Water deficit differentially impacts the yield and the accumulation of grape metabolites of new varieties tolerant to fungi

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Abstract

The use of resistant varieties is a long-term but promising solution to reduce chemical input in viticulture. Unfortunately, insufficient attention is paid by the breeders to the adaptation of these varieties to climatic changes, notably to increased water deficit (WD). Thus, prior to the adoption of such varieties by the wine industry in Mediterranean regions, there is a need to consider their suitability to WD. This study aimed to characterize the drought-strategies adopted by 6 new resistant varieties in comparison to Syrah, under a semi-arid Mediterranean climate. A gradient of WD was applied in the field and controlled through plant measurements at the single plant level, from 2019 to 2021. Bunches were harvested at the arrest of berry phloem unloading. The impacts of WD on berry size and composition (sugars, organic acids and potassium), were assessed at this specific stage. Under well-watered conditions, berry metabolites concentrations differed among the genotype, while yield, berry weight and accumulated pre-dawn water potential were mainly related to year. WD differentially reduced the berry size and the accumulation of primary metabolites at berry levels, but it little changed their concentrations in the fruits at the ripe stage.

Introduction

Climate change will lead to an increase in air temperature and reduction in rainfall regime, intensifying climatic demand and water deficits (Cardell et al., 2019; Santillán et al., 2020), which in turns will affect grapevine production and phenology, with negative impacts on grape and wine quality (Van Leeuwen et al., 2019). In addition, climate change may also increase disease pressure, leading to further yield and quality losses, besides increasing costs and reducing vineyard sustainability due to increased vineyard spraying (Salinari et al., 2007). Therefore, adopting new resistant varieties, may be a long-term solution for such future conditions, mainly by reducing diseases consequences.

Many breeding programs have been developed to attend such demand, in Europe (mainly in Germany, Italy, Switzerland and France) and abroad (Brazil, USA, China and Japan) (Yobrégat, 2018). However, up to now, most of the breeding programs of new resistant varieties do not consider drought tolerance as a primary attribute for selection. Characterizing and understanding the behaviour of these new varieties under WD conditions is a crucial point for their adoption in viticultural regions exposed to drought risks.

In this context the objectives of the present work were to study a set of new resistant varieties issued from INRAE breeding program and to describe and globally analyse the effects of water deficit on vine development, yield components and berry metabolites. Such characterization and analysis will provide a first set of data to compare these new varieties in terms of their suitability to regions where water supply could be limiting. This report presents the results regarding yield components, primary metabolites and potassium concentration in berries.

Materials and methods

The experiment was conducted at the INRAE experimental unit of Pech Rouge, in Gruissan, France in 2019, 2020 and 2021 seasons. Varieties studied consisted of two Bouquet (B1 and B2), two ResDur (RD1 and RD2),

two sugar-less Bouquet (SL1 and SL2) (Bigard et al., 2022) and one *V. vinifera* variety Syrah (Sy) as a control. All varieties coded with number '1' and '2' indicate vines producing white and red grapes, respectively. A total of 210 plants were followed (30 plants per genotype), where half of the plants, per genotype, were irrigated from flowering to harvest (40 L/week per plant). Water status and physiological measurements (leaf gas exchange) were performed weekly from flowering to harvest (pre-dawn water potential - Ψb) and 3 points of plant nitrogen status (SPAD) were conducted (flowering, bunch closure and veraison), for all plants. The accumulated Ψb (accΨb) was estimated for each plant as the area under the curve of the evolution of Ψb over time divided by the number of days of the period. All plants were then sorted in function of their accΨb into four different water level classes: mild WD (accΨb $\geq$ -0.3 MPa), moderate WD (-0.3 MPa > accΨb $\geq$ -0.6 MPa), high WD (-0.6 MPa > accΨb $\geq$ -0.8 MPa) and severe WD (accΨb < -0.8 MPa). The accumulated Ψb was calculated from flowering to harvest (accΨb) and from veraison to harvest (accΨb_ver).

Harvest date was defined at phloem unloading arrest, stage when berry reaches its maximum volume (Vmax) (Bigard et al., 2020). Vmax was estimated by Archimedes' method (Torregrosa et al., 2008) in 2019 and 2020 seasons and by image analysis in 2021 season. Image analysis consisted in counting the number of pixels per picture using ImageJ software (Lopes & Cadima, 2021). Then, all plants were individually harvested. The yield and number of clusters (Nb_clusters) per plant were directly assessed. A sample of 200 berries per plant were randomly weighted for assessing berry weight (BW). Then, the berries were pressed and the juice was analysed by HPLC analysis for soluble sugars: glucose (Glu) and fructose (Fru), organic acids: malic (H2M) and tartaric (H2T) acids, and potassium (K), as described previously (Alem et al., 2021; Bigard et al., 2020).

All variables were represented in a principal components analysis (PCA) and followed by an ANOVA and Tukey test (p-value < 0.05) to test the effects of genotype, year, water deficit and their interaction. The proportion of variance explained by each factor was estimated dividing the sum of squares for each group by the total sum of squares (η^2). Graphical processing and statistical tests were performed using R studio software (R Core Team, 2021).

Results and discussion

The accΨb during the season, per variety, was mainly explained by the year (η^2 of 57%) and less by the genotype (η^2 of 15%) (data not shown). Higher values were seen in 2019 (-0.36 MPa) and 2020 (-0.33 MPa) and lower in 2021 (-0.67 MPa), indicating a higher water deficit in 2021. This follows the same trend of the climatic demand (rainfall- ETo) of the season (April to October), which was stronger in 2021 (-716 mm) when compared to 2019 and 2020 (-596 mm and -539 mm, respectively). In general, SL1, RD2 and B2 showed higher accΨb values, and RD1 and Syrah lower, with B1 and SL2 showing intermediate values. However, there are other factors besides climate and genotype that may have affected accΨb, as soil water-holding capacity, soil depth and development of root system and canopy (Deloire et al., 2005; van Leeuwen et al., 2004).

A PCA explaining 60% of the variability is shown in figure 1, in which the first (Dim1) and second (Dim2) components explained 38.5% and 21.4% of the variability, respectively. Dim1 was mainly accounted by the years (fig.1b) and it was related at a higher extent by the accΨb, BW and H2T, and at a lower extent by yield and K (fig. 1a). Overall, 2021 showed lower accΨb and BW, and higher H2T than 2019 and 2020, indicating that more severe WD reflected in smaller berry size and higher H2T concentration.

Dim2 was related at a higher extent by Glu and at a lower extent by Fru and H2M (fig. 1a), and it was mainly accounted by the genotype factor, clearly separating the sugar-less genotypes (SL1 and SL2) (fig. 1b).

The effects of WD on yield formation depends on timing and intensity of WD. Number of clusters and the number of berries per cluster is defined by the previous year conditions, thus strong WD around flowering will decrease the yield for the next season (Guilpart et al., 2014; Levin et al., 2020). While, berry weight is mainly defined by the conditions occurring from anthesis to harvest of the same year (Levin et al., 2020; Ojeda et al., 2001; Wang et al., 2003). This may explain the orthogonal positioning of number of clusters and yield variables in relation to Dim1, and the strong correlation of BW and accΨb.

Interestingly, concentrations of berry metabolites (Glu, Fru, H2M) was not correlated with the water deficit factor (except to H2T), and most of berry variables were orthogonal to both axes. Such results are also seen when performing an ANOVA for berry metabolites concentration with genotype, year and water level as factors (all factors and interactions were significant, p-value < 0.05), where genotype had the highest proportion of variance explained on soluble sugars, organic acids and K (η^2 of 54%, 63% and 66%, respectively).

Despite differences observed for soluble sugars between water levels for some varieties in some years (table 1), such differences do not follow a clear trend, showing either an increase, decrease or no differences at all between

the severe and mild or moderate WD (table 1). Similar results were also observed for potassium, and organic acids (H2M + H2T).

The lack of response of berry soluble sugars and K concentration to water deficit agrees with previous works (Mirás-Avalos & Araujo, 2021; Pastenes et al., 2014), and opposes to other studies that observed either an increase (Cocco et al., 2020; Villette, 2020) or decrease (Wang et al., 2003) of berry sugar and K under WD. It also shows the importance of well defining sampling date (harvest) in function of a precise physiological or phenological development point. Harvesting at phloem unloading arrest, is as a way to decouple the effects that water deficit may have on metabolites biosynthesis from those of concentration, that usually takes place after Vmax, due to berry shrivelling (Alem et al. 2021). Furthermore, when analysing the same variables in a per berry basis (considering berry size), we can note a decrease in the quantity of berry primary metabolites and K (data not shown), highlighting the negative effect that WD has on plant carbon gain and on the translocation of those components into the fruit (Etchebarne et al., 2009; Pastenes et al., 2014; Wang et al., 2003).

Nevertheless, to define whether a genotype is more or less performant under WD conditions, it is necessary to measure different indicators that combine reproductive (yield and grape quality) and vegetative (annual and perennial organs) variables. Therefore, experiments are underway to include physiological and vegetative variables and berry secondary metabolites.

Conclusion

From a broad set of genotypes studied during 3 seasons, WD was inversely related to BW and metabolites quantity per fruit (soluble sugars, organic acids and potassium). However, when metabolites were expressed in concentration, no direct link with WD was observed, and differences were mainly genotype dependent. Yet, we could observe that the sugar-less trait was maintained regardless of WD level.

The differentiated effects on the concentration of molecules of interest versus their level of accumulation per plant or cultivation area are differently appreciated by the oenologist and the winegrower. From a strictly "oenological" point of view, the weak effect of drought on the composition of metabolites can appear as a positive factor. But in terms of profitability, for the winegrower, the reduction of berry components coupled with the decrease in yield, can lead to a considerable lost in the total production of fruit and also of molecules of interest.
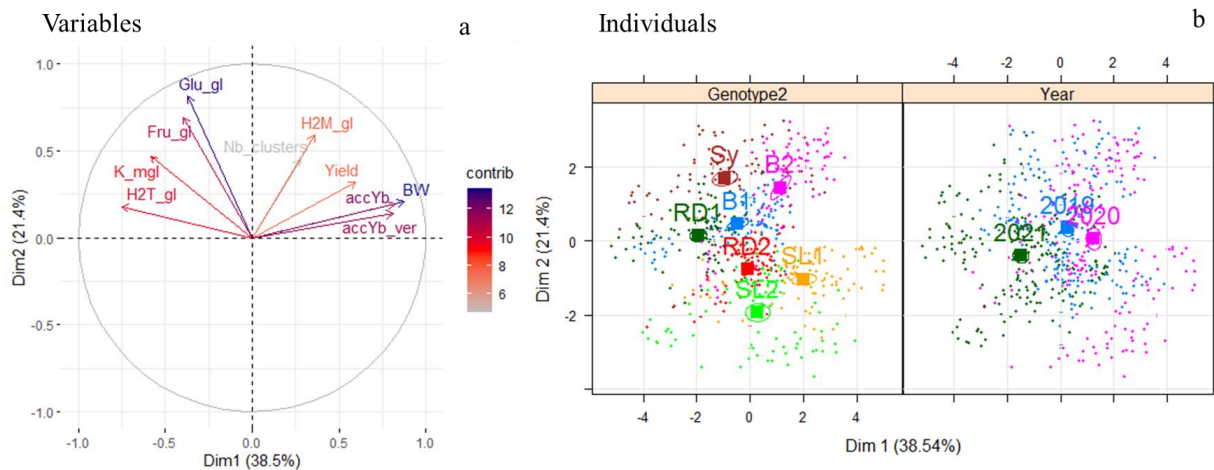

Figure 1. PCA for flowering to harvest (accΨb) and veraison to harvest (accΨb_ver), yield components, berry primary metabolites and potassium (a) for 6 resistant varieties and Syrah, from 2019 to 2021 (b), Gruissan – France.
Yield components (yield and berry weight) are in grams, berry primary metabolites (Glu, Fru, H2M and H2T) are in g/L and potassium (K) is in mg/L.

Table 1. Soluble sugars concentration means and standard deviations per water level after veraison for 6 resistant varieties and Syrah, from 2019 to 2021 Gruissan – France.

Year	WD	Genotypes						
		B1	B2	RD1	RD2	SL1	SL2	Sy
2019	Mild	n.a.	n.a.	n.a.	n.a.	218 ± 16 [ns]	n.a.	n.a.
	Moderate	251 ± 10 [ns]	253 ± 16 [ns]	267 ± 15 [ns]	221 ± 7	223 ± 10	226 ± 8 [ns]	271 ± 20 [ns]
	High	249 ± 13	254 ± 29	269 ± 8	n.a.	n.a.	220 ± 12	247 ± 26
	Severe	n.a.	n.a.	274 ± 12	n.a.	n.a.	n.a.	247 ± 17
2020	Mild	233 ± 33 [ns]	249 ± 8 [a]	n.a.	205 ± 5 [ns]	194 ± 18 [ns]	n.a.	n.a.
	Moderate	254 ± 11	258 ± 12 [b]	244 ± 16 [a]	208 ± 6	190 ± 12	162 ± 15 [ns]	225 ± 17 [ns]
	High	n.a.	n.a.	235 ± 13 [ab]	n.a.	n.a.	152 ± 5	228 ± 29
	Severe	n.a.	n.a.	219 ± 15 [b]	n.a.	n.a.	153 ± 8	n.a.
2021	Mild	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Moderate	256 ± 6 [b]	241 ± 8 [a]	233 ± 5 [b]	216 ± 7 [a]	217 ± 7 [ns]	215 ± 5 [a]	
	High	259 ± 3 [ab]	235 ± 16 [ab]	230 ± 9 [b]	203 ± 10 [b]	220 ± 10	211 ± 11 [a]	248 ± 27 [ns]
	Severe	266 ± 8 [a]	227 ± 10 [b]	247 ± 8 [a]	200 ± 16 [b]	221 ± 12	197 ± 6 [b]	255 ± 15

Different letters, in the same column, indicate statistical different between water level within each year and genotype (ANOVA + Tukey test, p-value < 0.05). 'ns' indicates not significance. 'n.a.' indicates not applicable.

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