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New Fungus-Resistant Grapevine *Vitis* and *V. vinifera* L. × *M. rotundifolia* Derivative Hybrids Display a Drought-Independent Response in Thiol Precursor Levels

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ABSTRACT: The use of new disease-resistant grapevine varieties is a long-term but promising solution to reduce chemical inputs in viticulture. However, little is known about water deficit effects on these varieties, notably regarding berry composition. This study is aimed at characterizing the primary metabolites and thiol precursors levels of 6 fungi-resistant varieties and Syrah. Vines were grown under field conditions and under different water supply levels, and harvested at the phloem unloading arrest. A great variability among varieties regarding the levels of thiol precursors was observed, with the highest concentration, of 539 μg/kg, being observed in the 3176-N, a hybrid displaying red fruits. Water deficit negatively and equally impacted the accumulation of sugars, organic acids, and thiol precursors per berry and per plant, with minor effects on their concentration. The observed losses of metabolites per cultivation area suggest that water deficits can lead to significant economic losses for the produce.

KEYWORDS: *grapevine, water deficit, berry composition, primary metabolites, secondary compounds, aroma*

1. INTRODUCTION

Viticulture is responsible for up to 60% of agrochemical use in Europe, in which most treatments are focused on the control of fungal diseases.[1] Thus, the adoption of disease-resistant varieties is a promising solution for reducing the use of pesticides. Many breeding programs have been developed to meet such demand, in Europe (mainly in Germany, Italy, Switzerland, and France) and abroad (Brazil, USA, China, and Japan). Unfortunately, insufficient attention has been paid by breeders to the performance of these varieties during challenging climate fluctuations, notably to increased water deficit (WD). Drought is one of the major limiting factors for the establishment of future viticulture that can alter grapevine development, yield, and durability besides affecting grape and wine quality.[2]

Indeed, water availability plays a major role in vegetative and reproductive developments, ultimately leading to negative impacts on yield and fruit composition. The effects of WD on berry growth are well-known, where it leads to decreases in berry volume, both by impaired cell expansion and water losses.[3] Moderate water deficit is also known to be beneficial to the accumulation of several secondary metabolites important in defining berry and wine quality, such as anthocyanins and polyphenols.[4] Nonetheless, WD effects on the aromatic potential are less clear and relative to each compound and its respective molecular group.[5] While WD is reported to promote concentration in monoterpenes, C13 norisoprenoids,[6] dimethyl sulfur potential,[7] and methoxypyrazines,[8] it decreases C6 compounds[9] and thiol precursors.[10,11]

Thiol precursors are odorless compounds being found in small concentrations in leaves and grapes, which during alcoholic fermentation are cleaved by yeast β-lyase activity, resulting in aromatic free molecules such as 3-sulfanylhexan-1-ol (3SH), 3-sulfanylhexyl acetate (3SHA), and 4-methyl-4-sulfanylpentan-2-one (4MSP), responsible for notes of grapefruit, passionfruit, and box tree, respectively.[12] Despite their small concentrations, these free molecules have a high contribution to wine aroma and typicity due to their large aromatic power (lower odor detection threshold).

Regarding 3SH precursors, these molecules are bound to amino acids (cysteine),[13] dipeptides (Cys-Gly and γ-Glu-Cys),[14,15] and glutathione (G).[16] It has been proposed that they derive from the reaction between 2-hexenal and G, catalyzed by glutathione *S*-transferases (GSTs), forming G3SH, which would subsequently cleave in either γ-Glu-Cys3SH (by glutamyltransferase) or CysGly3SH (by carboxypeptidases) and in Cys3SH, by combining both reactions.[17]

Thiol precursors levels are highly dependent on grapevine genotype with some varieties showing higher levels than others, as in *V. vinifera* cv "Sauvignon blanc", that has been reported to reach up to 1775 μg/L of glutathionylated precursor (G3SH) in grape musts[18] and where most precursors were first identified.[13] Yet 3SH precursors have been shown to be ubiquitously present in different *V. vinifera* cultivars.[19] Regarding grapevine hybrids, Nicolini et al. (2020)[20] studied 64 fungi-resistant varieties (red and whites) and identified eight varieties with high aromatic

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potential (>600 μg/kg of G3SH). Recently, another study has characterized the thiol aromatic potential of seven grapevine hybrids from French and American breeders, observing values up to 700 μg/kg of G3SH in berries.[21]

Besides genotype, thiol precursors concentration is developmentally modulated and dependent on biotic and abiotic factors and management practices. Their concentration increases during berry ripening,[22] and with incidence of *Botrytis cinerea*[23] and downy mildew.[17] Cultivation practices such as nitrogen fertilization,[24] pruning method,[25] and managing vineyards by organic or conventional methods[26] have also been shown to impact their levels in grapes. Yet, few studies have been conducted regarding how abiotic factors such as water availability, temperature, and light regulate their concentration.[17] Previous studies on Sauvignon blanc reported that mild WD was beneficial to the accumulation of thiol precursors when compared to high WD.[10,11] Moreover, Kobayashi et al. (2011)[17] observed that both G3SH and Cys3SH biosynthesis were upregulated by abiotic stresses such as water deficit in grape leaves and berries of Koshu, Chardonnay, and Merlot. However, all of these studies based their interpretations solely on concentration values, which may lead to confusion due to the double effect of berry water balance and actual metabolite synthesis. Indeed, much remains to be understood about how WD impacts accumulation and concentration of thiol precursors in the grapevine fruit. It is important to understand these regulations in order to anticipate the effect of pedoclimatic conditions and management practices, such as watering, on the type of metabolite accumulation and product profile. Yet, understanding the behavior of resistant varieties in front of WD is an important task, in view of future climate changes which they will also be subjected to. Thus, the aim of this work was to characterize the thiol aromatic potential in 6 new disease-resistant varieties and study the impact of WD on berry primary metabolites and thiol precursors accumulation and concentration.

2. MATERIALS AND METHODS

2.2. Plant Material and Growing Conditions. Experiments were performed with field-grown vines, during the 2021 season at the INRAE experimental unit of Pech Rouge, France (43.14° North | 3.14° East). The panel of the varieties included 2 already certified INRAE varieties: Artaban and Floreal and 4 new hybrids in the final stages of certification to be released from 2025:3159B, 3176N, G14, G5,[27] the 2 last ones carrying the sugarless trait,[28] and the *V. vinifera* var. Syrah. More details on the genotypes of this study (pedigree, fruit color, rootstock and year of plantation) are shown in Table S1.

The genotypes were located in close by plots where 30 plants per variety were selected and individually monitored and phenotyped. From those 30 plants, half were irrigated from flowering (June) until harvest (August). The water supply consisted of 40 L per plant per week. No fungicide was applied, with exception to the Syrah plot that was treated with Champ Flo (1.2 L/ha, 360 g/L of Cu) and Fluidosoufre S (5 L/ha, 700 g/L of S). All plots had the same planting density (4400 vines per hectare, 2.5 × 0.9 m) and orientation of rows (SW-NE) and were managed in VSP (vertical shoot positioning) based on the same pruning method,

2.3. Plant Water Status and Definition of the Harvest Date. The leaf predawn water potential (ψb) measurement was carried out weekly for each of the 30 biological replicates per genotype from June until the end of the experiment, between 3:00 h and 5:00 h, taking one leaf per plant, using a pressure chamber.

The accumulated ψb (acc ψb) before and after veraison was calculated as the area under the curve of evolution of ψb over time (number of days) per plant. All plants were then divided in function of their accψb into four different classes: mild WD (acc$\psi b \geq -0.3$ MPa), moderate WD ("M") (-0.3 MPa > acc$\psi b \geq -0.6$), high WD ("H") (-0.6 MPa > acc$\psi b \geq -0.8$ MPa) and severe WD ("S") (acc$\psi b < -0.8$ MPa).

Grape harvest date was defined as the time of phloem unloading arrest, the stage at which berry reaches the maximum water and soluble solid contents, which corresponds to the physiologically ripe stage.[29] The kinetics of berry volume was monitored through image analysis, carried out in 6 plants per variety, which were selected to cover a range of water status levels from mild to severe WD (see above). The images, taken once a week for 1 cluster per plant, were analyzed counting the number of pixels per cluster and following its increase over time.[30] The date of harvest was defined as the period where the number of grape pixels stopped increasing.

2.4. Primary Metabolites. At harvest 200 berries per plant were randomly sampled and weighed, and their juice was extracted (JumboMix mixer) and centrifuged (10 414 rcf for 5 min at 20 °C) for later composition analysis. Soluble sugars, glucose (Glc), and fructose (Fru), and main organic acids, i.e., tartaric (H2T) and malic (H2M) acids, assays were done by high-performance liquid chromatography analysis and a UV detector with a BIORAD Aminex HPX-87H column (7.8 × 300 mm), as previously described.[29,31] Amino acids and ammonium N concentrations (mg/L) were analyzed by a colorimetric method with *o*-phthalaldehyde (OPA) and *N*-acetylcysteine (NAC) (340 nm) and by an enzymatic method with α-céto-glutarate, NADPH, glutamate dehydrogenase (340 nm), respectively. Both were assessed with the Sequential Analyzer Gallery (Thermo Fisher Scientific, CERGY-PONTOISE, France). The yeast assimilable nitrogen (YAN) was calculated by the sum of amino acids and ammonium content.

2.5. Thiol Precursors Analysis. *2.5.1. Chemical Syntheses.* Chemical syntheses of natural and deuterated thiol precursors were performed as described in ref 26.

2.5.2. Sample Preparation and Analysis by LC-MS/MS. A sample of 50 berries per plant was taken, weighed, and stored at −20 °C for later analysis of thiol precursors. Prior to analysis, berries were unfrozen overnight at −4 °C and then crushed in a 250 mL mixer with sodium metabisulfite and benzene sulfinic acid (4.5 mg/mL $Na_2S_2O_5$ and 1 mg/mL of ABS of expected volume), and centrifuged (10 414 rcf, for 15 °C at 4 °C). The clear juice was filtered, and a 2 mL of solution was taken and stored at −20 °C prior to analysis. Thiol's precursors of 3SH (glutathionylated − G3SH, dipeptides − CysGly3SH and γ-GluCys3SH, cysteinylated − Cys3SH) and of 4MSP (glutathionylated − G4MSP and cysteinylated − Cys4MSP) were analyzed by a stable isotope dilution assay and LC-MS/MS through direct injection of grape must from the 6 resistant varieties studied and Syrah as previously reported.[26]

2.6. Data Representation and Statistical Analysis. All results were presented in mol per volume, berry, or plant, as well as in mol of C equivalents. The conversion for soluble sugars (glucose + fructose) and organic acids (malic + tartaric) was done considering their respective molecular masses (MW: 180, 180, 134, and 150 g/mol) and adjusted depending on the carbon skeleton structure of each compound, i.e., hexoses and organic acids with 6 and 4 atoms of carbon, respectively. For YAN, we considered the molecular masses of NH_4^+ (18.03) and an average of molecular masses (136.9) and number of C atoms (5.35) of all 20 proteinogenic nitrogen compounds (alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine) found in the grapevine fruit juice. Thiol precursors were expressed in mol per mass, mol per volume, mol per berry, as well as in mol of C equivalents per berry. The conversion was done considering their molecular masses and number of carbon (respectively), G3SH (407, 16), Cys3SH (221, 9), and CysGly3SH (278, 11).

The quantification of metabolites per berry was calculated as follows:

Table 1. Number of Plants and Means ± Standard Deviations for Accumulated ψb from Veraison to Harvest (Acc-ψb) and Berry Weight, Per Genotype and Water Deficit Class[a]

	Water deficit	Syrah	3176-N	Artaban	G14	Floreal	3159-B	G5
Number of plants	M	0	12	16	9	5	12	16
	H	5	8	9	6	9	3	5
	S	25	10	15	15	16	15	9
	Total	**30**	**30**	**30**	**30**	**30**	**30**	**30**
AccYb (MPa)	M	-	−0.51 ± 0.04	−0.52 ± 0.03	−0.49 ± 0.06	−0.57 ± 0.01	−0.54 ± 0.06	−0.51 ± 0.04
	H	−0.73 ± 0.04	−0.73 ± 0.07	−0.71 ± 0.04	−0.70 ± 0.08	−0.68 ± 0.05	−0.69 ± 0.06	−0.74 ± 0.05
	S	−1.02 ± 0.16	−0.85 ± 0.15	−0.89 ± 0.15	−1.05 ± 0.15	−1.00 ± 0.10	−0.93 ± 0.06	−0.92 ± 0.07
	Mean	-0.97 ± 0.20	-0.68 ± 0.16	-0.64 ± 0.16	-0.81 ± 0.20	-0.83 ± 0.20	-0.75 ± 0.19	-0.67 ± 0.20
G ***		d	abc	a	bc	cd	abc	ab
WD per genotype		a b	a b c	a b c	a b c	a b c	a b c	a b c
Berry weight (g)	M	-	1.9 ± 0.3	1.3 ± 0.1	1.6 ± 0.2	1.9 ± 0.03	1.8 ± 0.5	1.7 ± 0.2
	H	1.4 ± 0.1	1.5 ± 0.1	1.0 ± 0.1	1.2 ± 0.4	1.6 ± 0.04	1.4 ± 0.1	1.1 ± 0.2
	S	0.9 ± 0.3	1.2 ± 0.2	0.9 ± 0.1	0.8 ± 0.2	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
	Mean	**1.0 ± 0.3**	**1.6 ± 0.4**	**1.2 ± 0.2**	**1.1 ± 0.4**	**1.3 ± 0.4**	**1.3 ± 0.5**	**1.5 ± 0.4**
G ***		d	a	cd	bcd	ab	a	abc
WD per genotype		a b	a b b	a b b	a a b	a b c	a a b	a b b

[a]M, H, and S indicate moderate, high, and severe water deficit classes. Different letters indicate statistical difference (p-value < 0.05). ns indicates non-significance.

Table 2. Soluble Sugars (mol/L), Organic Acids (mmol/L), and Yeast Assimilable Nitrogen (mmol/L) Means ± Standard Deviations, Per Genotype and Water Deficit Class[a]

primary metabolites	Water deficit	Syrah	3176-N	Artaban	G14	Floreal	3159-B	G5
Soluble sugars (mol/L)	M	-	1.34 ± 0.04	1.20 ± 0.04	1.20 ± 0.03	1.29 ± 0.03	1.42 ± 0.03	1.21 ± 0.04
	H	1.38 ± 0.15	1.30 ± 0.09	1.13 ± 0.06	1.17 ± 0.06	1.28 ± 0.05	1.44 ± 0.02	1.22 ± 0.06
	S	1.42 ± 0.08	1.26 ± 0.06	1.11 ± 0.09	1.10 ± 0.03	1.37 ± 0.05	1.48 ± 0.04	1.23 ± 0.07
	Mean	**1.41 ± 0.09**	**1.30 ± 0.07**	**1.16 ± 0.07**	**1.14 ± 0.06**	**1.33 ± 0.06**	**1.45 ± 0.04**	**1.22 ± 0.05**
G ***		a	b	cd	d	b	a	c
WD per genotype		ns	a ab b	a b b	a a b	b b a	b ab a	ns
Organic acids (mmol/L)	M	-	58 ± 3	59 ± 2	49 ± 5	66 ± 3	59 ± 3	53 ± 4
	H	70 ± 2	57 ± 3	58 ± 4	48 ± 5	64 ± 2	60 ± 3	51 ± 3
	S	72 ± 4	57 ± 1	62 ± 5	54 ± 6	70 ± 2	61 ± 4	55 ± 4
	Mean	**71 ± 4**	**58 ± 2**	**60 ± 3**	**51 ± 6**	**68 ± 4**	**60 ± 4**	**53 ± 4**
G ***		a	c	bc	d	a	b	d
WD per genotype		ns	ns	ns	ab b a	b b a	ns	ns
YAN (mmol/L)	M	-	1.8 ± 0.6	0.6 ± 0.4	0.5 ± 0.3	2.3 ± 0.2	1.0 ± 0.5	1.7 ± 0.9
	H	2.6 ± 0.9	1.6 ± 0.3	0.7 ± 0.4	0.4 ± 0.2	2.1 ± 0.4	0.5 ± 0.2	1.7 ± 0.4
	S	1.3 ± 0.9	2.0 ± 0.4	1.6 ± 1.2	0.3 ± 0.2	1.9 ± 0.5	0.6 ± 0.2	1.5 ± 0.7
	Mean	**1.6 ± 0.9**	**1.8 ± 0.5**	**0.8 ± 0.6**	**0.4 ± 0.3**	**2.1 ± 0.4**	**0.7 ± 0.4**	**1.6 ± 0.7**
G ***		b	ab	c	d	a	c	b
WD per genotype		a b	ns	ns	a a b	ns	a b b	ns
pH	M	-	3.44 ± 0.04	3.42 ± 0.06	3.43 ± 0.08	3.40 ± 0.05	3.35 ± 0.06	3.34 ± 0.06
	H	3.37 ± 0.11	3.39 ± 0.04	3.34 ± 0.08	3.48 ± 0.07	3.41 ± 0.06	3.29 ± 0.04	3.42 ± 0.12
	S	3.37 ± 0.06	3.36 ± 0.05	3.34 ± 0.06	3.41 ± 0.06	3.45 ± 0.05	3.30 ± 0.06	3.41 ± 0.10
	Mean	**3.37 ± 0.07**	**3.40 ± 0.06**	**3.38 ± 0.08**	**3.43 ± 0.07**	**3.43 ± 0.06**	**3.32 ± 0.06**	**3.37 ± 0.09**
G ***		cd	abc	abc	a	ab	d	bcd
WD per genotype		ns	a b b	a b b	ns	ns	ns	ns

[a]M, H, and S indicate moderate, high, and severe water deficit classes. Different letters indicate statistical difference (p-value < 0.05). ns indicates non-significance.

$$\text{metabolite (mol, µmol, or nmol/berry)} = [\text{metabolite}]\ (\text{g, mg, or } \mu\text{g/kg} \times 1000) \times \text{BW (g/berry)} \div \text{MW}$$

The quantity per plant and cultivated area were then estimated by multiplying the metabolite per berry by the number of berries per plant and later by the number of plants per hectare.

All variables were analyzed with the nonparametric test Kruskal−Wallis (0.05 significance level) with genotype and water deficit level as factors. Corrections for multiple comparisons were performed with a Bonferroni adjustment. Correlations between variables were performed and taken into account when Pearson coefficients were higher than 0.40 (0.05 significance level). Graphical processing and statistical tests were performed using R Studio software. Image analysis was done using ImageJ software.

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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

3. RESULTS AND DISCUSSION

3.1. Climatic Conditions and Plant Water Status. The average of maximum and minimum temperatures for the 2021 cycle (April to October) were 28.8 and 8.3 °C, and a longer period (3 days) with extreme temperatures (T_{max} above 35 °C) was recorded in June. The annual rainfall in 2021 was 190 mm, resulting in a climatic water balance ($\sum$Rainfall − $\sum$Evapotranspiration) of −716 mm (Figure S1) and a calculated dryness index (DI,[32]) of −76 indicating a moderately dry year. The Winkler and Huglin indexes were respectively 2096° days and 2288 °C, which are typical of a warm temperate region.[32]

All plants (irrigated and nonirrigated) decreased their ψb from flowering to harvest, but nonirrigated plants showed a greater decrease (data not shown). In the period from veraison to harvest, plants in all varieties were differently distributed into three WD levels (moderate, high, and severe regarding their accψb) (Table 1). In general, Artaban and Syrah showed the highest (−0.64 MPa) and lowest (−0.97 MPa) accψb mean, while others showed intermediate values.

At the physiological ripe stage, the fresh berry weight varied from 1.0 to 1.6 g, respectively for Syrah and 3176-N (Table 1). Water deficit decreased the berry weight, from M to S treatments, while berries from H treatment were either different (Syrah, 3176-N, and G14) or equal (Artaban, Floreal, 3159-B and G5) to M and S. The negative effect of WD on berry size has been broadly reported[3,33] and is related to an impaired cell expansion due to a reduced water flow.[3]

3.2. Genotypic Variations of the Composition of the Fruits at the Physiological Ripe Stage. *3.2.1. Primary Metabolites.* Soluble sugars varied from 1.15 mol/L, in Artaban and G14, to 1.40 mol/L, in Syrah and 3159B, with a glucose to fructose ratio of 1 (Table 2). It represented a range of total soluble solids from 21°Brix to 25°Brix. Such values of concentration and composition are inside the expected range previously reported for *V. vinifera* and interspecific hybrids.[27,34]

The pH ranged from 3.32 in G5 to 3.43 in G14 and Floreal, with other varieties showing intermediate values. The organic acids concentration (H2M + H2T) varied from 52 mmol/L (in G14 and G5), to 70 mmol/L (in Syrah and Floreal) (Table 2), which represents a range of total acidity from 71.9 mequiv/L to 93.7 mequiv/L. Slightly lower organic acids concentrations were observed when comparing with values found by Bigard et al.,[34] but the proportion of H2M to H2T was found to be similar, varying from 0.18 to 0.39, in 3159B and G5, respectively.

In addition, both sugar-less varieties (G14 and G5) showed the lowest concentration of organic acids (51 mmol/L and 53 mmol/L, respectively) and soluble sugars (1.14 and 1.22 mol/L, respectively). Our results confirm their sugar-less trait[28] even though they show values slightly above those reported previously, which observed a maximum of nearly 1 M (1000 mmol/L).[27−29]

Variations observed among genotypes can also be related to an overestimation of V_{max}, i.e., harvesting after phloem unloading. The V_{max} is the moment the phloem stops loading water and solutes (mainly soluble sugars) into the berries, defining the moment of maximum volume and solutes. When V_{max} is estimated at the cluster level (due to intracluster heterogeneity) it averages berries from three developmental stages: (i) berries that are still on active loading, (ii) berries that are at their exact V_{max}, and (iii) berries that started to lose volume (water) and thus concentrate solutes.[29]

Yeast assimilable nitrogen concentration values in grape juices ranged from 0.4 mmol/L (37 mg/L) to 2.1 mmol/L (183 mg/L) in G14 and Floreal, respectively. YAN is linked to enological parameters such as yeast nutrition, fermentation kinetics, and wine aromas. YAN values from 140 mgN/L[35] to 267 mgN/L for a 200 g/L of glucose in the initial must (around 11.5° EtOH)[36] have been proposed to avoid stuck fermentations and wine defaults. All varieties (except Floreal) showed YAN values below 140 mgN/L, suggesting that a specific nitrogen supply, in grape must, would be necessary to successfully complete alcoholic fermentation.

3.2.2. Thiol Precursors. Varietal thiols such as 3-sulfanylhexan-1-ol (3SH), its acetate (3SHA) and the 4-methyl-4-sulfanylpentan-2-one (4MSP) are powerful aroma compounds in both red and white wines.[12] They came mainly from odorless compounds called thiol precursors, and up to now 4 different families have been identified in grapes: *S*-conjugate to glutathione, *S*-conjugate to dipeptides (γ-GluCys and CysGly for 3SH only), and *S*-conjugate to cysteine.[19,37]

To give a complete picture of the aromatic potential, we analyzed 6 thiol precursors (G3SH, CysGly3SH, γ-GluCys3SH, Cys3SH, G4MSP, and Cys4MSP) in 6 resistant varieties (3 displaying white fruits and 3 displaying red fruits) and Syrah. Among the samples, only 3 precursors were identified and quantified: G3SH, Cys3SH, and CysGly3SH (Figure 1 and Table S2). The absence of 4MSP precursors in the six resistant varieties here studied is in accordance with previous studies conducted with different grapevine hybrids.[20,21]

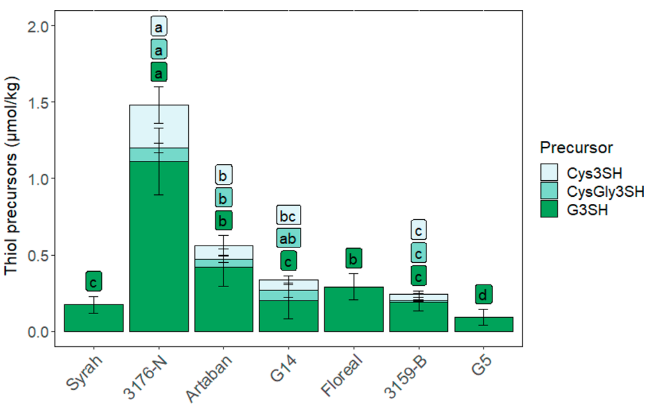

Figure 1. Thiols precursors (G3SH, Cys3SH, and CysGly3SH) mean concentration (μmol/kg) for Syrah and 6 resistant genotypes, Gruissan - France, 2021. Different letters with the same color indicate statistical difference (LSD, p-value < 0.05).

In general, the glutathionylated precursor G3SH contents represented between 70% to 100% of the total thiol precursors, followed by the cysteinylated (0−13%) and CysGly3SH precursor (0−17%). G3SH (identified in all varieties) ranged from 0.09 μmol/kg (G5) to 0.29 μmol/kg (Floreal), in white fruit varieties, and from 0.17 μmol/kg (Syrah) to 1.11 μmol/kg (3176N) in red fruit varieties. The cysteinylated (Cys3SH) and dipeptide precursor (CysGly3SH) were only identified in 3176N, Artaban, G14, and 3159B, where the former ranged from 0.04 μmol/kg to 0.28 μmol/kg, and the latter from 0.01 μmol/kg to 0.09 μmol/kg, in 3159B and 3176N, respectively (Figure 1 and Table S2). Both the quantities and proportion here reported were in accordance with previous studies conducted with *V. vinifera* varieties, with interspecific hybrids for studies considering all families of thiol precursors,[14,15,21,26]

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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

and with other fungi-resistant hybrids taking into account only G3SH and Cys3SH.[20] To our knowledge, this is the first time where one dipeptide precursor (CysGly3SH) has been identified and quantified in disease-resistant varieties. Thiol precursors for these hybrid varieties were below concentrations found in Sauvignon blanc (until 4.37 μmol/L according to ref 18) except 3176N, which demonstrated exceptional levels for a grapevine red fruit variety. Interestingly, this could be related to its genetic background, since 3176N results from the cross-breeding of Grenache and 3084-2-46.[27] It is well-known that Grenache rosé wines contain important levels of 3SH with concentrations reaching up to 675 ± 419 ng/L of 3SH in selected samples from Provence in France.[38] Red Grenache wines exhibited also significant levels of 3SH up to 854 ng/L in Coteaux du Languedoc wines (France),[39] and until 4 μg/L in Spanish Grenache red wines,[40] highlighting the link of such molecules with this specific cultivar. However, to date, no data on precursors in Grenache grapes are available to our knowledge. Considering these aspects, the cultivar 3176N seems interesting to be fermented as well as rosé or red wines.

Besides the varietal effect, the G3SH concentration may vary with vine and must nitrogen status, being affected by foliar and soil fertilization.[24,41] Among the 7 varieties studied, two with highest precursor levels showed positive correlations between YAN and G3SH, 3176N (0.44), and G14 (0.53) (Figure S3). Similar results were observed by Helwi et al.,[41] where a higher YAN was related to an increased G3SH concentration. However, such relations are not always so clear;[42] for example, it was found that correlations were dependent on the amino acid, where glycine, GABA, and isoleucine showed positive correlation, while glutamic acid and alanine showed negative correlations. All previous works were conducted with Sauvignon blanc grapes, and thus more studies concerning other varieties may be needed. Yet, a recent study reported no correlation between berry amino acids and the levels of thiol precursors on grapevine hybrids,[21] similar to the results obtained here for the Floreal, G5, 3159B, Artaban, and Syrah.

Several studies proposed that G3SH would derive from the junction of hexanal and glutathione, catalyzed by glutathione-*S*-transferases (GST).[16,17] Three genes were previously proposed to be involved in the biosynthesis of G3SH in grapevine and in the synthesis of GST's, VvGST1, VvGST3, and VvGST4, which are expressed under stress conditions in leaves and berry skin.[17] Both VvGST1 and VvGST4 were also observed to be related in the transport of anthocyanin into vacuole of grape cells.[43] A higher expression of those genes in red fruit varieties (for anthocyanin transportation) could explain the higher concentration of thiol precursors found in our red fruit varieties. Nicolini et al.[20] also observed higher concentrations of G3SH when comparing 23 red (0.82 μmol/kg) and 15 white (0.29 μmol/kg) resistant varieties.

3.3. Effect of the Water Deficit on the Fruit Composition at a Physiological Ripe Stage.

3.3.1. Methodology for Sampling. In the present study, the effects of WD on 6 new fungi-resistant genotypes were characterized on the basis of leaf predawn water potential (Table 1), of berry primary metabolites (Table 2, Figure 2) and thiol precursors (Figure 1, Figure 3, Table S2). The difficulty in deciphering water balance variations (accumulation and losses) and actual biosynthesis highlights the importance (i) to analyze berry metabolites content and concentration and (ii) to properly determine the sampling/harvest date as a function of the physiological development instead of the technological maturity.[5,29] Yet, analyzing results based solely on concentration values, sampled in different physiological stages, can lead to analytical bias and consequently opposite conclusions as observed previously.[10,44] Therefore, in the present study, to avoid any analytical bias, all samples were harvested at the same physiological stage (at the arrest of phloem unloading in the fruit, i.e., berry V_{max}[29,31]).

Besides, by analyzing the quantity of metabolites per plant, we can assess the global performance of the productive system when confronted with environmental variations. Thus, all the results hereafter are expressed in both quantity of molecules per berry (mol per berry) and per plant as already described for other secondary metabolites.[7,31,45,46] Yet, in order to evaluate the possible metabolic trade-offs between thiol precursors and primary metabolites, under WD conditions, ratios between the C equivalents of total thiol precursors and the sum of C equivalents accumulated in soluble sugars (SS), organic acids (OA), and YAN were also calculated.

3.3.2. Water Deficit Effects on Primary Metabolites. When analyzing the WD effects on soluble sugars, organic acids and YAN content per berry, we observed an important decrease between M and S treatments, for all varieties (p-value < 0.05, Figure 2). The reduction in soluble sugars content ranged from

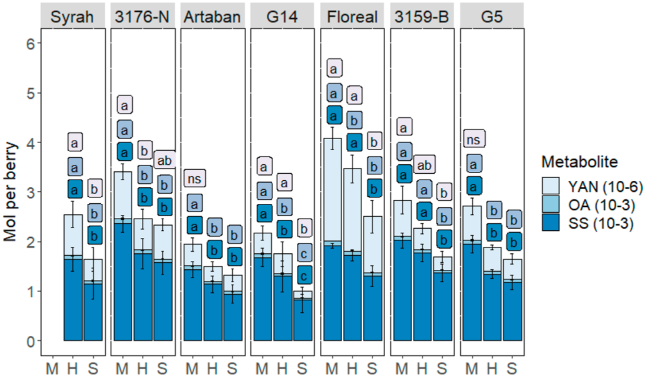

Figure 2. Soluble sugars (SS × 10^{-3}), organic acids (OA × 10^{-3}), yeast assimilable nitrogen (YAN × 10^{-6}) means ± standard deviations in mol per berry, for Syrah and 6 resistant genotypes, per water deficit class (M, H, and S indicate moderate, high, and severe water deficit classes), Gruissan - France, 2021. Different letters with the same color, within genotype, indicate a statistical difference (LSD, p-value < 0.05); ns indicates nonsignificance.

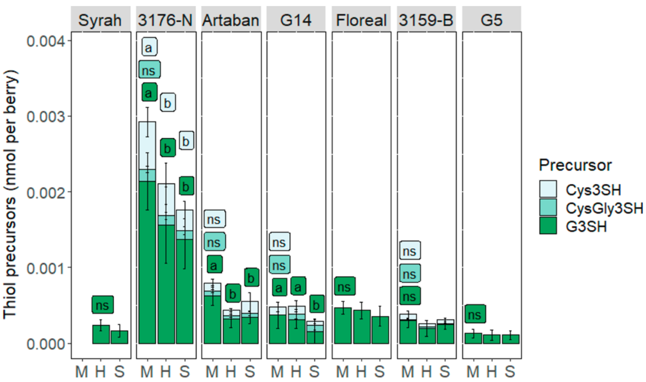

Figure 3. Thiols precursors (G3SH, Cys3SH, and CysGly3SH) mean in content per berry (nmol/berry) for Syrah and 6 resistant genotypes, per water deficit class (M, H, and S indicate moderate, high and severe water deficit classes), Gruissan - France, 2021. Different letters with the same color indicate statistical difference (LSD, p-value < 0.05); ns indicates nonsignificance.

−32% to −51% in Floreal and G14. Similar percentages were observed in the reduction of organic acids content, which ranged from −27% to −43% in Artaban and G14. These reductions are related to the negative effects of severe WD on the fruit sink strength (smaller berries) and on leaf carbon assimilation (i.e., photosynthesis), that may lead to lower carbon and water partitioning to berries.[47] Yeast assimilable nitrogen content showed a higher variation, ranging from −20% to −72% (in 3176-N and G14 respectively) between M and S treatments. Reductions in YAN content waere also observed in Pinot Noir and Arvine grapes grown in Switzerland.[44,48] However, other studies have shown that water deficit increased amino acids content,[49] mainly due to a higher accumulation of proline, arginine, alanine, GABA, and glutamic acid. However, the same authors emphasize that accumulations vary among grapevine variety, season, and rootstock.

Besides the negative effects observed at the fruit level, WD reduces yield, due to reduction in the number of clusters and of berries per plant, leading to further losses in the total yield of metabolites per cultivation unit area. When the total production of primary metabolites per plant was observed, WD strongly decreased the total SS, AO, and YAN per plant (p-value <0.05) in 3176-N, 3159-B, Floreal, and Syrah (Table S3). Floreal, which was the most affected genotype, showed reductions up to 70% in SS, AO, and YAN between M and S treatments, which resulted in losses of 5808 mol (1,043 kg), 300 mol (44 kg), and 13 mol (1 kg) per hectare, respectively. In contrast, 3176-N (the least affected genotype) showed losses of 3212 mol (530 kg), 123 mol (18 kg), and 2.2 mol (0.2 kg) per hectare, in SS, AO, and YAN, respectively (reduction of approximately 20%).

3.3.3. Water Deficit Effects on Thiol Precursors. In our study, grapes from S treatment showed strong reductions in the contents of G3SH in 3176-N, Artaban, and G14 (−36%, −46%, and −59% respectively) and in the contents of Cys3SH in 3176-N (−56%), per unit of fruit (Figure 3). Kobayashi et al.[17] proposed that abiotic stresses as radiance, temperature, and water deficit would increase thiols precursor synthesis due to a higher expression of VvGST's genes, and GST enzyme activity. However, such an expected increase was not observed in our study, and our results rather suggested that the synthesis of thiol precursors was negatively affected by WD.

Comparing our results with previous studies might be complex, since most are based on concentration values resulting from a pool of berries sampled at different physiological stages. Zufferey et al.[44] concluded that Cys3SH concentration on Arvine grapes was not affected by WD (ψb of −0.8 MPa). However, if considering the decrease of berry weight, and expressing their results on a per berry basis, a 30% reduction on the aromatic potential was observed. Other studies on Sauvignon Blanc grapes reported higher volumic concentrations of thiol precursors when vines were subjected to ψb higher than −0.40 MPa (mild to moderate WD).[10,11] In addition to different harvest reasoning, differences in experiment location, water deficit timing and intensity, leaf to fruit ratio, sample preparation, analytical methods and varieties studied can complicate comparisons between studies.

The total quantity of G3SH per plant was also negatively affected by WD in 5 of the 6 hybrid genotypes studied (3176-N, Artaban, G14, Floreal, 3159-B) and Syrah (Table S3). The highest reduction per plant was observed for Floreal and G14 (−60%) and the lowest for Artaban (−25%), equivalent to a loss of 1.2 μmol/ha (487 μg/ha) and 1 μmol/ha (389 μg/ha), respectively. Similarly, Cys3SH was decreased by 54% in 3176-N resulting in a loss per hectare of 1.9 μmol (Table S3). Overall, water deficit showed a negative effect on total accumulation of thiol precursors (sum of all precursors) per unit of fruit and per plant, for most of the genotypes studied.

3.3.4. Water Deficit Effects on the Proportion between Thiol Precursors and Primary Metabolites. In order to evaluate the possible metabolic trade-offs between thiol precursors and primary metabolites, under WD conditions, we estimated the mol of C equivalents from the molar concentrations of each metabolite (Table S4), and ratios between the total C allocated to total thiol precursors and total primary metabolites (the sum of soluble sugars (SS), organic acids (OA), and YAN) were calculated.

The major nonstructural C (NS-C) compartment was soluble sugars, followed by organic acids. The quantity of C allocated to YAN (amino acids) was a thousand times lower than SS and AO and to thiol precursors was even lower, with values on the order of 10^{-9} compared to SS and AO (Figure 2 and Table S4). Indeed, in the berry, considering the NS-C pool, sugars and organic acids are the main metabolic C sink[50] with secondary metabolites showing a low C sink strength, representing 1−2% of NS-C.[31] The ratio between thiol precursors and soluble sugars varied from 5.9 to 43.6×10^{-7} in the red fruit varieties Syrah and 3176-N, and from 3.4 to 1.0×10^{-10} in the white fruit varieties G5 and Floreal, respectively (Figure 4 and Table S4). In general,

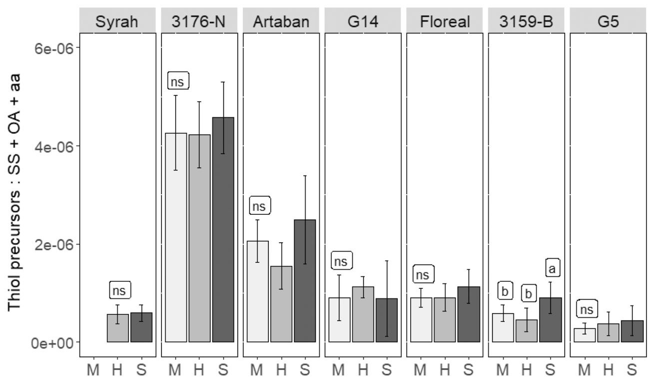

Figure 4. Ratio between total thiol precursors (Pthiols) and primary metabolites (Met1), in mol of Ceq/L, for Syrah and 6 resistant genotypes, per water deficit class (M, H, and S indicate moderate, high and severe water deficit classes), Gruissan - France, 2021. Different letters with the same color indicate statistical difference (LSD, p-value < 0.05); ns indicates nonsignificance.

water deficit had no significant effect on the ratio of total thiol precursors per total primary metabolites, despite the slight increase seen from M to S treatments. This shows that WD had similar negative impacts in both primary metabolites and thiol precursors accumulation. Interestingly, one exception was the white fruit variety, 3159-B, in which the increase in the ratio was significantly different (p-value <0.05). Such an increase indicates that under WD, the metabolic cost for these plants, to accumulate thiol precursors, was lower than that of sugars, acids, and amino acids all together. Indeed, changing the balance between secondary and primary metabolites is not obvious, and it seems to be more dependent on genotype and climatic variations than management practices.[31,46]

For the first time, fungi-resistant varieties have been characterized regarding berry primary metabolites and thiol precursors under different water supply levels. There were small differences regarding primary metabolites concentrations

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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

(soluble sugars, organic acids, and YAN) among genotypes, but a great variability among varieties regarding their levels on thiol precursors was found. From those, one red fruit variety, the 3176-N, was identified with very high levels of thiol precursors, showing a strong aromatic potential. Usually, moderate WD is seen as a positive factor in vineyards, based on the fact that it would increase the concentration of metabolites that contribute to wine quality. However, this general idea is often supported by studies that base their harvest date on parameters linked solely on metabolite concentration, rather than a specific and precise physiological development point. In the present study, grape sampling was targeted at berry phloem unloading stop, the moment at which maximum water and solute content is achieved, making it possible to discriminate accumulation from concentration. The lack of variability due to WD in the concentration of thiol precursors (an important factor contributing to grape quality) and the consistent decrease in content per berry, plant, and cultivation area unit suggest a significant economic loss for the producer, counterposing the supposed positive effect of WD. Yet, even though the greatest source of variation in thiol precursors levels is genotype related, further studies in different climatic conditions would be advised, to better understand how the interaction with the environment could potentially impact such metabolites.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.2c08595.

Additional plant material information (breeding, rootstock, and age of plants) (Table S1). Weather data during growing season (April to October, 2021) (Figure S1). G3SH, Cys3SH, and CysGly3SH means ± standard deviations in concentration (μg/kg), per genotype and water deficit class (Table S2). Thiol precursor (G3SH, Cys3SH, and CysGly3SH) mean concentration (μmol/L) for Syrah and 6 resistant genotypes (Figure S2). Pearson correlation between G3SH μmol/kg and YAN mmol/kg (Figure S3). Soluble sugars, organic acids, total amino acids, total thiol precursors mean ± standard deviations in mol per plant, per genotype and water deficit class (Table S3). Soluble sugars, organic acids, total amino acids, total thiol precursors, and calculated ratio means ± standard deviations in molar concentration of carbon equivalents, per genotype and water deficit class (Table S4) (PDF)

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Notes

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ABBREVIATIONS USED

WD, water deficit; G3SH, 3-sulfanylhexan-1-ol-L-glutathione; Cys3SH, 3-sulfanylhexan-1-ol-L-cysteine; CysGly3SH, 3-sulfanylhexan-1-ol-L-cysteinylglycine; G4MSP, 4-methyl-4-sulfanylpentan-2-one-glutathione; Cys4MSP, 4-methyl-4-sulfanylpentan-2-one-cysteine; YAN, yeast assimilable nitrogen; GST, glutathione-*S*-transferases; V_{max}, cluster/berry maximum volume; accψb, accumulated predawn water potential

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