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Intra-block spatial and temporal variability of plant water status and its effect on grape and wine parameters

Aladino Jasse[1], Anke Berry[1], Jose Alexandre-Tudo[1], Carlos Poblete-Echeverría[1,*]

[1] Department of Viticulture and Oenology, Faculty of AgriSciences, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa.

***** Correspondence: cpe@sun.ac.za; Tel.: +27-21-808-2747

Abstract

Improving wine composition is a critical factor for the wine industry. Phenolic compounds play an important role in wine composition contributing to its organoleptic characteristics. Although several factors can influence the phenolic concentration, plant water status in particular has shown to have a direct impact on the phenolic compounds. It is however complex to quantitate water deficit by plant water status measurements as they depend on the specific site (topography, viticultural management practices and soil characteristics) creating variable values within the vineyard block. This study focused on analysing the effect of natural spatial and temporal variability of plant water status on grape and wine parameters. A field experiment was done in a commercial Cabernet Sauvignon block to monitor the temporal and spatial intra-block variability of plant water status using a grid sample method. Soil analysis and topography were included in the evaluation. Each target vine was assessed for yield, ripeness as well as standard juice parameters. Micro-vinification was done for each target vine and the concentration of flavonoids (anthocyanins and tannins) analysed. The results showed that the spatial and temporal variability was evident along the season. Plant water status influenced changes in the concentration of phenolic compounds and grape parameters. The vines in the stressed class were associated with changes in soil texture and topography. These plants presented a significant increase in sugar content, anthocyanins and tannins and a strong decrease in yield when compared with the non-stressed classes. The results of this study may help to understand and quantify how spatial variability is naturally distributed and its effect on grape and wine parameters.

Keywords: Anthocyanins; Tannins; Yield; Cabernet Sauvignon, Stem water potential, Micro-vinification.

1. Introduction

Plant water status in a vineyard is variable inside of the blocks (intra-block variability) according to topography, viticultural management practices, and soil characteristics. Previous research has established that vine and bunch variability have a direct impact on the composition of the final wine (Kontoudakis et al., 2010). Although the grape parameters, such as yield, present spatial variability in the same block, traditional viticulture approaches the variability in the field in a uniform manner (Arnó et al., 2009). To this end, Precision Viticulture (PV) technologies aims at understanding and managing the spatial variability. PV covers a wide group of techniques and technologies with the objective of controlling spatial variability improving grape composition, adjusting crop management to field spatial variability (Santesteban, 2019). Irrigation strategies such as regulated deficit irrigation (RDI) largely affect plant water status (Acevedo-Opazo et al., 2010) when applied in specific areas of the vineyards. Interestingly, Brillante et al., (2017) demonstrated that spatial variability in plant water status still exist in a homogenous irrigation scheme.

Advances of wine production methods have led to the awareness of phenolic compounds impacting wine composition (Kennedy et al., 2006). Phenolic compounds are a crucial parameter of red wines contributing to their organoleptic characteristics, particularly to colour, flavour, texture and astringency of the wine and to its antioxidant properties (Teixeira et al., 2013). The sensory attributes are determined by the grape physiological and phenolic ripeness when the winemaker decides to harvest and are directly related with the level of phenolics in the berry (Adams, 2006). Standard physiological ripeness parameters such as sugar concentration, titratable acidity, pH, and sensorial taste are straightforward to evaluate at harvest, however, phenolic ripeness is complex, costly and time-consuming (Guidetti et al., 2010). In this sense, to answer wine consumer demands, winemakers and researchers have focused on controlling phenolic ripeness (Kontoudakis et al., 2010). When grapes reach the ripe stage (red grape varietals) skins are rich in flavonols, flavan-3-ols and anthocyanins, while seeds are known to be high in flavan-3-ols, which contributes to astringency and bitterness (Williams, 2012) of the wine. It has been demonstrated that the presence of phenolic compounds in the final wine is dependent on the biosynthesis in the grapevine, the phenolic ripeness process (Adams, 2006b), winemaking and ageing (Monagas et al., 2006). Different factors can therefore influence the concentration of phenolic compounds. Previous research has established the impact of viticulture practices, different oenological techniques (Nel, 2018), variety, vintage, and location on the phenolic composition of grapes and wines (Cliff et al., 2007).

Viticultural practices can influence berry secondary metabolite concentrations and therefore contribute to wine sensorial attributes, antioxidant capacity, stabilization, and protection during aging (Ferrandino & Lovisolo, 2014). This concept is supported by Vilanova et al., (2009) who noted that scientific literature perceives environmental conditions or viticultural practices as having a strong impact on the concentration of phenolic compounds. Guidoni et al., (2008) reported that berry phenolic ripening is directly impacted by seasonal climatic conditions and viticulture practices, such as cluster thinning and leaf removal. It has been widely reported that plant water status can influence the levels of phenolic compounds in grapes and wine (Deloire et al., 2004; Downey et al., 2006; Braidot et al., 2008). A detailed examination of the influence of viticulture practices on grape phenolic compounds was presented by Brillante et al., (2018). In this study, the authors determined that the application of water restrictions to grapevine has a higher influence on berry ripening than canopy management treatments with 3 levels of mechanically shoot thinning: heavy shoot thinning, light shoot thinning and no shoot thinning. Overall, there seems to be some evidence to indicate that reducing plant water status has an implication on skin concentration of tannins and anthocyanins in berries with the same size (Roby et al., 2004). Along with the level of water deficit, the period of water deficit also plays an important role. The application of water deficit before or after véraison, is significant in determining fruit and wine phenolic composition. Kennedy et al., (2002), Bindon et al., (2011) and Blancquaert et al., (2019) suggested that water deficit applied before or after véraison affects the biosynthesis of flavonoids in a different manner.

It is therefore important to understand the development of chemical compounds from grapes to wine in a spatial and temporal context with the aim of improving viticulture practices, such as irrigation, which can aid in achieving better wine composition. Therefore, this study is focused primarily on evaluating spatial and temporal natural intra-block plant water status variability as well as traditional harvest parameters to investigate the effect of these variations on grape and wine composition parameters by defining classes of water deficit.

2. Methods and material

2.1. Site description, soil, and weather data

The study was carried out during the growing season 2019-2020 at a commercial vineyard. The experiment site is located in the Stellenbosch Wine of Origin district, Simonsberg-Stellenbosch ward – South Africa (SAWIS, 2014), 33°54'11.8"S - 18°55'12.4"E and 430 m above sea-level. The geology of the region is characterized by compacted sedimentary formations of the Malmesbury Group from the Precambrian Era (King 1983; Carey 2005).

The climate in the area is Mediterranean with winter rainfall. According to Köppen-Geiger climate classification, it presents a Dry and Warm Summer -Csb (Peel et al., 2007), receiving summer morning Northerly hot winds and afternoon cooler South-Westerly breezes originating from False Bay (Morgenthal, 2004). The seasonal (from September 2019 to February 2020) maximum average temperature was 33.03°C and minimum average temperature was 10.65°C, and the seasonal effective rainfall is approximately 639.05 mm for the same period (Tokara weather station).

The experiment block is composed of 2.24 ha of sandy loam soil with a pH of 5.7, planted in 2003 with vines cv. Cabernet Sauvignon (clone CS 338 C), grafted on 101-114 Mgt rootstock - Vitis riparia x Vitis rupestris. The 4,840 planted vines in the block have an inter row spacing of 2.5 m and 2 m of vine spacing. The vines were trained to a Vertical Shoot Positioning (VSP) trellis system with bi-lateral cordon and spur pruned. The block has an orientation facing South. The vineyard was drip-irrigated with emitters spaced at 0.6 m and totally delivering 2.3 L/h per vine. The historical average yield in the block is 8.24 Ton/ha with an average of 4.0 kg/vine.

2.2. Selection of the target vines

A combination of plant, soil and water deficit variables were measured and analysed to select the target vines, covering all the spatial variability conditions (different levels of vigour) presented in the experimental block. Soil surface electrical conductivity (ECa), Trunk circumference (TC), and Normalized difference vegetation index (NDVI) (from previous seasons) were used to locate the target vines. The implemented methodology is described in the following sections

2.2.1. Soil surface electrical conductivity (ECa)

At the beginning of the experiment, (September 2019), ECa was measured, using an electromagnetic sensor (EM38, Geonics Ltd., Mississauga, Canada) together with a Garmin eTrex 10 GPS. The GPS locations of each vine in the block were manually geo-corrected using the number of vines per row, distance between vines and length of the rows. The entire block was scanned in a row alternate sequence, with a sampling frequency of 5 reads per second on vertical dipole mode.

2.2.2. Trunk circumference

At the same period that ECa was measured (September 2019), trunk circumference (TC) of all vines in the block were determined by manually measuring the circumferences of the trunk at an average section between 10 cm above the graft union and 10 cm below the cordons.

2.2.3. Normalized difference vegetation index

The standard normalized vegetation index (NDVI) from 2 previous seasons (2017-2018 and 2018-2019) was calculated for the maximum vegetative growth period (January to February) using Sentinel-2A images corresponding to T34HCH tile. Free-cloud atmospherically corrected images were downloaded from the European Space Agency (ESA) Copernicus project website. NDVI was calculated in R (R Core Team, 2014) using the standard equation proposed by Rouse et al., (1974).

2.2.4. k-means analysis

Data from ECa, TC and NDVI was analysed generating classes of variability using the standard unsupervised k-means function in R (R Core Team, 2019). The k-means algorithm is one of the most used methods for clustering and it partitions the data by minimizing the within-cluster sum of squares to achieve higher within-cluster similarity. In k-means the number of clusters is defined by the user and the algorithm minimize the variation intra-clusters (Zhao et al., 2010). In this study, the experiment vineyard block was divided arbitrarily into 4 spatial clusters for each analysed variable. The clusters were ordered in ascending way and then in the matching areas the target vines were located considering a separation of 5 vineyard rows to cover the whole vineyard block. As a result, a total of 43 target vines were selected (Figure 1). The block edges were avoided in order to decrease the influence of winds, solar exposition, and water streams on vine measurements. All target vines were subjected to the same canopy management, vine treatments, and irrigation schedules along the growing season.

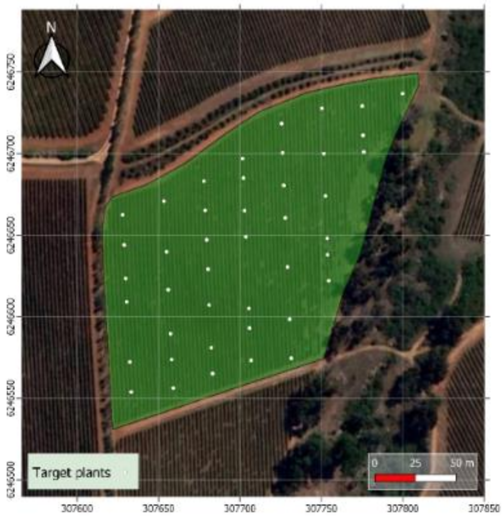

Figure 1: Location of the target vines and distribution in the experimental block.

2.3. Field measurements on the target vines during the growing season

2.3.1. Soil physical and chemical characteristics

At the beginning of the season, soil samples were extracted at a depth of 50 cm, distancing 30 cm from the target vines at the beginning of the season. The samples were analysed at the Directorate Plant science of Elsenburg for texture, resistance, macro/micronutrients, cation exchange capacity (CEC), particle size and ammonium (NH+).

2.3.2. Plant water status

Stem water potential (SWP) was measured weekly from budburst on mid-November to harvest at the end of February (Table 3), using a pressure chamber (PMS Instrument Company, model 1505D, Albany, USA). SWP was measured around midday (12:00 to 13:00 local time) on every target vine. Two mature and healthy leaves were selected from the middle of the canopy of each vine, facing the shaded side of the canopy to avoid overheating. To diminish leaf transpiration each selected leaf was covered with aluminium foil inside a plastic zip bag at least 1 hour before midday measurements (Choné et al., 2000).

2.4. Harvest measurements

2.4.1. Yield measurements components

All target vines were harvested 1 day before the commercial harvest of the block, with an average 23.8 Brixº for the entire block. All bunches were carefully handpicked.

2.4.2. Standard berry composition

The bunches of each target vine were destemmed, and the berries crushed, separately, for micro-vinification. A sample of 4 mL of juice for further analysis was collected after crushing the berries. From the crushed berries, the juice was immediately analysed to determine sugar concentration (PAL-1 Digital Pocket Refractometer, Atago, USA), pH and titratable acidity (TA) (Metrohm, model 702 SM Titrino, Switzerland).

2.5. Micro-vinifications

Harvest date was determined by sugar concentration and berry physical evaluation by the farm winemaker. Each target vine was harvested and kept separately. After physical measurements were complete, bunches were destemmed and berries were mixed for each target vine. In order to make fermentation relatable, all fermentations were scaled to a standardized 2 kg CONSOL jar. The berries were hand crushed and standard experimental winemaking procedures specified by the Department of Viticulture and Oenology at Stellenbosch University, were followed. The must was inoculated with Saccharomyces cerevisiae (Lalvin ICV D21, Lallemand, France) and a blended complex yeast nutrient (Fermaid K Lallemand France) was added. After 10 days of fermentation in a temperature-controlled room at 23ºC, all wines had a minimum residual sugar of 2.5 g/l and grapes were manually pressed.

2.6. Phenolic analysis in wine

Wine phenolic compounds were calculated using spectrophotometric analysis with a Multiskan GO Microplate Spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA), following the protocol proposed by Aleixandre-Tudo et al., (2019). The wine was diluted 50 times with HCL 1M, kept in the dark for 1 hour and analysed. The absorbance was measured in the wavelengths of 270 nm, 290 nm, 500 nm, 520 nm, 540 nm. The results were uploaded to the online web-based phenolic analysis platform - Phenolab.co.za - to calculate the content of anthocyanins and tannins (Aleixandre-Tudo et al., 2017; Aleixandre-Tudo et al., 2019).

2.4.5. Data analysis

Basic descriptive analysis, principal component analysis (PCA) and correlation analysis were applied to the variables measured during the growing season. PCA supported the search for patterns in the data and the classification of any combination of variables that could explain the impact of plant water status on the wine and grape parameters analysed. The correlation analysis was used to evaluate the strength of relationship between the analysed variables and the final result was presented as a correlation matrix. The non-parametric Kruskal-Wallis Rank Sum Test was used to determine whether the variables belonging to the water deficit classes were different. Moreover, a Pairwise-Wilcox Test was used to determine which classes differed statistically according to the level of water deficit for the analysed grape and wine variables. All data processing, statistical analyses and graphical results were performed using R software (R Core Team, 2014).**3.**

Results

3.1. Weather conditions and Total water application

In a historical context of 9 seasons, the season 2019-2020 presented maximum values similar to the historical records, however the minimum temperature was higher than the historical average by 1.36°C. Considering an equivalent growing season period, the season from September 2019 to March 2020 registered the lowest accumulated precipitation (639.05 mm - historical average 888.63 mm). The precipitation was low resulting in a noticeable water deficiency during the grapevine growing season. From budburst to berry setting the experimental site received 176.5 mm of rain (average of last 8 seasons was 180.9 mm). However, between Berry setting and Harvest only 27 mm of precipitation was registered (average of last 8 seasons was 74.9 mm) (Table 1). After véraison, almost no precipitation was registered (only one event of 1.01 mm). According to these conditions, two irrigations were scheduled by the farm manager.

Table 1. Summary of climatic data of the experimental site per week during the study period.

W	**T (°C)**	**T_{max} (°C)**	**T_{min} (°C)**	**RH (%)**	**Pp (mm/week)**	**I (m^3/ha)**	**SR (MJ/m^2/day)**	**ET_0 (mm/week)**	**VPD (kPa)**
1	18.66(±20.1)	29.53	10.66	65.71(±20.2)	8.12	-	22.03(±21.2)	25.64	0.74
2$^+$	16.01 (±15.4)	25.00	9.72	68.92(±12.6)	0.00	-	26.94(±4.4)	27.38	0.57
3	19.69 (±6.3)	33.36	12.38	65.18(±6.7)	0.00	-	24.75(±20.7)	34.30	0.80
4	20.83(±13.8)	32.86	10.01	51.49(±16.7)	6.59	-	26.72(±11.9)	36.43	1.20
5	20.34(±17.03)	29.48	10.89	68.21(±23.8)	9.13	-	28.63(±6.3)	28.95	0.76
-	17.76(±18.3)	27.24	11.50	69.32(±18.0)	3.15	-	23.45(±25.0)	25.12	0.62

-	17.95(±11.5)	29.97	10.31	66.90(±14.1)	7.11	-	20.93(±38.5)	28.91	0.68
-	21.51(±20.2)	27.45	16.09	57.80(±19.0)	0.00	-	23.36(±37.9)	10.98	1.08
6++	20.11(±8.6)	28.01	15.00	75.17(±14.5)	0.00	-	24.61(±32.0)	22.27	0.59
7	21.37(8.5)	31.40	14.77	69.16(±8.8)	0.00	-	24.87(±18.4)	30.01	0.79
8	24.32(±18.8)	34.33	15.70	60.07(±9.7)	0.00	123.04	24.17(±35.8)	34.70	1.22
9	20.90(±17.2)	28.62	12.44	66.20(±19.2)	1.01		23.14(±23.8)	29.87	0.84
10	22.21(±12.3)	33.45	12.66	62.84(±20.9)	0.00	184.56	25.65(±6.7)	33.53	1.00
11	22.50(±11.0)	33.57	16.02	65.48(±19.8)	0.00		23.73(±17.2)	31.91	0.94
12+++	24.68(±16.0)	34.27	15.08	58.18(±27.3)	0.00	-	23.46(±15.2)	34.43	1.30

W corresponds to the week number from 04/11/2019 to 17/02/2020, numbered weeks indicated the weeks with stem water potential measurements, no numbered weeks indicates that stem water potential was not measured due to cloudiness, + indicates Setting, ++ indicates véraison, +++ indicates harvest, T is the average ambient temperature, Tmin is the minimum ambient temperature, Tmax is the maximum ambient temperature, Pp is the precipitation, RH is the relative humidity, I is the irrigation amount, SR is the solar radiation, ET0 is the reference evapotranspiration, VPD is the average vapor pressure deficit. Values in brackets indicate the coefficient of variation expressed in percentage.

The plant water status analysis was done during the course of 12 weeks with a gap between the Week 5 and 6, because during this period the weather conditions (light rain and cloudiness at midday) were not suitable to measure the maximum level of water stress during this period. The maximum temperature in January and February was 34.3°C and 37.1°C, respectively. High values of vapor pressure deficit (VPD) were calculated for the corresponding higher values of temperature and evapotranspiration (ETo). The highest atmospheric demand was registered at post-véraison, with a maximum in week 8 (ETo = 34.7 mm/week and VPD = 1.22 kPa) (Table 1).

3.2. Descriptive analysis of grape and oenological variables

The results obtained from the juice and wine analysis are summarized in Table 2. Block scale represents the value reported by the winemaker at commercial harvest. As was expected the block values registered for sugar content, TA and yield were between the minimum and maximum values and close to the averages calculated for the 43 target vines.

Table 2. Descriptive analysis of Anthocyanins, Tannins, Sugar content, Titratable acidity and Yield.

	Anthocyanins+ (mg/L)	**Tannins+ (mg/L)**	**Sugar content++ (°Bx)**	**TA++ (g/L)**	**Yield (kg/plant)**
Block scale	-	-	25.00	5.43	4.90
Avg.	590.8	1119.8	23.87	4.50	5.23
Max	817.8	1911.2	26.53	5.83	9.20
Min	323.6	577.4	19.90	3.87	2.01
CV	19.02	26.28	5.41	10.35	34.34

+ Indicates phenolic compounds measured in wine, ++ Indicates chemical analysis measured in juice, block scale is the value reported by the winemaker at commercial harvest, Avg is the average value for the target vines, Max is the maximum value for the target vines, Min is the minimum value for the target vines, CV is the coefficient of variation (%), TA is the titratable acidity (g/L).

The target vines registered high variability for yield, tannins and anthocyanins with CV values of 34.34%, 26.28% and 19.02%, respectively (Table 2). The lowest variability was registered for sugar content (5.41%). Only 3 plants registered a juice sugar content below 22° Bx and 7 plants presented values higher than 25°Brix.

Table 3 indicates the values of SWP along the growing season from November to February (12 weeks). SWP values increased as grapes reached closer to the maturity stage, reaching a maximum water deficit period during Week 11 (post-véraison period). The CV was around 17% with higher values at the beginning of the season (lower water deficit). From week 1 to week 6 the average of SWP varied between -0.49 MPa and -0.73 MPa, showing low to medium limitation in plant water availability before véraison. A slight decrease in water availability is observed in week 5, after the maximum daily temperatures increased significantly in the previous weeks. From the initial phase of véraison (week 6) the steady decrease in average SWP values (from -.732 to -1.090 MPa) indicates a decrease in the plant water status as shown in Figure 2, spatial variability of SWP changed along the season as well as the level of water deficit reached by the plants. Plants with lower values of SWP (higher water deficit) are spatially concentrated in the central, top central and top right areas of the block. This distribution is in line with the topographic inclination of the block and the soil texture analysis.

Table 3. Variation of the SWP per Week during the studied season.

	W_1	**W_2**	**W_3**	**W_4**	**W_5**	**W_6**	**W_7**	**W_8**	**W_9**	**W_{10}**	**W_{11}**	**W_{12}**	**W.d**
	MPa	MPa	Mpa	MPa	MPa	MPa	MPa	MPa	MPa	MPa	MPa	MPa	MPa
Avg.	-0,49	-0,41	-0,49	-0,53	-0,65	-0,73	-0,89	-1,02	-0,87	-1,06	-1,14	-1,09	-0,78
Diff	-	-0,07	0,07	0,05	0,12	0,08	0,16	0,13	-0,15	0,19	0,08	-0,05	-
Min	-0,69	-0,60	-0,74	-0,67	-0,89	-1,02	-1,16	-1,38	-1,17	-1,38	-1,41	-1,39	-0,93
Max	-0,31	-0,23	-0,23	-0,37	-0,34	-0,51	-0,68	-0,66	-0,61	-0,63	-0,65	-0,69	-0,62
CV	-20,45	-20,76	-20,07	-14,95	-20,05	-14,85	-14,38	-18,07	-16,1	-17,87	-14,96	-16,76	-10,77
		Setting				Véraison						Harvest	

W.d is the whole dataset, Avg. is the average value for the target vines (MPa), Max is the maximum value for the target vines (MPa), Min is the minimum value for the target vines (MPa), Diff is the difference between consecutives weeks (MPa), CV is the coefficient of variation (%), W indicates weeks.

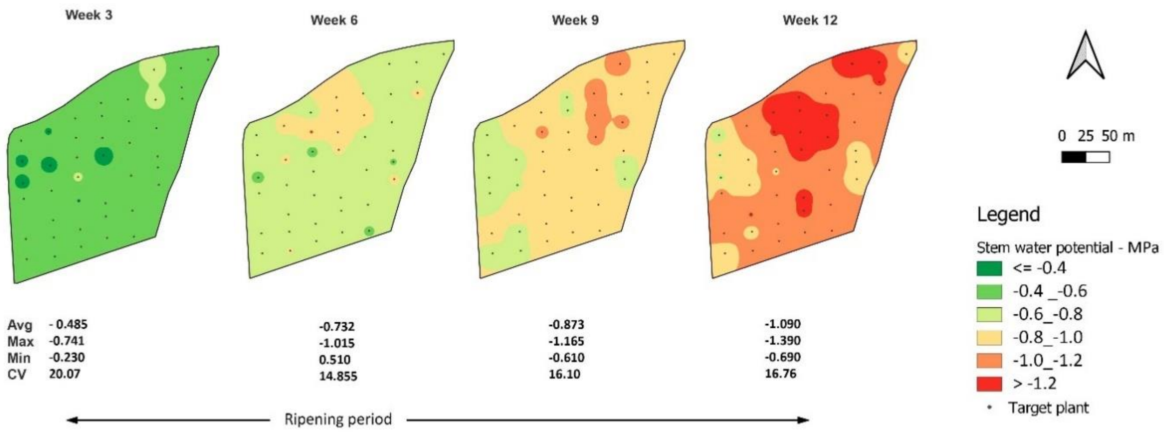

Figure 2. Temporal and spatial variability of SWP for the target vines from pre-Veraison (Week 3) to Harvest (Week 12).

3.3. PCA and correlation analysis

A PCA analysis was done to explore the effect of the SWP measured during 12 weeks over the 43 target plants on grape and wine parameters (Figure 3). The first 2 dimensions explained 53.9% of the variation in the data, with 40.3% explained by Dimension 1 and 12.5% explained by Dimension 2. As expected, anthocyanins and tannins were directly correlated with the Sugar concentrations and indirectly correlated with yield and acidity. The 12-SWP weekly measurements presented different associations with the phenolic compounds (anthocyanins and tannins) showing an effect of the time progression. This time effect is evident in the correlation matrix (Figure 4) where higher correlation values between phenolic compounds and SWP were registered from week 7 onwards. Also, a positive significant correlation was indicated between phenolic compounds and sugar concentration. As anticipated, the total acidity and yield had a strong negative correlation with sugar content and the phenolic compounds.

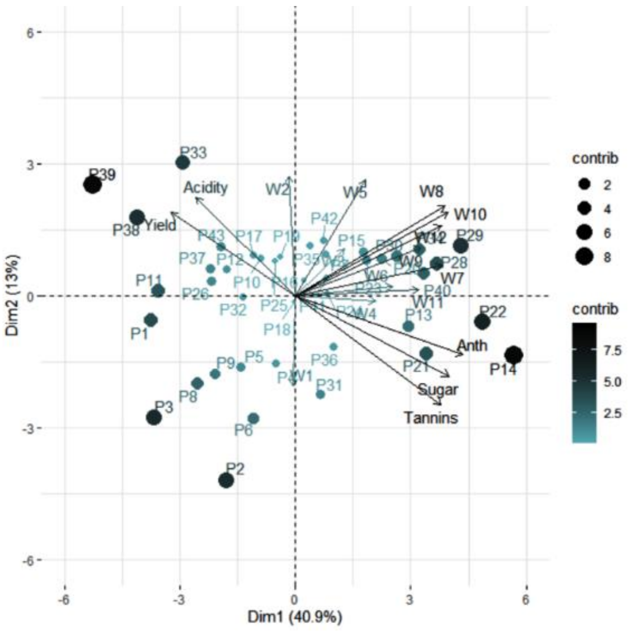

Figure 3. PCA analysis considering all the data set from the 2019 growing season for cv. Cabernet Sauvignon. Nomenclature used: P represent the target plants, W represent SWP for the 12 weeks, Acidity is the titratable acidity (TA in g/L), Sugar (°Balling) is the grape juice sugar content, Anth. is the anthocyanins concentration in mg/L measured in wine, Tannins is the tannin concentration in mg/L measured in wine, Yield is the yield per vine, contrib is the level of contribution each variable had to the dimensions. Size and colour intensity of the circles indicate the relationship of the individuals (target plants) with each factor.

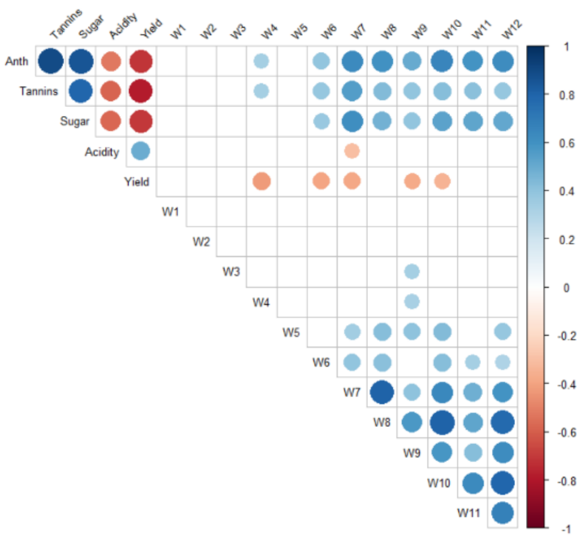

Figure 4. Correlation matrix of the studies variables. W represent SWP for the 12 weeks. Colour and the size of the circles are proportional to the correlation coefficients between the variables.

3.2. Classification of the target vines according to the plant water status

From the SWP measured values along the season, the target plants were grouped according to the level, period, and duration of the waters deficit in 3 classes: i) Class 1 - low water deficit, ii) Class 2 - moderate water deficit and iii) Class 3 - severe water deficit (Figure 5). As benchmarks, the period between week 6 to 8 (véraison) was used to discriminate the plant water status level. The general thresholds indicated for Class 1, 2 and 3 were < 9 bar, between 9 to 12 MPa and > 12 bar, respectively. These values were converted to MPa: > -0.9 bar, between -0.9 to -1.2 MPa and < -1.2 bar for further analysis.

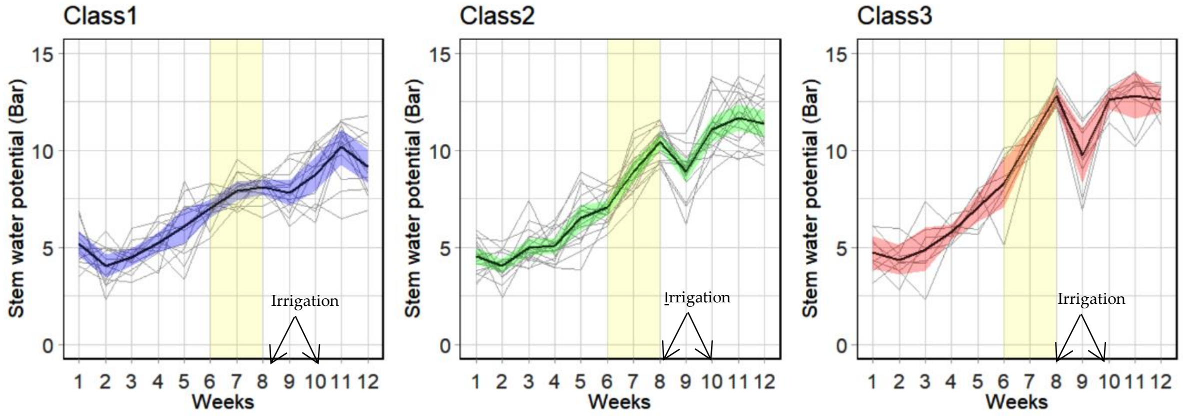

Figure 5. Classification of the seasonal evolution of SWP along the growing season. Yellow area represents the Veraison period and arrows indicate the irrigation events.

The analysis of Figure 5 shows that plants in Class1 have a more homogeneous variation along the season, with a low amplitude of SWP values from Week 1 to Week 12. On the other side, plants from Class 3 presented a greater amplitude of SWP during the season. The progression of water deficit in the plants until véraison is similar in all three classes, however, between week 6 and week 8 (véraison period) the plants in Class 3 rapidly reached the point of severe water deficit (Van Leeuwen et al., 2009). This trend in Class 3 was only diminished for a week, with the first irrigation event, to return rapidly to the same level of water deficiency until harvest (Table 3). The irrigation events had a lighter effect on SWP values in Class 1 and Class 2 than in Class 3, where vine water status was severely affected.

Table 4. Descriptive analysis water, soil, grape and wine variables in the 3 water stress classes

	SWP (Bar)*	**Sand (%)****	**Clay (%)****	**Carbon (%)****	**Anth (mg/L)+**	**Tann (mg/L)+**	**SC (°B)++**	**TA (g/L)++**	**MA (g/L)++**	**Yield (Kg/v)**
					Class 1					
Avg	7.04	24.86	41.00	8.43	523.4	1028.4	23.18	4.55	3.38	5.03
Max	11.80	50.00	46.00	14.00	673.1	1454.3	24.7	5.6	5.4	9.2
Min	2.32	8.00	36.00	6.00	323.6	577.4	19.9	4.1	2.4	8.4
CV	7.63	40.29	8.12	21.18	17.1	26.6	6.0	9.3	27.0	31.4
					Class 2					
Avg	7.89	40.58	8.32	1.78	583.5	1041.2	23.77	4.56	3.72	5.88
Max	13.90	48.00	12.00	2.30	757.7	1464.2	25.4	5.7	5.3	8.3
Min	2.43	34.00	6.00	1.35	440.5	764.0	22.0	4.0	2.6	3.0

CV	7.45	8.31	23.05	13.78	16.10	21.92	3.67	9.07	21.51	28.17
Class 3										
Avg	8.86	39.11	8.89	1.71	711.1	1427.7	25.17	4.29	3.50	3.56
Max	14.10	44.00	12.00	2.03	817.8	1911.2	26.5	5.8	5.1	5.8
Min	2.30	32.00	8.00	1.35	544.3	1049.1	23.7	3.9	2.6	2.0
CV	13.35	9.08	19.84	13.54	12.21	18.46	3.76	14.26	21.75	34.08

[*]Indicates field measurements of SWP (Stem Water potential) average value, [**]Indicates soil analysis (surrounding soil) from each target vine, [+] Indicates phenolic compounds measured in wine, [++]Indicates chemical analysis measured in juice, Avg is the average value for the target vines, Max is the maximum value for the target vines, Min is the minimum value for the target vines, CV is the coefficient of variation (%), TA is the total acidity, MA is the malic acid, SC is the sugar content in grape juice, Anth is the anthocyanins concentration in mg/L in wine and Tann is the tannin concentration in wine.

The box plots presented in Figure 6, gives an indication of the dispersion of the variables inside the water deficit classes and the statistical differences between classes (Wilcoxon Rank Sum test). In general, acidity presented lower differences, no statistical differences among the classes were registered for malic acid and a single difference between Class 2 and Class 3 was registered for titratable acidity (Figure 6). The analysis of anthocyanins, tannins, sugar content and yield, presented significant statistical differences for Class 3 in all cases. For anthocyanins, Class 3 was significantly higher than Class 1 and 2 (Figure 6). The positive skew in Class 3 reveals that 50% of the observations in this group were higher than 711.1 mg/L (Table 4). Likewise, tannins presented significantly higher values in Class 3 (Figure 5). However, Class 3 demonstrates a much smaller 2nd quarter, indicating that 50% of the plants presented a tannin content superior to 1427.7 mg/L and a highest mean value of 1911.2 mg/L (Table 4). The sugar content was similar for Class 1 and 2, however, Class 3 was significantly higher (Figure 5) ranging from 23.7°Brix to 26.5°Brix (Table 4). The yield of the plants in Class 3 was significantly lower than the yield in the remaining 2 classes (Figure 6). In fact, 50% of the plants in Class 3 had a yield lower than 3.56 kg/plant. Conversely, more than 75% of the plants in Class 1 and 2 had a yield superior to 4.26 kg/plant.

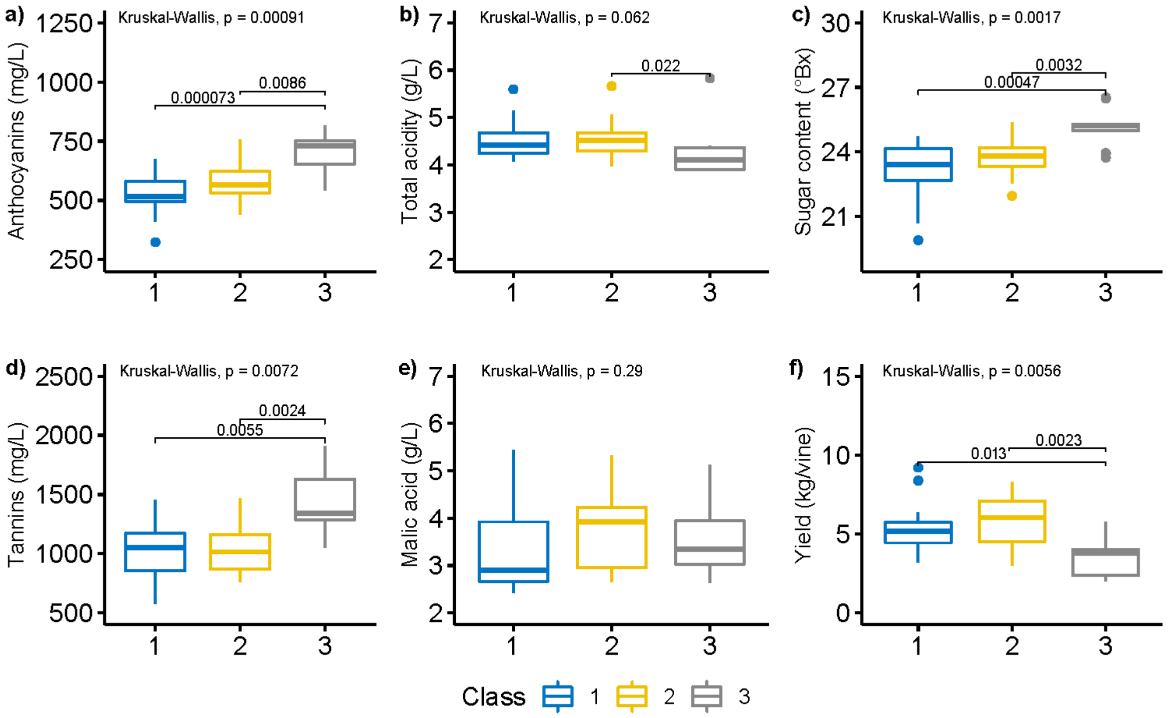

Figure 6. Boxplots of grape and wine parameters within each water stress class, a) Anthocyanins, b) Titratable acidity, c) Sugar content, d) Tannins, d) Malic acid, e) Yield per vine. p-values for Kruskal-Wallis tests indicate a difference in means within a group. Lines with p-values indicate statistically significant (p-value < 0.05) in the Wilcoxon Rank Sum test for respective pairwise differences in means.

4. Discussion

4.1. Temporal variability of SWP

Water potential is a consequence of soil water availability, evaporative demand and vine canopy structure (Van Leeuwen et al., 2006). It is an inclusive measurement that incorporates soil, plant, and atmospheric conditions related to the available water in the plant (McCutchan & Shackel, 2019). Stem water potential is a method usually applied in grapevines to provide an indication of the plant capacity to conduct water from the soil to the atmosphere, throughout the leaves. SWP is a reliable indicator of the early water deficit in plants (Choné et al., 2001).

In this regard, when compared to other methods, SWP has been described as the most convenient way to measure vine water status as an aid in vineyard irrigation management decisions (Williams, 2012; Choné et al., 2000), because it reflects whole vine water status during the day, when the vine is physiological active. In opposition, leaf water status only reflects the water potential of one individual leaf, turning it difficult to relate leaf water potential with leaf transpiration, if it is measured during the day or to photosynthesis if measured at pre-dawn (Van Leeuwen et al., 2006). In our experiment the temporal variability of SWP during the growing season was evident. Water deficit measured by SWP increased along the season, indicating a decrease of the plant water status as a consequence of high atmospheric demand and lower soil water availability. This temporal pattern has been reported in several studies (Hardie and Considine, 1976; Van Leeuwen et al., 2009; Brillante et al., 2016).

The atmospheric demand characterized by VPD and ETo increased along the season reaching maximum values in the post-véraison period where the maximum water deficit was also evidenced by SWP measurements (Table 3 and 4). It has been previously reported that water potential, in drip irrigated vineyards, are more closely correlated to the atmospheric demand (e.g. VPD) than to soil moisture (Rogiers et al., 2012). SWP values registered in this study were positively impacted by the irrigation events (higher SWP values) in the post-véraison period (Figure 5). This finding is consistent with other studies that reported a clear impact of irrigation on plant water status (Chaves et al., 2007; Acevedo-Opazo et al., 2010; Zúñiga et al., 2018; Brillante et al., 2018).

4.2. Spatial intra-block variability of SWP

When viticulture practices, such as vine spacing, training and trellising, shoot positioning, pruning are independently and equitably applied to all plants in a block, soil type and topography are factors with an important role in the natural spatial variability in vineyards (Yu et al., 2020). As temperature increased, the relative humidity diminished and the soil became drier, in the absence of rain and even with two irrigation treatments, consequently plant water status established a clear spatial pattern in our experimental block (Figure 7). Therefore, spatial variability of plant water status was noticed with an average season CV value of 18% (Table 3). Brillante et al., (2016) indicated the existence of big natural spatial variations in grapevine water status within short distances in vineyards. In the mentioned study plant water deficit of Chardonnay grapevines was monitored by stem/leaf water potential in two plots with different soil properties. The singularity of water variation in shallow layers was attributed to soil characteristics affecting soil evaporation, grapevine root density and cover crop uptake. Significant analysis concluded that soil properties is the main factor for plant water status differences between plants over short distances.

Topography was another important factor to explain the spatial variability. Kitchen et al., (2003) has established that topography is a measure to study availability of water in the soil. In accordance with this concept, Van Leeuwen et al., (2004) concluded that the intensity of vine water deficit depends on the water-holding capacity of the soil. In our study, plants with lower SWP were more concentrated at the top central area of the block (figure 7). The top central area is characterized by a steeper slope than the remaining of the block. Plants located in areas with steeper inclination registered less water content than the remaining plants. This result could be associated with a lower penetration of water in slopes due to run-off and soil texture, therefore, a lower level of available water in the soil. In accordance with these results, Koundouras et al., (2006) investigated the influence of site (flood plain, hill slope and plateau) on grape and wine composition from uniformly viticultural conditions of *Vitis. vinifera L.cv*. Agiorgitiko vines. Plant water deficit became significantly higher during grape maturation in grapevines planted on the hill slope plot, suggesting that plant water uptake is dependent on soil capacity to retain and supply water to grapevines. When combining these two factors soil water availability is the major factor of spatial variability of plant water status. In this sense, Ledderhof et al., (2017) in an attempt to assess the relationship between vine water status and yield and berry composition, found that the amount of clay in the soil texture composition is the main driver of variability. This idea is supported by our classification, where Class 1 (Table 4) presented an average value of clay content 5 times higher than Class 2 and 3.

Figure 7 Spatial representation of 3 classes defining the spatial variability of plant water status in the intra-block

4.3. Classification based on plant water status

In our experiment the target plants were grouped into 3 classes according to level, period, and duration of the water deficit using the véraison period as benchmark. Véraison is the beginning of berry ripening and it is in this stage that berries start a variety of physical and chemical changes. According to several authors (Ferrandino & Lovisolo, 2014; Gambetta et al., 2020) sugar accumulation and abscisic acid (ABA) concentration are responsible for berry composition modifications during this period. The resultant immediate effect of the sugar and ABA accumulation in the berry is, first the change of skin colour for red varietals, and then, the softening of the berry and resume of berry growth. Other changes during véraison include slow increase of berry volume, biosynthesis of skin and pulp compounds, an increase of pH and a decrease of acidity. The increase of berry size during ripening starts after the lag phase when the incorporation of proteins aids the stretch of the skin and it peaks at the end of the lag phase (Keller, 2015). The growth is mainly related to the amount of water accumulated in the mesocarp vacuoles and it has been suggested that water is the most important component for berry maturation. Until véraison the xylem flow is the main transporter of water to berries, however at véraison phloem flow becomes the only source of water to grape berries (Keller, 2015). Because of the different effects of water deficit on berry development around véraison, it was hypothesised that véraison is a critical period on the impact of water status on berry development. The proposed classes categorize target vines under different water conditions according the SWP values registered during the season (Table 3).

Grape vine water status can be different among cultivars when evaluated in the similar atmospheric and soil conditions. In some cultivars the plant water potential is not perturbed by soil and atmospheric water conditions and they are referred as "isohydric". The *Vitis vinifera* cultivar Cabernet Sauvignon is a "anisohydric" grapevine that is affected by modifications of soil water content, which decreases the plant water potential as a response to soil water depletion (Myburgh, 2018). Vine water status can be monitored by means of SWP. The level of water deficit can be classified according to the water potential measured on leaves. A significant study on the subject was presented by Van Leeuwen et al., (2009). In the study an investigation into the vine water deficit impact on the overall vintage, average thresholds for plant water status have been proposed: no water deficit > -0.6 MPa, weak water deficit: -0.6 to -0.9 MPa, moderate to weak water deficit: -0.9 to -1.1 MPa, moderate to severe water deficit: -1.1 to -1.4 MPa, severe water deficit: < -1.4 MPa. Similarly, Myburgh, (2018) found exactly the same thresholds in a study defining plant water potential on Cabernet Sauvignon cultivar. In another major study Gambetta et al., (2020) determined that, in some varietals in irrigated and non-irrigated vineyards, SWP lower than -1.5 MPa lead to leaf shedding and risk of vine mortality due to cavitation or turgor loss. Furthermore, the SWP in viticultural management for premium wine is normally targeted between -1.2 to -1.4 MPa.

In our study the proposed classes were determined considering the level of water deficit and the evolution of plant water status from véraison onwards. Therefore, target plants with a SWP below -0.9 MPa at véraison were categorized as Class 1 (moderate water deficit). Plants with a SWP between -9 and -12 were classified as Class 2 (moderate to severe water deficit) and vines with SWP higher that -12 Bar were classified as Class 3 (severe water deficit) (Table 4). Additionally, the level of deficit of the plants in post-véraison until harvest was also considered. Acevedo-Opazo et al., (2010) found that in an experiment of cv. Cabernet Sauvignon under three different regulated deficit irrigation (RDI) treatments, the most effective irrigation treatment optimizing grape composition was the event that restricted SWP to values below -1.2 MPa.

Even though before véraison the plants in the three classes maintain close values of stem water potential, a steady decrease of stem water potential is already perceptible in this period. Furthermore, target plants rapidly reached a medium level of water deficit at véraison, until achieving the maximum deficit four weeks later at harvest. The reason for this rapid water deficit development in the targeted plants is generally related with the immediate increase of VPD, the depletion of soil moisture and the subsequent there could be a possibility of a reaction from the plant opening the stomata, increasing transpiration (Choné et al., 2001; Phogat et al., 2017).

The temporal and spatial variability is more pronounced between véraison and harvest. However, the spatial variability in classes can be partially explained in part by the land elevation, due to the position of target plants in the block and to, the exposition of plants to sun and wind. When target plants are located in a higher inclination receive more sun exposition and are affected by stronger wind, this translates into the plants developing a higher deficit condition than plants located in a flat area of the block, with low sun exposition and less wind. These results reflect those of Brillante et al., (2017) who also found that despite vineyards being irrigated, spatial variability in water content still exists and vineyard irrigation management should be executed differently for the different SWP classes of the block.

Temporal variability in grapevine water status has been well documented (Chaves et al., 2007; Acevedo-Opazo et al., 2010; Zúñiga et al., 2018; Brillante et al., 2018). The evolution of plant water status along the season is a result of soil, weather, and plant management factors. However, the plant water status pattern is that the available soil water progressively contain less water until harvest (Chaves et al., 2007) and that has a determinant impact on grape composition (Acevedo-Opazo et al., 2010). The evaluation of plant water status during the period of water deficit in post-véraison is a complex task. It is dependent of the analysis of changing weather temporal factors and spatial pedological factors. Our water deficit classes were defined following the logic behind the concept of water stress integral proposed by Myers (1988) where a cumulative evolution of water potential along the water deficit period is considered.

4.4. Impact of water stress on yield, grape juice composition and wine phenolics

It has been proved that water deficit has an impact in the physiology of the vines. In this sense, irrigation techniques have been implemented and analysed to modulate vines response under specific water stress conditions. Among these techniques regulated deficit irrigation (RDI) is the most followed by producers and researchers (Ruiz-Sanchez et al., 2010).

As a common rule, grape producers apply water deficit in grapevines in order to improve grape composition and concentration and to control vegetative growth. However, water limitation to grapevines at the end of the growing season has different effects in grape composition depending on application timing and amount. Plant water responses to water deficits is markedly different before and after véraison. Before, berries are more sensitive to alterations in plant water content (Gambetta et al., 2020). In this phase plant water deficit affects the berry capacity of cell division and expansion, and therefore, berry size and structure (Bondada & Shutthanandan, 2012). After véraison, the cuticular berry transpiration decreases progressively and the sugar transport and accumulation increase rapidly and the berry is more resistant to shrivelling (Gambetta et al., 2020).

Our results follow an opposite tendency. Plants which reported lower levels of water deficit (Class 1 and 2) in post-véraison, reported significantly higher yield values (av. 5,46 kg/plant). Conversely, plants from Class 3 with higher water deficit reported a lower yield (3.56 kg/plant). A possible explanation for these results may be the existence of a threshold of plant water deficit that promotes the yield component (berry weight) in grapevines. It can thus be suggested that plant water status in post-véraison is a determinant factor for yield. These results therefore need to be interpreted with caution. This study has been unable to demonstrate a difference in number of clusters between the classes. However, the average plants with a critical water deficit in Class 3 registered clusters with a lower yield (103 g/cluster) in comparison to plants in Class 1 and 2 (av. 136 g/cluster) with a moderate water deficit in post-véraison. As noted by Lovisolo et al., (2010) yield and berry composition depend strongly on vine adaptability to drought. The level of drought recovery in grapevines is related with the cultivar and environmental conditions affecting plant water deficit.

Optimum production of wines is dependent on the grape composition at harvest. As a standard in wineries, measurement of grape parameters is done by means of visual and sensorial evaluation of the grapes and the analysis of total soluble solids (TSS) and acidity (Guidetti, 2010). High sugar, lower acid, rich colour and full varietal fruitiness are criteria for harvesting the fruit at desirable ripeness (Boulton et al., 1999). However, depending on the style and level of composition intended to be achieved there are several potential harvest dates.

Regarding literature about the effects of water deficit on berry juice composition parameters (pH, TA and sugar content), some authors suggest that plant water status derived from deficit irrigation has a substantial impact on soluble solids of grape composition (Esteban et al., 2001; Yu et al., 2020). Others have found no evidence of significant water deficit irrigation effect on grape soluble solids composition of pH, TA (Acevedo-Opazo et al., 2010) and sugar accumulation (Chaves et al., 2007). In our study the values of berry juice composition parameters analysed at harvest demonstrated no significant differences between classes for pH, however, a small decrease of TA was registered in Class 3.

Leeuwen et al., (2009) argues that mild conditions of water deficit are beneficial to grape composition, decreasing malic acid and increasing sugar, anthocyanins and tannins of grape content. In our study the sugar content at harvest was positively correlated with the level of plant water deficit in post-véraison. Grape berry sugar content increases with the level of deficit between classes. The observed increase in sugar could be attributed to the decrease of water content in the berry, therefore berry size, which leads to the increase of sugar concentration in the berry. There are, however, other possible explanations. The chemical composition of grape juice and wine correlation analysis illustrated a strong positive relationship (r= 0.9) between anthocyanins, tannins, and sugar. The analysis demonstrated that anthocyanins and tannins in wine were highly correlated with the sugar concentrations. This supports the idea that sugar may be useful as an indicator of wine phenolic content. A note of caution is due here since further studies should investigate this idea. The impact of water deficit in the increase of Brix° has been reported by Roby et al., (2004). Brix° values were higher for water stressed plants in Class 3, but this was already expected since sugar content is a component to determine Brix°.

4.5. Wine phenolics

The phenolic content and composition of red grapes are the main attributes to wine sensory properties and wine colour. Anthocyanins are phenolic compounds that impart red colour to wine and tannins contributing to the mouth feel properties (Aleixandre-Tudo et al., 2018). Although extensive research has been carried out on flavonoid composition of wines, few studies have investigated the relationship between plant water deficit and flavonoid composition of wines. A study conducted by Downey et al., (2006) suggests that plant water status is a common denominator for flavonoids in wine. In our study a positive relationship was noticed between the increase of water deficit in plants of Class 3 and the increase of anthocyanins content of fermented wines.

Even though some authors argue that wine tannin concentrations can only be altered with higher values of water deficits (Yu et al., 2020), our experiment demonstrated that plant water deficit can be one of the factors that impact tannin and anthocyanin concentration in wines. The experiment shows that tannin and anthocyanin concentration increased 27% and 23%, respectively, from Class 1-2 to Class 3. This finding was also reported by Kennedy et al., (2002) who found an increase of tannin concentration in response to water deficit. In our study, total phenols content in wine was 24% lower in Class 1. A similar result was presented by Nadal & Arola (1995) who reported a decrease of 22% of total phenols in wines produced from irrigated vines as compared to rainfed vines.

In our experiment, the values of tannins, anthocyanins and sugar (brix°) are higher in wines from Class 3, suggesting that canopy temperature had a high influence on the synthesis of these compounds during grape development. These data must be interpreted with caution because other factor could have affected the parameters, such as soil water content. If so, this observation may support the hypothesis that the average temperatures in pre- and post-véraison periods were very high, mainly affecting the plants with lower soil water content located in the steepest areas of the block.

There is still uncertainty, however, whether plant water deficit has an impact on the increase of wine phenolic concentration because of alterations occurring through grape biosynthesis or due to the enhanced concentration. It is also uncertain how this change will influence phenolic extraction during fermentation (Casassa et al., 2015). Ojeda et al., (2002) confirmed two types of berry responses to water deficit: an indirect and always positive effect on the concentration of phenolic compounds due to berry size reduction, and a direct action on biosynthesis that can be positive or negative, depending on the type of phenolic compound, period of application, and severity of the water deficit (Kennedy et al., 2002; Ojeda et al., 2002). Other than a direct stimulation of biosynthesis, water deficits could also increase the concentrations of skin tannins and anthocyanins due to the differential growth responses of the skin and inner mesocarp tissue to water deficits, resulting in greater skin mass and relative skin mass per berry, and therefore greater amounts of skin-localised solutes (Roby et al., 2004). Further studies, which take these variables into account, will need to be undertaken.

5. Conclusion

The present study highlights the complex response of wine phenolic content (anthocyanins and tannins) and grape parameters (sugar content, TA, MA, and yield) to the temporal and spatial variability of plant water status in a commercial block induced by natural conditions of topography and soil characteristics.

In this study all target plants were treated with the same viticultural management practices and only the natural effects were accountable to the spatial variability. Plant water status variability occurred along the season, suggesting soil texture and topography as key factors for this effect. Plant water status influenced changes in the concentration of phenolic compounds in the final wines (anthocyanins and tannins) and grape parameters particularly yield, and sugar content.

The results of this investigation show that plants located in a higher inclination, receive more sun exposition and are affected by stronger wind and develop a higher deficit condition, in contrast to plants located in flatter areas of the block, with low sun exposition and less wind exposure. This results indicate that variation of soil texture between the different water deficit classes can exist. The results support the idea that vineyard irrigation management should be executed differently for the different SWP classes of the block.

The research presented here may help to understand and quantify how spatial variability is naturally distributed in a vineyard and ultimately impacts the style of wine that is possible to produce from a section or the entire block. Further studies at intra-block scale are needed to understand the implications of plant water status in the phenolic content and composition of the final wines as well as the drivers associated with the variability.

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