented the lowest amounts (12.91 and 266.92 mg L^{-1}, respectively). For total anthocyanins, the values ranged from 596.33 mg L^{-1} (Merlot Khorus) to 133.07 mg Kg^{-1} (Carbenet Eidos). Julius presented the highest concentration of caftaric acid (113.67 mg L^{-1}), while Cabernet Eidos had the lowest amount (38.12 mg L^{-1}). The variations of wine composition were due to genetic factor of each cultivar, which can be considered well adapted to the pedoclimatic conditions at Serra Gaúcha, except for Cabernet Eidos which presented very low yield, considering it to be explained by the low adaptation of the variety to the climatic conditions of the region or management adopted

Conclusion:
In 2021 vintage, Cabernet Eidos presented very low productivity and, this variety is not recommended for commercial use in the region. Other varieties showed very good yields and could be used by wineries. Differences were observed in the cycle duration, TSS, TTA and pH at harvest date, which suggest many possibilities for winemaking. Physico-chemical composition of skin+pulp and seed extracts, and wines, allowed us to determine the different oenological potential of the grapes and wines, in terms of flavanols, flavonols and anthocyanins. It is possible to recommend varieties to elaborate different kind of wines, for example, among young or aging wines. However, more study and harvests are needed to further validate the adaptation of these varieties in the Serra Gaúcha region.

Table 1. Agronomical and oenological characterization of red Italian varieties in 2021 vintage.

Varieties	Weight per plant (Kg)	Yield (Kg ha-1)	Cycle duration (DAP)	Total soluble sugars-TSS (°Brix)	Total acidity (g L^{-1} tartaric acid)	pH
Merlot Kanthus	2.3^{ab}±0.1	8,505.0^{ab}±315.0	160	19.6^{a}±0.2	5.3^{ab}±0.2	3.52^{ab}±0.02
Cabernet Volos	2.3^{ab}±0.1	8,548.0^{ab}±1,847.0	160	19.5^{a} ±0.4	6.7^{a}±0.1	3.31^{b}±0.04
Julius	3.0^{a}±0.4	11,245.5^{a}±1,06.5	168	22.5^{a} ±0.5	5.2^{b}±0.0	3.40^{ab}±0.03
Merlot Khorus	2.7^{a}±0.2	10,080.0^{a}±630.0	183	20.4^{a} ±2.6	6.7^{a}±0.0	3.71^{ab}±0.08
Cabernet Eidos	0.6^{b}±0.0	2,226.5^{c}±179.0	183	21.1^{a} ±2.7	5.2^{b}±0.0	3.79^{a}±0.16

*Averages followed by the same lowercase letter in the line do not differ by the Tukey test at the 5% probability level. DAP: days after pruning.

Table 2. Characterization of total and individual phenolic compounds from skin+pulp, seeds and wines extracts by UPLC/MS of Italian red varieties resistant, 2021 vintage.

Parameters*	Merlot Kanthus	Cabernet Volos	Julius	Merlot Khorus	Cabernet Eidos
Skin+pulp**					
Total flavonols + stilbenes	9.08^{ab}±1.54	14.47^{a}±2.27	13.42^{a}±1.25	7.18^{b}±1.40	12.69^{ab}±1.91
Total flavanols	265.81^{c}±30.42	782.85^{a}±33.27	503.47^{b}±58.36	789.75^{a}±233.86	250.82^{c}±34.47
Total anthocyanins	87.25^{b}±13.23	99.37^{ab}±8.27	83.58^{b}±11.00	57.06^{c}±2.47	125.76^{a}±13.94
Caftaric acid	18.59^{a}±2.26	16.56^{a}±2.77	13.26^{ab}±0.76	2.71^{c}±0.43	5.35^{c}±1.09
Seeds**					
Total flavonols + stilbenes	5.39^{c}±1.23	6.02^{c}±0.68	5.05^{c}±1.15	10.80^{ab}±4.22	11.98^{a}±1.40
Total flavanols	3390.56^{bc}±171.38	4777.63^{a}±208.64	3006.00^{c}±567.85	3732.62^{bc}±180.25	4159.15^{ab}±176.36
Caftaric acid	4.22^{b}±0.62	4.04^{b}±0.65	5.52^{ab}±1.31	9.25^{a}±2.02	6.96^{sb}±0.40
Wines**					
Density (20°C)	0.9974b±0,0002	0.9993a±0.0000	0.9958d±0.0002	0.9960c±0.0000	0.9974b±0.0008
Alcoholic Content (%)	11.41c±0.01	10.96d±0.01	12.86b±0.00	13.28a±0.02	10.22e±0.02
pH	3.54b±0.01	3.59b±0.01	3.73a±0.05	3.27c±0.01	3.83a±0.01
Total acidity (g L-1)	7.5c±0.0	7.8b±0.0	7.0d±0.0	9.4a±0.0	6.4e±0.1
Total flavonols + stilbenes	55.74c±2.24	45.40d±1.20	84.96a±0.93	68.86b±0.91	12.91e±0.98
Total flavanols	342.28c±6.50	377.99bc±2.61	566.13a±6.99	401.96b±0.04	266.92d±19.54
Total anthocyanins	340.33b±3.48	339.61b±4.99	329.79b±1.36	596.33a±0.15	133.07c±0.83
Caftaric acid	64.37c±0.27	67.62bc±1.28	113.67a±3.00	74.67b±1.60	38.12d±0.30

*Averages followed by the same lowercase letter in the line do not differ by the Tukey test at the 5% probability level. ** For skin+pulp and seeds, results are expressed in mg Kg^{-1} of grapes, while for wines, in mg L^{-1}; flavanols and stilbenes (expressed in mg Kg^{-1} of quercetin-3-O-glucoside) and total flavanols (expressed in mg Kg^{-1} of epicatechin).

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