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Carbon trade-offs in the fruits of fungus-tolerant *Muscadinia* × *Vitis* hybrids exposed to water deficit

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ABSTRACT

Adopting disease-tolerant grapevines is an efficient option to implement a smarter management strategy limiting the environmental impacts linked to pesticide use. However, little is known on their production of fruit metabolites regarding expected future climate fluctuations, such as increased water shortage. Moreover, previous studies about how water deficit impacts grape composition, lack accuracy due to imprecise timing of fruit sampling. In this study, we phenotyped six new fungus-tolerant genotypes exposed to varying water status in field-grown conditions. The accumulation of water, main cations, primary and secondary metabolites were precisely monitored at the arrest of phloem unloading in fruits, which was targeted at the whole cluster level. The goal was to decipher the effects of both genotype and water deficit on the allocation of carbon into soluble sugars, organic acids, amino acids and anthocyanins. The results revealed that the effect of decreased water availability was specific to each berry component. While fruit sugar concentration remained relatively unaffected, the malic/tartaric acid balance varied based on differences among genotypes. Despite showing contrasted strategies on carbon allocation into berry metabolites, all genotypes reduced fruit yield and the amount of compounds of interest per plant under water deficit, with the extent of reduction being genotype-dependent and correlated with the response of berry volume to plant water status. This first set of data provides information to help reasoning the adaptation of these varieties according to the expected risks of drought and the possibilities of mitigating them through irrigation.

1. Introduction

Grapevine is one of the main fruit crops, with a total surface area of 7.3 million hectares worldwide (OIV, 2023b). However, viticulture faces the challenge of coping with increased air temperature and fluctuated rainfall due to climate change, while at the same time is expected to reduce the use of pesticides. It is estimated that viticulture accounts for 60% of pesticide use in Europe, mainly to control fungal diseases, such as downy and powdery mildew (Muthmann and Nadin, 2007). In this context, the introduction of disease-tolerant grapevine genotypes is a solution with a high potential for achieving a more sustainable and smart grape production.

Berry composition is important in defining grape and wine quality and mainly depends on the balance of primary and secondary metabolites and inorganic cations. During the first phase of development, berries grow due to cell division and expansion, accumulating mostly tartaric and malic acids (Conde et al., 2007). The second growth phase is marked by the cessation of xylem nourishment and a shift of phloem unloading from symplasmic to the apoplastic pathway (Zhang et al., 2006). This shift is accompanied by an intensive importation of sugars and the activation of secondary metabolites biosynthesis, such as anthocyanins. During berry development, the concentration of metabolites varies, depending on their synthesis, respiration rates and the water fluxes in and out of the berries (Bigard et al., 2018; Keller et al., 2015). Berry growth stops at the arrest of phloem unloading, which coincides with maximum water and sugar contents in the grapevine fruit (Shahood et al., 2020). After this stage, berries shrivel as they are unconnected from vines, but are still prone to water loss and to further concentrate the solutes.

Water deficit is a major factor underlying berry metabolite accumulation through its effects on berry cell expansion and berry size (Ojeda et al., 2001), on berry water balance (water inflow and berry transpiration), carbohydrate supply (photosynthetic activity and source: sink competition) (Pastenes et al., 2014) and activation of secondary

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metabolites biosynthesis through hormonal gene regulation (Rienth et al., 2021). Severe WD invariably alters berry size and composition, by strongly reducing carbon assimilation and water transport, which in turn lower sugar imports (Pastenes et al., 2014) and anthocyanins biosynthesis in berries (Ojeda et al., 2002). Mirás-Avalos & Intrigliolo (2017), suggested that decreases in leaf water potential (up to a threshold of −1.3 MPa of stem water potential) had positive effects on total soluble solids (TSS) but decreased total acidity, while the effects on anthocyanins were highly dependent on genotype. However, most previous studies phenotyped populations of berries randomly sampled and often surpassed the point of maximum contents in water and soluble solids. Without precise scaling of fruit physiological ripeness, there is a risk of confusing the effects of WD on both the volume of the berry (due to reduced cell-expansion and/or dehydration) and the actual biosynthesis and import of metabolites. Tracking individual fruit growth through non-destructive methods, such as time-lapse RGB imaging (Daviet et al., 2023) is then of great importance, but very difficult to implement in field trials.

In the present study, we investigated whether decreased carbon assimilation and water absorption in grapevines under water deficit alter the allocation of carbon among berry primary metabolites (soluble sugars, malate, tartrate, amino acids) as well as between berry primary and secondary metabolites. The aim was to elucidate how new disease-tolerant genotypes prioritize the production of specific metabolites over others during stress conditions. In a former study, we characterized and compared their different plant acclimation strategies to WD at leaf and plant levels and proposed an original methodological approach to reduce experimental noise commonly present in open-field experiments (Wilhelm de Almeida, Pellegrino, et al., 2023). The first strategy was to consider each vine as a single biological replicate, as a way to avoid mixing plants displaying different water status. Secondly, the physiological ripe stage, i.e. when phloem unloading into berries stops, was targeted at the cluster level for all genotypes and water treatments, to reduce biases related to the dual effect of WD on metabolite synthesis and berry shriveling (Bigard et al., 2019; Shahood et al., 2020). More precisely, the goal of this study was to decipher genotype and WD effects on the allocation of carbon toward soluble sugars, organic acids, amino acids and anthocyanins.

2. Material and methods

2.1. Plant material and growing conditions

Experiments were performed with field-grown vines from 2019 to 2021 at the INRAE experimental center of Pech Rouge, Gruissan, France (43.14° North | 3.14° East), under a Mediterranean climate. A total of 6 fungus disease-tolerant genotypes deriving from *Vitis vinifera* L. × *Muscadinia rotundifolia* hybrids were studied. The genotypes included (i) 3176N and 3159B, red and white fruited genotypes, respectively (grafted onto 140Ru and planted in 2012), (ii) Artaban and Floreal, red and white fruited genotypes, respectively (grafted onto 110R and planted in 2015) and (iii) G14 and G5, red and white fruited genotypes, respectively (grafted onto 140Ru and planted in 2015). These last two genotypes carry the sugarless trait, previously reported in Ojeda et al. (2017) and described by Bigard et al. (2022). The *V. vinifera* var. Syrah was used as a control (grafted onto 140Ru and planted in 2012) (Wilhelm de Almeida, Pellegrino, et al., 2023; Wilhelm de Almeida, Torregrosa, et al., 2023).

A total of 30 plants per genotype were individually phenotyped, where half were manually irrigated (I) from flowering until harvest, while the other half was not irrigated (NI) and exposed to water deficit (WD), which fluctuated yearly. The same individuals were followed from year to year. The water supply consisted of 20 L per plant applied once a week in 2019 and 2020 (i.e. 96 mm year $^{-1}$) and twice a week (3–4 days apart) in 2021 (i.e. 184 mm year $^{-1}$) for each individual plant. More details and information regarding the experimental design are described in (Wilhelm de Almeida, Pellegrino, et al., 2023).

2.2. Plant water status and sampling strategy

Leaf predawn water potential (**Ψ**b) was weekly monitored (at least 48 h after irrigation) for all 30 plants per genotype, from flowering until final fruit sampling. The accumulated **Ψ**p from flowering to harvest (Acc-**Ψ**p) was then estimated for each plant in each year as the area under the curve of **Ψ**p over time. The Acc-**Ψ**p served as the reference variable to integrate variabilities linked to plot, vine age and rootstock. Besides the Acc-**Ψ**p, the fruit carbon isotope composition (δC13) was also analyzed to assess the plant water status (Couchoud et al., 2020). For this analysis 6 plants per genotype per year were selected to cover a range of Acc-**Ψ**p.

Harvest was performed when the physiological ripeness stage was achieved. At fruit level, the physiological ripeness is defined as the point at which phloem unloading ceases and berries reach their maximum water and solute contents (Bigard et al., 2019; Shahood et al., 2020). The determination of this stage was performed at the cluster level by monitoring the cluster volume increase of three clusters per genotype, weekly, by Archimedes method (Torregrosa et al., 2008), in 2019 and 2020. In 2021 the grape volume increase was followed in 6 plants per genotype (3 irrigated and 3 non-irrigated) by image analysis as described previously (Wilhelm de Almeida, Pellegrino, et al., 2023; Wilhelm de Almeida, Torregrosa, et al., 2023). All clusters per plant were harvested when cluster volume stopped increasing.

2.3. Metabolites analysis

All primary and secondary metabolites assayed, as described below, were expressed in mol of C equivalents per volume (mol C-eq L^{-1}) and per berry (mol C-eq berry^{-1}). The former takes into account the accumulation of carbon and water into fruits (refer here-in as 'concentration'), while the latter considers the quantity of metabolite accumulated per fruit (refer here-in as 'content').

The C equivalent conversion was done considering the respective molecular masses (MM) of each metabolite and the number of atoms of carbon per molecule (*N° of C*) (Eq. (1)):

$$Metabolite\ (mol\ or\ mmol\ Ceq/L) = [Metabolite]\ (g\ or\ mg/L\ ) \div MMg\ or\ mg/mol) \times N°\ of\ C \quad \text{EQ.1}$$

The metabolites content per berry was estimated from berry weight and must density, which was recalculated in function of soluble sugars (SS) concentration (density (g/mg) = 0.0004 × SS (g/L) + 1.0056) from tabulated values of the Standard and technical documents of the International Organisation of Vine and Wine (OIV, 2023a).

2.3.1. Primary metabolites and main cations

At the physiological ripe stage, 200 berries per plant were randomly sampled and weighed. Then, their juice was extracted and centrifuged for later composition analysis as described in Wilhelm de Almeida, Torregrosa et al. (2023). Glucose (Glu), fructose (Fru), tartaric (H2T) and malic (H2M) acids concentrations were analyzed by high-performance liquid chromatography (HPLC) and UV detector as reported in (Bigard et al., 2019). Amino acids (aa) and ammonium concentrations were analyzed as in Wilhelm de Almeida, Torregrosa et al. (2023). The yeast assimilable nitrogen (YAN) was calculated as the sum of amino acids and ammonium concentrations. C equivalent conversion was done for Glu, Fru, H2T and H2M, using MM of 180, 180, 150, 134 g mol^{-1}, respectively. N° of C was 6 and 4 atoms for SS and organic acids, respectively. For amino acids, an average MM of 136.9 g mol^{-1} and N° of C of 5.35 were used for all 20 proteinogenic nitrogen compounds mostly found in grapevine berries as proposed in Wilhelm de Almeida, Torregrosa et al. (2023). Lastly, the main fruit inorganic cations concentrations, K^+ and Ca^{++} were analyzed by HPLC and measured

using a conductometer, as described in (Bigard et al., 2020). Cation concentrations were expressed in mol per volume, considering their respective molecular mass (MM: 39.1 and 40.08 g mol^{-1}).

2.3.2. Anthocyanins

For the four red fruited genotypes, the total extractable anthocyanins concentration was measured as described in (Bigard et al., 2019). The anthocyanins conversion to mol of C-eq per volume was done considering the MM and the N° of C atoms of malvidin-3-O-glucoside (331.3 and 17 respectively) as in Alem et al. (2021).

2.4. Statistical analysis

All graphical processing and statistical tests were performed using R studio software. The total average yield, SS (Glu + Fru), H2M, H2T, total acidity, YAN and anthocyanins produced per plant over the period from 2019 to 2021 were calculated, and later compared with genotype and irrigation treatment as factors (ANOVA, p-value <0.05).

To evaluate the response of berry weight and of each berry component (in concentration and in content per berry) to Acc-**Ψ**p variations, multiple linear regressions were fitted considering each plant as one biological replicate (Eq. (2)).

$$Variable = Acc-\Psi_p + Acc-\Psi_p : Genotype + Genotype + Year + Genotype : Year + error \quad \text{EQ.2}$$

Slopes were settled to vary only with genotype because of the low range of Acc-**Ψ**p variations in 2019 and 2020. However, intercepts were settled to vary not only with genotype, but also with year and the interaction between genotype and year. The contribution to the model of the slopes ('Acc-**Ψ**p'), of the effect of Genotype on the slopes ('Acc-**Ψ**p: Genotype') and of the effects of Genotype, Year and their interaction ('Genotype:Year') on the intercepts (Acc-**Ψ**p = 0) were tested for berry weight and for each berry components including Glu, Fru, H2M, H2T, amino acids, anthocyanins and K^+. For Ca^{++} (only measured in 2021) the effects of Genotype on the intercept and slopes were also tested. The proportion of variance explained by each factor (η^2) was estimated as in Wilhelm de Almeida, Pellegrino et al. (2023).

Multiple comparisons were performed with Bonferroni adjustment. The significance levels of 0.001, 0.01 and 0.05 are indicated by '***', '**', '*', respectively, while no significance is represented by 'ns'. To decipher the genotypic and pluriannual responses to WD, multivariate analyses (PCA) were conducted considering the estimated values of the variables at moderate (Acc-**Ψ**p = −15 MPa) and high WD (Acc-**Ψ**p = −40 MPa).

3. Results

3.1. Weather conditions and plant water status across seasons

Weather (temperature and rainfall) conditions varied throughout the three years of the experiment. The 2021 season had three days under extreme temperatures (daily Tmax >35 °C), while 2019 had two days and 2020 none. In addition, 2021 season was characterized as the driest, showing the lowest climatic water balance from April to October ($\sum$Rainfall - $\sum ET_0$) of −716 mm, while the highest, of −596 mm was reached in 2019 (Wilhelm de Almeida et al., 2023b). Accordingly, the lowest Acc-**Ψ**p and highest δC13 were reached in 2021 season (−37.9 MPa, −24.9 ‰), while the highest in 2019 (−19.4 MPa, −26.6 ‰) and 2020 (−18.0 MPa, −26.7 ‰). Strong correlations were found between Acc-**Ψ**p and δC13 for all combinations of years x varieties x treatments (r^2 = 0.72, p.value = 0) (Fig. S1).

Water status also varied among the varieties. After three years of experiment, the sum of Acc-**Ψ**p was the highest (lowest WD) in G5 and Artaban, and the lowest (highest WD) in Syrah and Floreal (Table 1). The highest relative difference between NI and I vines was seen in both sugarless genotypes, reaching −43% and −45% in G14 and G5 respectively. Artaban displayed the lowest relative difference between NI and I vines (−20%) (Table 1).

3.2. Genotype and irrigation effect on berry components after three successive years

In order to assess the effects of irrigation on the general production per plant, the accumulated values of Acc-**Ψ**p (MPa), yield (kg per plant), soluble sugars, H2T, H2M, YAN and anthocyanins (in g per plant), were calculated as the sum of values in 2019, 2020 and 2021.

After three years of experiment, both genotype and irrigation had an important effect on the total accumulated values of yield and metabolites per plant. Globally, 3176N presented the highest yield and metabolites values per plant, while those variables were the lowest for Floreal. An exception was observed for YAN values, which were the highest in Syrah and the lowest in 3159B, Artaban and G14 (Table 2).

For all varieties, NI vines showed lower average accumulated values of yield per plant (−16%, 1.2 kg), SS (−17%, 250 g), H2M (−27%, 4.1 g), H2T (−13%, 5.7 g), total acidity (−18%, 101 mEq), YAN (−23%, 0.1 g) and anthocyanins (−10%, 1.1 g) per plant. The most affected genotype was Floreal, for which relative differences between NI and I vines varied from 28% for H2T to 46% for H2M. Although G14 showed low reductions between NI and I vines for most berry components, it displayed the greatest loss in anthocyanins accumulation per plant (−24%) (Table 2).

Table 1
Estimated marginal means, standard error (SE) and relative difference (Rel. Diff. %) between non irrigated (NI) and irrigated (I) treatments for the accumulated pre-dawn water potential (Acc-**Ψ**p), for 6 fungus disease-tolerant genotypes and Syrah, from 2019 to 2021, Gruissan - France.

	Acc-**Ψ**p (MPa)							
	I		NI		Relative diff. (%)	G	I	G:I
	Av	*SE*	Av	*SE*		***	***	***
Syrah	−81.6[a]	*1.3*	−102.4[ab]	*1.4*	−25	*a*		[a]
3176N	−58.3[cd]	*0.9*	−73.5[d]	*0.9*	−26	*d*		[a]
3159B	−61.4[bc]	*1.0*	−82.3[c]	*0.9*	−34	*c*		[a]
Artaban	−54.4[de]	*0.5*	−65.3[e]	*2.8*	−20	*e*		[a]
Floreal	−78.2[a]	*1.1*	−107.1[a]	*1.6*	−37	*a*		[a]
G14	−68.0[b]	*1.8*	−97.3[b]	*3.0*	−43	*b*		[a]
G5	−49.9[e]	*0.6*	−72.5[d]	*2.0*	−45	*de*		[a]
Mean	−64.5	*1.2*	−85.8	*1.7*	−33			

[a] Relative difference was calculated as Rel. Diff. % = (NI–I)/I * 100). '***', '**', '*' indicates 0.001, 0.01 and 0.05 significance levels and 'ns' indicates no significance. Different letters in the same column indicate statistical differences among genotypes within treatment (superscript letters) and regardless of treatment (normal case letters) (Bonferroni adjustment).

Table 2
Estimated marginal means, standard error (SE)) and relative difference (Rel. Diff. %) between non irrigated (NI) and irrigated (I) treatments for the accumulated values of yield, soluble sugars (Glu + Fru), malic acid (H2M), tartaric acid (H2T), yeast assimilable nitrogen (YAN) and total anthocyanins per plant, for 6 fungus disease-tolerant genotypes and Syrah, from 2019 to 2021, Gruissan - France.

	Yield (kg)								Soluble sugars (kg)								H2M (g)							
	I		NI		Relative diff. (%)	G	I	G:I	I		NI		Relative diff. (%)	G	I	G:I	I		NI		Relative diff. (%)	G	I	G:I
	Av	*SE*	Av	*SE*		***	***	*ns*	Av	*SE*	Av	*SE*		***	***	*ns*	Av	*SE*	Av	*SE*		***	***	**
Syrah	8.9	*0.5*	7.4	*0.6*	−17	*e*			1.9	*0.09*	1.6	*0.12*	−17	*b*			25.6^{c}	*1.4*	18.9^{d}	*1.6*	−26	*d*		[a]
3176N	12.4	*0.5*	10.8	*0.4*	−13	*f*			2.8	*0.10*	2.5	*0.10*	−12	*c*			31.6^{d}	*1.8*	24.0^{e}	*1.3*	−24	*e*		[a]
3159B	7.0	*0.4*	6.8	*0.4*	−3	*de*			1.6	*0.08*	1.5	*0.09*	−4	*b*			9.8^{a}	*0.6*	8.4^{bc}	*0.6*	−15	*bc*		*ns*
Artaban	5.5	*0.3*	4.4	*0.2*	−20	*b*			1.1	*0.06*	0.8	*0.04*	−21	*a*			8.3^{a}	*0.6*	5.0^{ab}	*0.3*	−40	*ab*		[a]
Floreal	4.4	*0.2*	2.9	*0.2*	−34	*a*			1.0	*0.04*	0.6	*0.05*	−36	*a*			6.2^{a}	*0.4*	3.4^{a}	*0.3*	−46	*a*		*ns*
G14	6.8	*0.5*	6.0	*0.5*	−12	*cd*			1.1	*0.08*	1.0	*0.08*	−16	*a*			10.1^{a}	*1.1*	9.8^{c}	*1.2*	−3	*c*		*ns*
G5	6.3	*0.4*	4.6	*0.3*	−27	*bc*			1.2	*0.07*	0.9	*0.06*	−26	*a*			14.8^{b}	*1.1*	8.2^{bc}	*0.8*	−45	*c*		[a]
Mean	7.3	*0.3*	6.1	*0.3*	−16				1.5	*0.07*	1.3	*0.07*	−13				15.2	*1.0*	11.1	*0.8*	−27			

	H2T (g)								YAN (g)								Anthocyanins (g)							
	I		NI		Relative diff. (%)	G	I	G:I	I		NI		Relative diff. (%)	G	I	G:I	I		NI		Relative diff. (%)	G	I	G:I
	Av	*SE*	Av	*SE*		***	***	*ns*	Av	*SE*	Av	*SE*		***	***	***	Av	*SE*	Av	*SE*		***	***	*ns*
Syrah	59.4	*3.1*	50.5	*4.5*	−15	*d*			1.0^{d}	*0.07*	0.7^{d}	*0.08*	−28	*d*		[a]	8.5	*0.6*	8.0	*0.5*	−6	*a*		
3176N	69.5	*2.9*	62.2	*2.9*	−11	*e*			0.5^{bc}	*0.04*	0.5^{c}	*0.04*	−5	*c*		*ns*	18.8	*0.8*	17.8	*0.9*	−6	*b*		
3159B	42.0	*2.2*	38.8	*2.3*	−8	*c*			0.2^{a}	*0.02*	0.2^{ab}	*0.01*	8	*a*		*ns*								
Artaban	34.8	*1.9*	28.7	*1.7*	−18	*ab*			0.3^{a}	*0.02*	0.2^{a}	*0.01*	−29	*a*		*ns*	7.1	*0.4*	6.3	*0.4*	−11	*a*		
Floreal	29.8	*1.4*	21.5	*1.6*	−28	*a*			0.6^{c}	*0.03*	0.4^{bc}	*0.03*	−39	*c*		[a]								
G14	37.9	*3.0*	38.0	*3.0*	1	*bc*			0.1^{a}	*0.02*	0.2^{a}	*0.01*	18	*a*		*ns*	8.5	*0.7*	6.5	*0.5*	−24	*a*		
G5	28.5	*1.7*	21.8	*1.6*	−24	*a*			0.5^{b}	*0.04*	0.3^{ab}	*0.03*	−31	*b*		[a]								
Mean	43.1	*1.7*	37.4	*1.7*	−13				0.5	*0.03*	0.4	*0.02*	−20				10.7	*0.7*	9.7	*0.7*	−9			

[a] Relative difference was calculated as Rel. Diff. % = (NI–I)/I * 100). '***', '**', '*' indicates 0.001, 0.01 and 0.05 significance levels and 'ns' indicates no significance. Different letters in the same column indicate statistical differences among genotypes within treatment (superscript letters) and regardless of treatment (normal case letters) (Bonferroni adjustment).

3.3. Genotype, year and Acc-Ψp contributions in berry components responses to water deficit

Berry weight and components (content, concentration and ratio) were fitted in function of the variations in Acc-Ψp. Five parameters were considered, two related to the slopes ('Acc-Ψp', 'Acc-Ψp:Genotype') and three to the values at Acc-Ψp = −15 MPa ('Genotype', 'Year', 'Genotype: Year'). The η^2 of each parameter was determined for each berry component (Fig. 1).

When considering berry component per unit of fruit, the contribution of 'Acc-Ψp' (parameter 'Acc-Ψp') reached the maximum values mainly due to its high impacts on berry weight (η^2 = 60%) (Wilhelm de Almeida et al., 2023a). Genotype contribution (parameter 'Genotype') was the most important on amino acids (68%) while it showed little effect on H2T (17%). The effects of Year and its interaction with genotype (parameters 'Year' and 'Genotype:Year') showed little contributions to the model, reaching a maximum of 5% in Anthocyanins and 18% in H2T, respectively (Fig. 1).

The contributions of the parameters to the model highly differed when considering the components per volume instead of unit of fruit. Yet, the contribution of the parameter 'Acc-Ψp' was minimum, reaching a η^2 maximum of 25% in H2T and minimum of 0.3% in Fru. The variations in components per volume were mostly explained by the genotype effect ('Genotype'), whose contribution ranged from 48% to 76% in H2T and amino acids concentrations, respectively. However, for anthocyanins concentration, the 'Genotype' parameter had lower impact (22%). The interaction between genotype and year ('Genotype:Year') was the most important for this component (36%) (Fig. 1).

Similarly, to the berry components per volume, the 'Acc-Ψp' contributions on the ratios were minimum, and the contributions of the genotype and its interaction with year were maximum. The 'Acc-Ψp' parameter reached a maximum of 11% in H2M:H2T, while 'Genotype' and 'Genotype:Year' parameters reached up to 60% and 32% in H2M: H2T and Antho:H2T ratios.

Interestingly, the contribution of the parameter 'Ψp:Genotype' on the model was shown to be the lowest, regardless of the variable considered (component per unit or per volume of fruit and ratios). The η^2 values, reached a maximum of 2.5% in H2M per unit of fruit, 3.1% in anthocyanins per volume and 4.0% in Antho:H2T ratio (Fig. 1).

Based on the above results, it seemed most relevant to consider the effects of WD on the distribution of the different berry metabolites in concentration rather than their contents per unit of fruit. Indeed, the effect of WD on metabolite content was found to be intricately linked to the changes in berry weight across the seven studied genotypes. By focusing on metabolite concentrations and ratios, we can gain a clearer understanding of the specific effects of Acc-Ψp and genotype on the metabolic responses, without the confounding influence of changes in berry weight.

3.4. Genotype sensitivity of metabolites accumulation to water status (slopes)

The variation of the sensitivity of metabolite accumulations to water deficit among the genotypes was analyzed by comparing the slopes of the regressions. Different global trends were observed, depending on the compound: the concentration of some components decreased (H2M and amino acids), increased (H2T, anthocyanins and Ca++), were only slightly affected (soluble sugars) or were highly dependent on genotype (K+) as Ψp decreased (Fig. 2, Table S3). However, a great variation among berry component concentration responses among genotypes was observed.

From all primary metabolites concentrations only H2T and H2M showed a consistency increase and decrease (respectively) among all genotype as Acc-Ψp became more negative. G14 and 3159B showed the highest (1.95 mmol C-eq L^{-1} per 1 MPa accumulated) and lowest (0.94 mmol C-eq L^{-1} per 1 MPa accumulated) increases of H2T, with other genotypes showing intermediate behaviors (Fig. 2, Table S3). Such increases represented, a gain in H2T of 34% for G14 and 14% for 3159 B between Acc-Ψp = −15 MPa and Acc-Ψp = −40 MPa (Table 3). The H2M concentration decrease was the most important in 3176N, Artaban and G5 (−1.87 mmol C-eq L^{-1} per 0.1 MPa, on average), while Syrah, 3159 B, Floreal and G14 showed lower reductions (−0.63 mmol C-eq L^{-1} per 1 MPa accumulated, on average) (Fig. 2, Table S3). Such decrease led to H2M differences between Acc-Ψp = −15 MPa and Acc-Ψp = −40 MPa up to −91% for Artaban (Table 3).

Soluble sugars concentrations were either positively (3159B, 16.8 mmol C-eq L^{-1} per 1 MPa accumulated), negatively (3176N, Artaban and G14; −27.83 mmol C-eq L^{-1} per 1 MPa accumulated, on average) or not impacted (Syrah, Floreal and G5) by Acc-Ψp decrease (Fig. 2, Table S3). Despite these trends, the relative differences between Acc-Ψp = −15 MPa and Acc-Ψp = −40 MPa were relatively weak, i.e. below 12% (decrease or increase) for all genotypes (Table 3).

Amino acids were decreased by WD only in Syrah, 3159B and Floreal (−0.069, −0.052 and −0.029 mmol C-eq L^{-1} per 1 MPa accumulated), while other genotypes were not impacted (Fig. 2, Table S3). Yet, only Syrah and 3159 B showed important losses between Acc-Ψp = −15 MPa and Acc-Ψp = −40 MPa, of −35% and −43%, respectively (Table 3). Anthocyanins showed opposite responses in Syrah and G14, increasing in the former (1.35 mmol C-eq L^{-1} per 1 MPa accumulated) and decreasing in the latter (−0.53 mmol C-eq L^{-1} per 1 MPa accumulated) (Fig. 2, Table S3). These responses led to a gain of 73% and a loss of 16%, in Syrah and G14 respectively between Acc-Ψp = −15 MPa and Acc-Ψp = −40 MPa (Table 2). No significant responses were observed for 3176 N and Artaban anthocyanins' response to WD (Fig. 2, Table S3).

Potassium concentrations increased in G14, Floreal, Syrah and G5 (0.22 mmol L^{-1} per 1 MPa accumulated, on average) and decreased in 3176N and Artaban (−0.40 mmol L^{-1} per 1 MPa accumulated, on average). Relative differences of K+ between Acc-Ψp = −15 MPa and

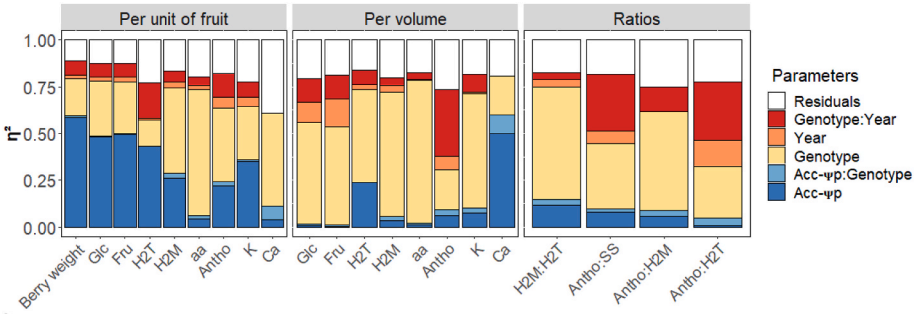

Fig. 1. Proportion of variance explained (η^2) by each factor (parameters) and its interactions (p-value <0.05), on slopes (blue colours) and intercepts at mild WD (Acc-Ψp = −15 MPa) (yellow, orange and red colours), in berry weight, each berry component per unit of fruit and per volume, and for the ratios of malic acid to tartaric acid (H2M:H2T), anthocyanins to soluble sugars (Antho:SS), anthocyanins to malic (Antho:H2M) and to tartaric acid (Antho:H2T). Only significant parameters are shown (p.value < 0.05).

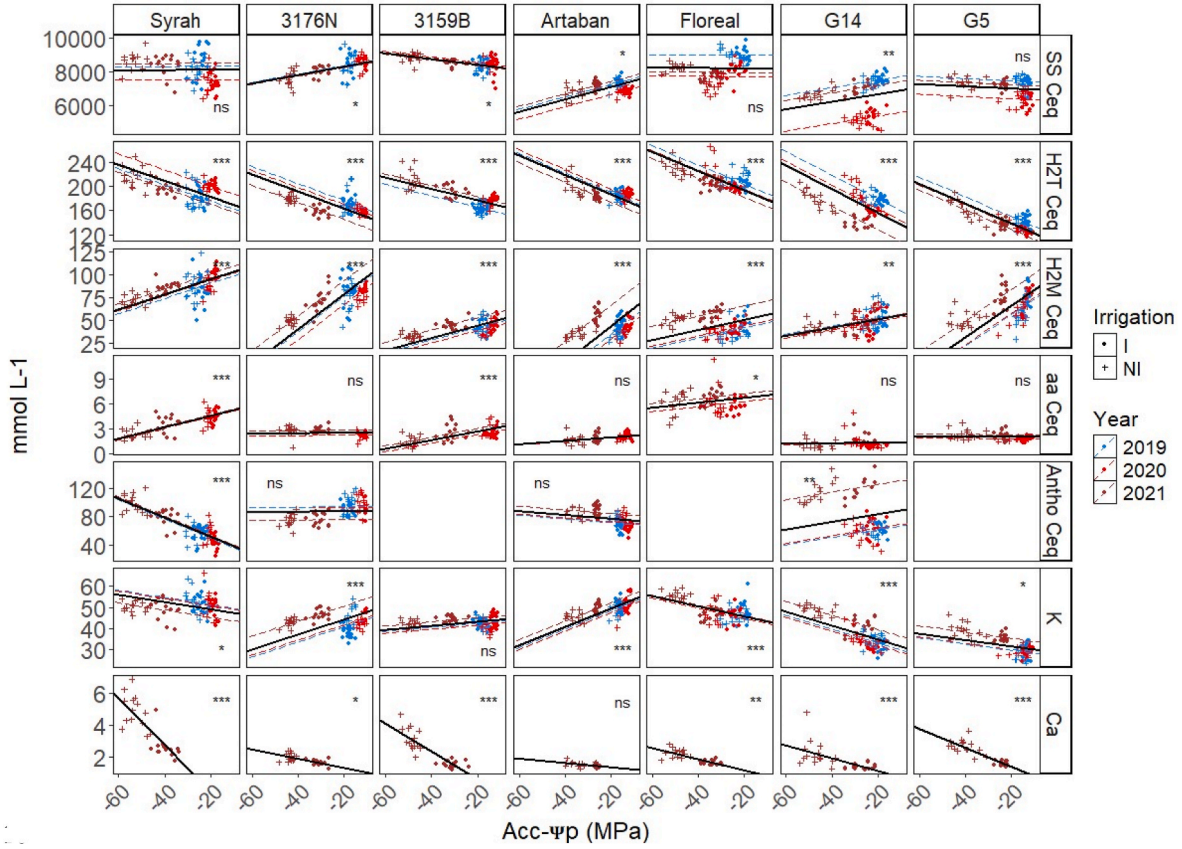

Fig. 2. Soluble sugars (SS), tartaric acid (H2T), malic acid (H2M), amino acids (aa) and anthocyanins (Antho) in mmol C-eq L^{-1}, and main cations (K^{+}, Ca^{++}) in mmol L^{-1}, response to accumulated **Ψ**p (Acc-**Ψ**p) for Syrah and 6 fungus disease-tolerant genotypes, Gruissan -France.
* Each point represents one plant measured in that specific year (blue, red and brown colours for 2019, 2020 and 2021, respectively). Point shapes '●' and '+' indicate irrigated and non-irrigated plants, respectively. Solid black lines are multiple linear regressions ([component] = Acc-**Ψ**p + Genotype*Year + Acc-**Ψ**p:Genotype). Dashed lines indicate intercepts for each year within each genotype. '***', '**', '*' indicates 0.001, 0.01 and 0.05 significance levels and 'ns' indicates no significance of slopes for each genotype.

Acc-**Ψ**p = −40 MPa ranged from 24% for G14 to −12% for Artaban (Table 2). 3159B was the only genotype that showed no differences in K+ concentrations as WD increased (Fig. 2, Table S3). Calcium (Ca++) showed higher concentrations with WD, at a higher extent in Syrah (0.15 mmol L^{-1} per 1 MPa accumulated) and lower in 3176N, Floreal and G14 (0.03, 0.04, 0.04 mmol L^{-1} per 1 MPa accumulated, respectively) (Fig. 2, Table S3).

When considering the ratio between metabolites, different response trends to WD were noticed. All genotypes reduced the H2M:H2T ratio as WD increased, with higher decreases being observed for G5 and 3176N (16.23 x 10-3 and 14.62 x 10-3 per 1 MPa accumulated, respectively), and lower for 3159B and Floreal (4.33 x 10-3 and 4.24 x 10-3 per 1 MPa accumulated, respectively) (Fig. 3). In contrast, anthocyanins ratios highly varied with genotype. Anthocyanins to SS ratio increased in Syrah and Artaban at similar rates (0.16 x 10-3 and 0.12 x 10-3 per 1 MPa accumulated) while it was not affected in 3176N and G14. Yet, anthocyanins to H2T ratio decreased in 3176 N (-3.7×10^{-3} per 1 MPa accumulated) and G14 (-10.4×10^{-3} per 1 MPa accumulated), while it increased in Syrah (4.1 per 1 MPa accumulated) and was not affected in Artaban. Anthocyanins to H2M ratio consistently increase in all genotypes, at a higher extent in Artaban (95.2×10^{-3} per 1 MPa accumulated) than in Syrah, 3176N and G14 (27.3×10^{-3}, 35.7×10^{-3} and 12.9×10^{-3} per 1 MPa accumulated) (Fig. 3).

*3.5. Genotype metabolites accumulation under high water deficit (Acc-**Ψ**p = −40 Mpa)*

Berry components concentrations at Acc-**Ψ**p = −40 MPa varied among genotypes and years (Fig. 2). Considering all genotypes combined, 2021 displayed the highest concentrations of H2M, amino acids, anthocyanins and K+ (51 mmol C-eq L^{-1}, 2.9 mmol C-eq L^{-1}, 90 mmol C-eq L^{-1} and 45 mmol L^{-1}, respectively) while presenting the lowest H2T concentrations (189 mmol C-eq L^{-1}). The seasons of 2019 and 2020 showed the highest (7.9 mol C-eq L^{-1}) and lowest (7.0 mol C-eq L^{-1}) SS concentrations, respectively (Fig. 2). The high effect of genotype and year interaction on anthocyanins was mostly seen for G14, which showed low values in 2019 and 2020 (51 and 53 mmol C-eq L^{-1}, respectively), but the highest value in 2021 (115 mmol C-eq L^{-1}) when compared to other genotypes (Fig. 2).

Regardless of the years, genotypes could be divided into two different groups regarding the total C concentration in SS and H2T at Acc-**Ψ**p = −40 MPa. A first group formed by 3176 N, 3159 B and Floreal, with similar or higher values than those of Syrah, showing values of SS and H2T of 8.1 mol C-eq L^{-1} and 209 mmol C-eq L^{-1}, respectively. And a second group formed by both sugarless varieties, G5 and G14, which consistently showed lower values for SS (7 mol C-eq L^{-1}, on average) and H2T (183 mmol C-eq L^{-1}, on average) (Fig. 2, Table 3). Although Artaban was grouped with the first group in terms of H2T concentrations (219 mmol C-eq L^{-1}), it showed similar SS to the two sugarless genotypes (6.4 mol C-eq L^{-1}) (Table 3).

Regarding H2M concentrations, all genotypes showed lower values than Syrah (79 mmol C-eq L^{-1}). However, while all hybrids (3176N, 3159B, Floreal, G14 and G5) displayed values around 37 mmol C-eq L^{-1} (on average), Artaban reached extremely low values, of 5 mmol C-eq L^{-1} (Fig. 2, Table 3).

Amino acid concentrations were the highest in Floreal (6.1 mmol C-

Table 3
Estimated marginal means (av) and standard error (SE) calculated values of soluble sugars (SS, mol C-eq L^{-1}), tartaric acid (H2T, mmol C-eq L^{-1}), malic acid (H2M, mol C-eq L^{-1}), amino acids (aa, mmol C-eq L^{-1}), anthocyanins (mmol C-eq L^{-1}) and main cations (Ca++ and K+, mmol L^{-1}) at mild (Acc-Ψp = −15 MPa) and high (Acc-Ψp = −40 MPa) water deficit for 6 fungus disease-tolerant genotypes and Syrah, Gruissan-France.

Genotype	Soluble sugars (mol C-eq L^{-1})					H2T (mmol C-eq L^{-1})					H2M (mmol C-eq L^{-1})					Amino acids (mmol C-eq L^{-1})				
	−15 MPa		−40 MPa		Relative Diff (%)	−15 MPa		−40 MPa		Relative Diff (%)	−15 MPa		−40 MPa		Relative Diff (%)	−15 MPa		−40 MPa		Relative Diff (%)
	av	SE	av	SE		av	SE	av	SE		av	SE	av	SE		av	SE	av	SE	
Syrah	8.1^{c}	0.2	8.1^{d}	0.1	0	176^{de}	4	209^{ce}	2	19	99^{c}	3	79^{d}	2	−20	4.9^{d}	0.3	3.2^{c}	0.2	−35
3176N	8.4^{c}	0.1	7.8^{cd}	0.2	−7	157^{c}	2	192^{bc}	5	22	89^{c}	2	41^{bc}	4	−54	2.6^{bc}	0.2	2.5^{bc}	0.3	−4
3159B	8.3^{c}	0.1	8.8^{e}	0.1	6	172^{d}	2	196^{bd}	4	14	48^{a}	2	31^{b}	3	−35	3.0^{c}	0.2	1.7^{ab}	0.2	−43
Artaban	7.3^{b}	0.1	6.4^{ab}	0.3	−12	179^{de}	2	219^{def}	7	22	54^{a}	2	5^{a}	6	−91	2.1^{ab}	0.2	1.6^{ab}	0.5	−24
Floreal	8.2^{c}	0.1	8.2^{d}	0.1	0	186^{e}	3	226^{f}	2	22	54^{a}	3	40^{bc}	2	−26	7.0^{e}	0.3	6.1^{d}	0.1	−13
G14	6.8^{a}	0.1	6.3^{a}	0.1	−7	146^{b}	2	195^{b}	2	34	54^{a}	2	43^{c}	2	−20	1.4^{a}	0.2	1.3^{a}	0.1	−7
G5	7.0^{ab}	0.1	7.2^{bc}	0.2	3	131^{a}	2	171^{a}	4	31	75^{b}	1	32^{bc}	4	−57	2.0^{a}	0.2	2.1^{ab}	0.2	5

Genotype	Anthocyanins (mmol C-eq L^{-1})					K^{+} (mmol L^{-1})					Ca^{2+} (mmol L^{-1})				
	−15 MPa		−40 MPa		Relative Diff (%)	−15 MPa		−40 MPa		Relative Diff (%)	−15 MPa		−40 MPa		Relative Diff (%)
	av	SE	av	SE		av	SE	av	SE		av	SE	av	SE	
Syrah	45^{a}	0.4	79 ^{ns}	0.3	73	48^{bcd}	1.2	53^{c}	0.8	10	0^{a}	0.3	2.5^{c}	0.1	∞
3176N	88^{c}	0.2	87	0.5	−1	46^{bc}	0.7	37^{ab}	1.6	−20	1.2^{b}	0.3	1.8^{ab}	0.1	50
3159B						44^{b}	0.7	41^{b}	1.5	−7	0.2^{ab}	0.3	2.2^{bc}	0.1	1000
Artaban	76^{b}	0.2	82	0.8	8	52^{d}	0.7	41^{ab}	2.3	−21	1.3^{b}	0.3	1.6^{a}	0.2	23
Floreal						45^{bc}	1.1	51^{c}	0.7	13	1.0^{b}	0.3	1.8^{ab}	0.1	800
G14	87^{c}	0.3	73	0.3	−16	33^{a}	0.8	41^{b}	0.7	24	0.9^{b}	0.2	1.8^{ab}	0.1	100
G5						31^{a}	0.5	35^{a}	1.4	13	1.1^{b}	0.2	2.5^{c}	0.1	127

*Relative difference was calculated as Rel. Diff. % = (NI–I)/I * 100). Different superscript letters in the same column indicate statistical differences among genotypes within Acc-Ψp level (Bonferroni adjustment).

eq L^{-1}) and lowest in G14 (1.3 mmol C-eq L^{-1}). No differences in anthocyanin concentrations between the red fruit genotypes were observed at high WD, where genotypes averaged 80 mmol C-eq L-1 (Fig. 2, Table 3).

Potassium (K^{+}) concentrations were the highest for Syrah and Floreal (52 mmol L^{-1}, on average) and the lowest for G5 (35 mmol C-eq L^{-1}, on average) (Fig. 2, Table 3). In general, 3176 N, Artaban, Floreal and G14 showed lower concentrations of Ca++ than Syrah, 3159B and G5 (Fig. 2, Table 3).

Lastly, the ratios between berry metabolites also showed variations among the genotypes. The highest H2M:H2T ratio at Acc-Ψp = −40 MPa was observed in Syrah (0.38), and the lowest in Artaban (~0) (Fig. 3, Table S1). For anthocyanins ratios with SS and H2M, Artaban showed the highest values (of 13.2 and 3.73, respectively), while Syrah the lowest (9.6 and 1.06, respectively). However, when considering anthocyanins ratios to H2T, 3176N showed the highest values (0.46), while G14 the lowest (0.35) (Fig. 3, Table S1).

3.6. Genotypic variations in the hierarchy of metabolite accumulation under contrasted water status (Acc-Ψp = −15 and Acc-Ψp = −40 MPa)

In order to analyze the overall hierarchy of metabolites accumulation under WD and its variations among the genotypes, two principal component analyses of berry components concentration were performed under mild WD (MWD, Acc-Ψp = 15 MPa, Fig. 4A) and high WD (HWD, Acc-Ψp = −40 MPa) (Fig. 4B). Under MWD variables retained in the PCA explained up to 77% of the total variation, where first (Dim1) and second (Dim2) dimensions accounted for 52% and 25% respectively (Fig. 4A). Three distinct groups of variables were formed, with the first (left side of Dim1) being formed by H2T, K+, aa and SS. Orthogonal to the first, the second group (right side of Dim1) was mainly formed by anthocyanins (Antho), its ratios with H2M (Antho.H2M), H2T (Antho. H2T) and SS (Antho.SS) and Ca++. Orthogonal to both groups (bottom side of Dim2), H2M and its ratio with H2T formed the third group (Fig. 4A). Genotypes were mostly distributed vertically along Dim2, separating those with high H2M and H2M:H2T but low H2T and K (G5 and 3176N) from those with an opposite response (Floreal, 3159B and Artaban). Syrah was horizontally separated from G14, due to their contrasting responses on anthocyanins accumulation (Fig. 4A).

The PCA of variables under HWD explained 70%, with Dim1 and Dim2 accounting for 46% and 24% of the variation (Fig. 4B). Although the three groups of variables were mostly kept under HWD, the variables within groups one and two were more separated from each other. Genotypes kept their distribution, apart from 3159B, which was distanced from Floreal, due to a lower H2T accumulation (Fig. 4B). In both PCA analyses, the two sugarless genotypes, G5 and G14, and Artaban were separated from the other genotypes due to their contrasting SS levels (Fig. S3).

4. Discussion

4.1. Pros and Cons of the methodological approaches

In the present study, each vine was considered as a single biological replicate, taking into account the accumulated WD perceived by each plant (Acc-Ψp) and possible heterogeneity of soil water, root distributions and/or canopy development as previously reported (Wilhelm de Almeida, Pellegrino, et al., 2023). This approach allowed us to build quantitative relationships between berry component variables and actual vine water status, and later to compare genotype responses.

Although the trends were fairly linear for the observed range of values, extrapolation beyond this range assumes that the relationship continues linear, which may not be accurate. Indeed, plant responses to variations in water availability and other factors usually follow more complex non-linear patterns, often characterized by curves with thresholds (Archontoulis and Miguez, 2015; Lebon et al., 2006). Thus,

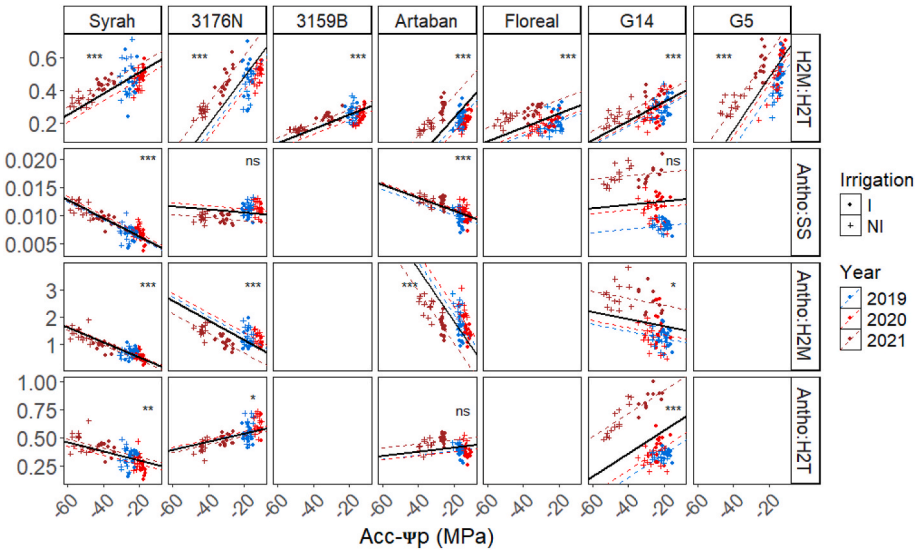

Fig. 3. Malic to tartaric acid (H2M:H2T), anthocyanins to soluble sugars (Antho:SS), anthocyanins to malic acid (Antho:H2M) and anthocyanins to tartaric acid (Antho:H2T) ratios response to accumulated **Ψ**p (Acc-**Ψ**p) for Syrah and 6 fungus disease-tolerant genotypes, Gruissan -France.
* Each point represents one plant measured in that specific year (blue, red and brown colours for 2019, 2020 and 2021, respectively). Point shapes '●' and '+' indicate irrigated and non-irrigated plants, respectively. Solid black lines are multiple linear regressions ([component] = Acc-**Ψ**p + Genotype*Year + Acc-**Ψ**p:Genotype). Dashed lines indicate intercepts for each year within each genotype. '***', '**', '*' indicates 0.001, 0.01 and 0.05 significance levels and 'ns' indicates no significance of slopes for each genotype.

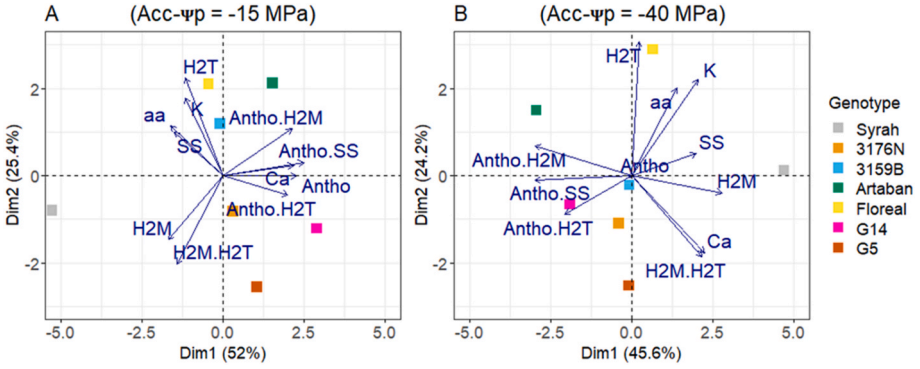

Fig. 4. PCA (Dim1, Dim2) of plant responses to mild WD (MWD, Acc**Ψ**p = −15 MPa) (A) and high WD (HWD, Acc**Ψ**p = −40 MPa) (B), for soluble sugars (SS), tartaric acid (H2T), malic acid (H2M), amino acids (aa), anthocyanins (Antho), the ratios of anthocyanins to soluble sugars (Antho.SS) and to malic acid (Antho.H2M) per genotype, and the cations Ca^{++} (Ca) and K^{+} (K), Gruissan - France (2019–2021).

only the linear part of the curve observed for all genotypes below the threshold of −15 MPa was considered. Then, this threshold Acc-Ψp values of −15 MPa and −40 MPa were adopted to analyze and understand the strategies adopted by genotypes under mild and high WD (respectively MWD & HWD; Fig. S4, (Deloire et al., 2020; Van Leeuwen et al., 2009), and to the reality of WD generally experienced by the vineyards in semi-arid Mediterranean regions. It is important to point out that Acc-**Ψ**p was closely correlated to seasonal average instantaneous **Ψ**p (Fig. S4) and minimum **Ψ**p (data not shown), other common parameters to represent plant perception to WD.

In the three years of experiment, all plants decreased the **Ψ**p over the season at higher intensities in NI than I vines (data not shown). Among the three years, 2021 led to the highest drop in Acc-**Ψ**p for NI, from moderate at flowering (ca −28 MPa, ~ −0.5 MPa, Fig. S4) to a severe WD at harvest (ca −55 MPa, ~ −1.0 MPa, Fig. S4). Such a difference was mostly due to the lower level of rainfall during the winter and the growing season in 2021. However, it is crucial to highlight that Acc-**Ψ**p, being an accumulated **Ψ**p value, may not necessarily account for the diurnal fluctuations in WD that plants may experience when vapor pressure deficits are elevated throughout the day.

Phenotyping at physiological ripeness allowed a gain in precision besides helping to distinguish the accumulation of metabolites per unit of fruit or per volume, while avoiding interpretative bias due to berry shriveling. However, this approach still has limitations. Indeed, the physiological ripeness at the population level was determined by the average of individual berries. These berries are either (i) still increasing in volume, thus accumulating water and carbon, or (ii) they have reached the physiological ripeness stage, thus at maximum water and C or (iii) they have surpassed this stage and started to lose volume, losing water and concentrating C. Therefore, this approach may underestimate the value of an individual berry in terms of water and carbon content (Shahood et al., 2020). However, considering the available phenotyping tools, this was the most suitable method to compare different genotypes at equivalent stages for their ability to accumulate water, primary and secondary metabolites under drought conditions in the field.

Doing so, we observed that plant water status variation mainly impacted berry weight and thus indirectly regulated berry components per unit of fruit, but had limited effects on berry component concentrations (Fig. 1). Besides water availability, climatic factors such as light, air moisture and temperature, can also play a major role on berry

components concentration. Indeed, after genotype, year was a major factor contributing to the maximum concentration of berry components. Climatic factors impact several levels of berry metabolism, but also influence berry water balance and grapevine phenology and thus the duration of berry development and ripening. For instance, ripening duration, defined as the time from veraison to phloem unloading arrest, in 2021 was up to 11–16 days shorter compared to 2019 and 2020 seasons, respectively (Wilhelm de Almeida, Pellegrino, et al., 2023). Such differences might have influenced the different metabolites and cations levels observed among these seasons, which were notably particularly important for anthocyanins.

Finally, the concentrations of SS, H2T, H2M, K+, Ca++ and anthocyanins in the 6 fungus-tolerant genotypes here studied were in agreement with those observed in a other sets of *V. vinifera* L. and interspecific varieties, including sugarless genotypes, at phloem unloading arrest (Bigard et al., 2018, 2019, 2022; Wilhelm de Almeida, Torregrosa, et al., 2023), and at technological maturity (Dai et al., 2011).

In spite of showing reduced SS concentrations, both sugarless genotypes, G5 and G14, displayed similar SS content per berry, due to bigger berries (Table S2, Table S3, Table S4) (Wilhelm de Almeida, Pellegrino, et al., 2023), to other genotypes (Table S4), as also reported by (Bigard et al., 2022). In addition, the low H2T concentrations observed in these genotypes are related to lower H2T levels straight from the onset of ripening (véraison), as previously observed by Bigard et al. (2022). The same authors have shown a greater expansion of the pericarp during the ripening phase for those genotypes lowering even more the H2T concentrations. This higher cell expansion occurs at a comparable osmotic pressure and leads to a higher dilution of H2T and SS in these genotypes. The reduced SS concentrations in sugarless genotypes had no negative effect on anthocyanins levels. Indeed, regardless of the water level, G14 showed higher anthocyanins concentrations and content per berry than Syrah.

4.2. Genotypic responses of berry primary metabolites and cations concentrations to WD

The impact of high WD on SS concentration remained minimal across genotypes (<12%, Fig. 2, Table 3) despite significant reductions in photosynthetic activity, which ranged from −51% to −76% in Syrah and G5, respectively, as evidenced in our previous study (Wilhelm de Almeida, Pellegrino, et al., 2023). This observation aligns with a meta-analysis by Gambetta et al. (2020), which indicated a limited effect of WD on berry SS concentrations (<8%), even when photosynthesis experiences a substantial reduction of up to 70% (Hewitt et al., 2023). Various structural and transcriptional adjustments occur at the onset of ripening to maintain a consistent level of sugar supply to the berries until physiological ripeness, despite the limitations of photosynthesis. Some changes that contribute to building up berry sink strength and an important SS accumulation in fruits include the shift from symplastic to apoplastic phloem unloading (SS is exclusively imported by phloem) (Keller et al., 2015; Zhang et al., 2006), expression of sugar transporters and specific proteins (Rienth et al., 2016) and the activation of H+/sugars exchangers in the tonoplast (Bigard et al., 2022; Shahood et al., 2020). Furthermore, the increase in osmotic pressure due to phloem apoplastic unloading is genotype dependent, which might explain the different requirements of SS concentrations among the genotypes (Keller et al., 2015).

Although WD had little effect on SS concentration, the decrease in water availability strongly reduced the sugar content per unit of fruit in all genotypes (Fig. S2, Table S4), which was strongly linked to the response of fruit size to plant water status. In general, the genotypes that showed greater reductions in the amount of SS per fruit (3176N, G5 and G14), were also those that previously showed greater reductions in fruit size to WD (Table S2, Table S3) (Wilhelm de Almeida, Pellegrino, et al., 2023).

The consistent and similar increase of H2T concentration and decline in content (per unit of fruit) (Fig. 2 and S1) among genotypes, might be attributed to a reduction in fruit size caused by decreased water flow due to WD (Ojeda et al., 2001). The increase in the concentration (Table S3) and decrease in the content per berry of Ca++ (Fig. S2, Table S4) across most genotypes highlights this concentration effect. The reduction in size concentrates the H2T and Ca++, particularly accumulated during the early herbaceous phase of fruit development (Bigard et al., 2018; Cabanne and Donèche, 2003; Champagnol, 1984; Rogiers et al., 2006). Yet, the low content per berry may suggest an earlier effect of WD on organic acid synthesis and Ca++ accumulation. Ca++ is transported via xylem (Hocking et al., 2016) and its concentration in berries is directly dependent on the total number of cells, which is defined around the berry set and is normally not affected by WD (Ojeda et al., 2001). As H2T and Ca++ levels remain constant throughout ripening (Bigard et al., 2022; Rogiers et al., 2006), their concentration may increase during late ripening due to water loss via transpiration. This is particularly true in conditions of high Vapor Pressure Deficit (VPD), which were common during the study, where VPD frequently reached 3–4 kPa in the afternoon throughout ripening (data not shown). It should be noted that low evaporative demand during ripening can result in an excess of water discharge from the phloem that is not transpired. This excess water may be recycled through the xylem, also enhancing concentration (Keller et al., 2015).

In contrast, the response of K+ to WD varied significantly depending on genotype. K+ is accumulated during both the berry's herbaceous growth phase and ripening (Mpelasoka et al., 2003). The concentration increase observed in most genotypes (Syrah, Floreal, G14 and G5) may be attributed to a concentration effect, such as that of H2T and Ca++, and possibly amplified by increased activity of K+ channels (Nieves-Cordones et al., 2019). Only Artaban and 3176N showed a decrease in K+ concentration under WD, possibly buffering the low H2M concentrations, especially in Artaban. This could be due to a negative effect of WD on the uptake and mobilization of inorganic compounds, such as K+, by roots (Dundon and Smart, 1984; Oddo, 2020); or due to lower phloem flow into berries (Mpelasoka et al., 2003), supported by the higher decrease in SS concentrations observed in these genotypes. Other factors such as soil physico-chemical properties, mineral availability, genotype, rootstock, and temperature during ripening were proposed to impact K+ levels in berries (Mpelasoka et al., 2003; Ruhl, 1989; Villette, 2020).

Among all genotypes, the H2M concentrations and content decreased under WD (Fig. 2 and S1). The decrease in H2M may be attributed to a lower initial H2M pool, related to a lower C and water imports into berries as a consequence of WD. This results in diminished H2M accumulation during the herbaceous phase of berry growth, when grapes are not the primary sink for photoassimilates (Ollat and Gaudillère, 2000). Additionally, a lower C import into berries during ripening, due to WD, may have a further negative effect, potentially leading to increased malate respiration as an alternative C source in berries. Malate breakdown is known to increase in berries when plants are subjected to increased temperatures (Kliewer, 1964; Sweetman et al., 2014). This is linked to an increased pumping out of malate in cells to maintain the energetic homeostasis of berries (Rienth et al., 2016). Indeed, recent studies have observed that metabolic and transcriptomic regulations involved in malate metabolism were activated under heat stress but also under water deficit, leading to the induction of several known to be linked to malate respiration (Hewitt et al., 2023; Zhan et al., 2023). These observations might suggest that a general mechanism of malate consumption during the first phase of sugar loading (Savoi et al., 2021) is activated under both thermal and water constraints.

In addition, the investigation into malate metabolism on various *V. vinifera* cultivars, unveiled a genotypic effect on the initial pools and consumption patterns of malate in berries (Bigard et al., 2018; Frioni et al., 2023; Torregrosa et al., 2017). In the present study, Artaban and Syrah showed the most contrasting consumption of H2M (91% vs 20% reductions, respectively, Table 3). This inherent variability among

genotypes presents as a compelling selection criterion that significantly impacts adaptation and the preservation of berry quality.

Despite a potentially different regulation in malate respiration, such responses might also be related to their contrasting responses of photosynthesis and canopy development under WD. In our previous study (Wilhelm de Almeida, Pellegrino, et al., 2023) we observed that, at high WD, Artaban exhibited greater reductions in both photosynthesis and canopy size when compared to Syrah vines. One possible explanation for the observed differences is that the higher regulation of photosynthesis in Artaban vines may have resulted in lower C gain and flow to berries, leading to lower initial malate pools. Another hypothesis is that the differences in canopy size may have led to variations in microclimate conditions within the cluster zone, potentially influencing temperature dynamics. Indeed previous studies have suggested that higher bunch exposure to light and warm temperatures conditions may stimutale malate respiration (Kliewer, 1964; Kliewer and Schultz, 1964; Rienth et al., 2016; Sweetman et al., 2014). Furthermore, high temperatures were observed to elicit greater effects on malate respiration in berries relative to water deficit (Hewitt et al., 2023).

4.3. Carbon trade-offs between berry primary and secondary metabolites among genotypes under WD

In order to assess the carbon trade-off between metabolites under WD conditions, the H2M to H2T ratio and the anthocyanins to SS, H2M or H2T ratios were analyzed (Fig. 3, Table S1). The H2M to H2T ratio decreased as WD increased for all genotypes due to the coordinate respective decrease and increase of these acids. Yet, the genotypic differences were mainly dictated by the consumption of H2M under WD, which was higher in 3176N, G5 and Artaban than for others. Duchêne et al. (2014) proposed that genotypes that maintain a low H2M:H2T could be valuable in the context of climate change specifically regarding the increase in air temperatures. However, as these two organic acids result from very different metabolic pathways (DeBolt et al., 2006) and can be differentially accumulated according to the genotype (Bigard et al., 2018), the level of each is also a relevant parameter to quantify. Genotypes with high levels of H2M and H2T and eventually a low H2M:H2T would have the ability to maintain a more stable pH, as long as K+ and Ca++ are not excessively high. As the free form of both H2T and H2M react with these cations to form their respective precipitated salts, impacting acidity and pH and consequently wine flavor and aroma (Conde et al., 2007). In this regard, varieties such as G14, 3159B and Floreal would be of interest, due to their relatively small values and fluctuations in H2M:H2T under WD. Although Syrah showed low variations in H2M:H2T ratio, it exhibited high levels of K+ and Ca++.

In WD conditions, both Syrah and Artaban exhibited a notable rise in the Antho:SS ratio, although for distinct reasons. The increase in Syrah was attributed to a significant augmentation in anthocyanins without significant changes in SS levels. Conversely, Artaban's ratio increase was mainly a result of SS reductions. Furthermore, the increase in Antho:H2M for all genotypes can be attributed to the overall consumption of H2M, with Artaban demonstrating the most substantial reduction and thus exhibiting the highest gain in Antho:H2M ratio. Although all genotypes experienced elevated H2T concentrations, primarily caused by a decrease in berry size (as previously mentioned in the discussion topic 4.2), it is noteworthy that Syrah and Artaban did not exhibit any decrease in the Antho:H2T ratio, unlike 3176N and G14. This indicates that the impact of WD on primary metabolites and anthocyanins was not equally distributed across those two groups of varieties. It seems that in the former group (Syrah and Artaban), H2M and/or SS were more affected by WD when compared to anthocyanins. Moreover, the up-regulation of anthocyanins under WD appeared to be more pronounced in Syrah compared to Artaban (Fig. 2).

It is widely accepted that plants have a trade-off dynamic in resource allocation between primary metabolites, which are mainly for growth, and secondary metabolites which are for plant defense and thus synthesized under stress conditions, but also to attract seed-dispersing animals. Anthocyanins are phenolic compounds mainly accumulated in berry skins, after veraison, and are end-products of the phenylpropanoid metabolism. They are up-regulated under abiotic stresses, such as WD, happening either before or after veraison (Castellarin et al., 2007; Gambetta et al., 2020; Ojeda et al., 2002; Savoi et al., 2016). Their synthesis is modulated by the expression of flavonoid genes (UFGT, CHS and F3H), metabolism and ABA signaling at veraison (Castellarin et al., 2007; Ferrandino and Lovisolo, 2014; Savoi et al., 2016).

Shellie & Bowen (2014) previously observed pronounced differences in anthocyanin concentrations and responses to vine water status (**Ψ**stem) between Malbec and Cabernet Sauvignon. It is possible that similar inherent genotypic factors are responsible for the differences observed between Syrah and Artaban. Variations in the intensity of WD experienced by both genotypes could also play a role. The average Acc-**Ψ**b from flowering to physiological ripeness was −30.7 MPa for Syrah and −20.2 for Artaban (across all years and irrigation treatments). An earlier onset of WD in Syrah could have triggered earlier abscisic acid (ABA) signaling. Previous research has shown that ABA application before or after veraison can significantly increase anthocyanin levels at harvest (Villalobos-González et al., 2016) to a greater extent than sugars levels (Wang et al., 2021), possibly modifying the ratio between these two compounds.

Besides genotype and water availability, light and temperature also play a major role in anthocyanins accumulation and biosynthesis (Azuma, 2018). Such environmental factors are taken into account in the 'Year' effect. Indeed, genotype interaction with year showed the highest contribution in anthocyanins concentration and its respective ratios with primary metabolites (Fig. 1). In particular, the two genotypes, G14 and 3176N, displayed respectively the highest and lowest anthocyanins concentrations in 2021, while they did not differ from other genotypes in 2019 and 2020 (Fig. 2). Inherent differences in sugar loading durations may have contributed to the observed variation, as 3176N required more than 15 days to attain physiological ripeness when compared to G14 (data not shown). This longer period may have resulted in a longer exposure to WD, which negatively impacted anthocyanins accumulation in 3176N, particularly in the driest year of 2021.

The C allocation into different berry metabolites varied among genotypes, but it remained consistent within each genotype under different water conditions (Fig. 4). Independently of water conditions Floreal prioritized allocating C to H2T, SS and amino acids, while low H2M concentration and consumption, but still keeping rather stable H2M:H2T ratios. Conversely, G5 had a contrasting approach, favoring high H2M concentration and consumption, low H2T, SS and amino acids, and thus favoring higher H2M:H2T ratio.

Red varieties Syrah and 3176N showed similar strategies with regards to most primary metabolites, i.e. both displayed high C distribution to SS, H2T and H2M. However, Syrah decreased C allocation to amino acids but increased to anthocyanins, while 3176N decreased C distribution to anthocyanins but kept the same C levels in amino acids. G14 and Artaban showed different responses to WD compared to Syrah and 3176N. While both displayed low C in all primary metabolites at both water conditions, G14 reduced C partitioning to anthocyanins while Artaban impaired C allocation to H2M and SS. Interestingly, Artaban showed characteristics closer to sugarless genotypes than to typical wine varieties such as Syrah, in spite of having higher H2T levels. The low SS C-values in the berries may be due to several factors. Firstly, it could be attributed to a higher level of other substances, such as H2T and cations (other than K^+ and Ca^{++}). These cations are present at low concentrations in grapevine fruit (<50 mmol L^{-1}) and help maintain the osmotic balance in the berries. Secondly, it could be due to higher expansion of the berry's skin. Finally, it could be due to higher sensitivity of photosynthetic regulation in response to WD, which limits the supply of sucrose to the fruit.

4.4. Long-term impacts of WD on berry metabolites accumulation at the plant level

Currently, irrigation serves as a pivotal instrument in mitigating the impacts of drought in dry areas. Its importance is clearly seen by the strong reductions in the accumulated values of metabolites yielded per plant at the end of 3-year study for all genotypes (Tables 1 and 2). In our previous study we observed that besides showing contrasting regulations under WD, these genotypes were also contrasted in regards to the short and long-term strategies adopted (Wilhelm de Almeida, Pellegrino, et al., 2023).

In the present study, although genotypes regulated differently berry component concentrations, these variations did not impact the average total accumulated values per plant over 3 years. Instead, the yield per plant primarily influenced the metabolite quantities. The genotypes with the highest (Syrah and 3176N) and lowest (Artaban and Floreal) yields were also those with the highest and lowest quantities of metabolites accumulated in the ripe grapes. The same pattern was observed when comparing the values of total metabolites under I and NI conditions. Floreal and G5 showed the greatest yield reductions between NI and I vines, and consequently the highest losses of all berry primary metabolites (Table 2). Two exceptions to this general trend were observed, where the overall genotype strategy observed for metabolite concentrations was reflected in the long-term metabolite quantities produced. The first exception concerns the strong genotype effect of Floreal in the accumulation of amino acids, both in concentration and in quantity, as indicated by the high total amounts of amino acids per plant after 3 years of study. The second was the high H2M consumption observed in Artaban, which resulted in one of the highest total H2M losses between NI and I vines (38%), despite only exhibiting a reduction of 18% in yield (Table 2).

5. Conclusion

Water deficit critically hampers the import of water into the grapevine fruits, impacting fresh weight yield, metabolite accumulation and distribution. This study's findings reveal that new disease-tolerant genotypes adopt diverse acclimation strategies, at the fruit level, to accumulate cations and allocate C into primary and secondary metabolites. Decreased water availability minimally affected sugar concentrations but elevated H2T and Ca^{++} concentrations, in all genotypes. Confronted with a limited photo-assimilate flow to the fruit under drought, some genotypes exhibited higher malate decrease rate, which altered the balance between primary metabolites (malate/sugars or malate/tartrate) and between secondary and primary metabolites (anthocyanins/malate). Besides the metabolic trade-offs, water stress has a genotype-dependent impact on fruit volume reduction, specifically influencing tartaric acid levels, ultimately determining the proportion of organic acids contributing to the overall acidity of ripe grapes. These changes demonstrate that water stress compromises the composition of grapevine fruit, thereby impacting potential quality, beyond the general response to yield reduction. Another important point to avoid confounding the effects of concentration during fruit overripening is to objectify the stage of phenotyping; in grapevines, the only ripening stage that correspond to a precise physiological stage is the point at which water and solute import stops due to phloem unloading arrest. Outdoor, at plant level, this can be non-destructively approached through grape growth monitoring. This study, which experimented with new phenotyping methodologies and yielded original data about the adaptability of new disease-tolerant hybrids to drought conditions.

Assessing fruit crop yield on both individual plant and hectare scales is crucial for productivity evaluation and resource optimization. Understanding how climate influences water and solute accumulation in fruit, and identifying trade-offs in carbon allocation to key compounds, is essential for aiding winegrowers in informed decision-making and enhancing agricultural practices. Furthermore, integrating these insights into breeding selection processes can facilitate the development of varieties better suited to cope with climate change challenges.

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CRediT authorship contribution statement

Luciana Wilhelm De Almeida: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hernán Ojeda:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Anne Pellegrino:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Laurent Torregrosa:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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