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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**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. 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. 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. 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. Nonetheless, WD effects on the aromatic potential are less clear and relative to each compound and its respective molecular group. While WD is reported to promote concentration in monoterpens, C13 norisoprenoids, dimethyl sulfon pollutant, and methoxypyrazines, it decreases C6 compounds and thiol precursors. 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. Despite their small concentrations, these free molecules have a high contribution to wine aroma and typically due to their large aromatic power (lower odor detection threshold).

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 and where most precursors were first identified. Yet 3SH precursors have been shown to be ubiquitously present in different *V. vinifera* cultivars. Regarding grapevine hybrids, Nicolini et al. (2020) studied 64 fungus-resistant varieties (red and whites) and identified eight varieties with high aromatic...
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.14–16 Besides genotype, thiol precursors concentration is developmentally modulated and dependent on biotic and abiotic factors and management practices. Their concentration increases during berry ripening,17,18 and with incidence of Botrytis cinerea19–21 and downy mildew.22 Cultivation practices such as nitrogen fertilization,23 pruning method,24 and managing vineyards by organic or conventional methods20 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. Previous studies on Sauvignon blanc reported that mild WD was beneficial to the accumulation of thiol precursors when compared to high WD.20,21 Moreover, Kobayashi et al. (2011)17 observed that both G3SH and Cys3SH biosynthesis were up-regulated 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, and the genotypes of this study (pedigree, fruit color, rootstock and year of plantation) are shown in Table S1.

2.2. Data Preparation and Statistical Analysis. 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 unfrenced overnight at −4 °C and then crushed in a 250 mL mixer with sodium metabisulfite and benzene sulfinic acid (4.5 mg/mL Na2S2O5 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 − Cys3SH, cysteinylated − Cys3SH) and of 4MSP (glutathionylated − GluCys3SH, cysteinylated − Cys3SH and of 4MSP (glutathionylated − GluCys3SH, cysteinylated − Cys4MSP) were analyzed by a stable isotope dilution assay and LC-MS/MS through direct injection of grape juice from the 6 resistant varieties studied and Syrah as previously reported.26

2.6. Data Representation and Statistical Analysis. All results were present 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., 184 hexoses and organic acids with 6 and 4 atoms of carbon, respectively. For YAN, we considered the molecular masses of NH3 (18.03) and an average of molecular masses of (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, and 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 ψ\textsubscript{b} from Veraison to Harvest (Acc-ψ\textsubscript{b}) and Berry Weight, Per Genotype and Water Deficit Class\textsuperscript{a}

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

\textsuperscript{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\textsuperscript{a}

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

\textsuperscript{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.

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.

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.

metabolite (mol, μmol, or nmol/berry) = [metabolite] (g, mg, or μg/kg × 1000) × BW (g/berry) ÷ MW

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.
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 (Tmax above 35 °C) was recorded in June. The annual rainfall in 2021 was 190 mm, resulting in a climatic water balance \((\Sigma \text{Rainfall} - \Sigma \text{Evapotranspiration})\) of −716 mm (Figure S1) and a calculated dryness index \((\text{DI}, {32})\) 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 \(\psi_b\) 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 \(\psi_{acc}/\psi_b\)) (Table 1). In general, Artaban and Syrah showed the highest (−0.64 MPa) and lowest (−0.97 MPa) \(\psi_{acc}/\psi_b\), 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 reported33,34 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 3159-B, 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 3176B and Floreal), 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.,27 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 trait33,34 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 \(V_{\text{max}}\) i.e., harvesting after phloem unloading. The \(V_{\text{max}}\) 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 \(V_{\text{max}}\) 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 \(V_{\text{max}}\) 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/L35 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 nutrient supply, in grape must, would be necessary to successfully complete alcoholic fermentation.

3.2.2. Thiol Precursors. Varietal thios such as 3-sulfanylhexa-1,5-dione (3SH), its acetate (3SHA) and the 4-methyl-4- sulfanylpentan-2-one (4MSP) are powerful aroma compounds28 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 (\(\gamma\)-GlucCys 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, Cys3SH3, \(\gamma\)-GlucCys3SH, Cys3SH, G4MSP, and Cys4MSP) in 6 resistant varieties (3176-N 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](https://doi.org/10.1021/acs.jafc.2c08595)

Figure 1. Thiols precursors (G3SH, Cys3SH, and CysGly3SH) mean concentration (\(\mu\)mol/kg) for Syrah and 6 resistant genotypes, Grussan - 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 \(\mu\)mol/kg (GS) to 0.29 \(\mu\)mol/kg (Floreal), in white fruit varieties, and from 0.17 \(\mu\)mol/kg (Syrah) to 1.11 \(\mu\)mol/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 0.09 \(\mu\)mol/kg to 0.28 \(\mu\)mol/kg, and the latter from 0.01 \(\mu\)mol/kg to 0.09 \(\mu\)mol/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.28,29,30,31
and with other fungi-resistant hybrids taking into account only 333 G3SH and Cys3SH. To our knowledge, this is the first time 334 where one dipeptide precursor (CysGly3SH) has been 335 identified and quantified in disease-resistant varieties. Thiol 336 precursors for these hybrid varieties were below concentrations 337 found in Sauvignon blanc (until 4.37 μmol/L according to ref 338 18) except 3176N, which demonstrated exceptional levels for a 339 grapevine red fruit variety. Interestingly, this could be related to 340 its genetic background, since 3176N results from the cross- 341 breeding of Grenache and 3084-2-46. It is well-known that 342 Grenache rosé wines contain important levels of 3SH with 343 concentrations reaching up to 675 ± 419 ng/L of 3SH in 344 selected samples from Provence in France. Red Grenache 345 wines exhibited also significant levels of 3SH up to 854 ng/L in 346 Coteaux du Languedoc wines (France), and until 4 μg/L in 347 Spanish Grenache red wines, 40 highlighting the link of such 348 molecules with this specific cultivar. However, to date, no data 349 on precursors in Grenache grapes are available to our 350 knowledge. Considering these aspects, the cultivar 3176N 351 seems interesting to be fermented as well as rose or red wines. 352

Besides the varietal effect, the G3SH concentration may vary 353 with vine and must nitrogen status, being affected by foliar and 354 soil fertilization. Among the 7 varieties studied, two with 355 the highest precursor levels showed positive correlations between 356 YAN and G3SH, 3176N (0.44), and G14 (0.53) (Figure S3). 357 Similar results were observed by Helwet et al., where a higher 358 YAN was related to an increased G3SH concentration. 359 However, such relations are not always so clear; for example, 360 it was found that correlations were dependent on the amino acid, 361 where glycine, GABA, and isoleucine showed positive 362 correlation, while glutamic acid and alanine showed negative 363 correlations. All previous works were conducted with Sauvignon 364 blanc grapes, and thus more studies concerning other varieties 365 may be needed. Yet, a recent study reported no correlation 366 between berry amino acids and the levels of thiol precursors on 367 grapevine hybrids, similar to the results obtained here for the 368 Floreal, G5, 3159B, Artaban, and Syrah.

Several studies proposed that G3SH would derive from the 369 junction of hexanal and glutathione, catalyzed by glutathione-S- 370 transferase (GST). Three genes were previously proposed 371 to be involved in the biosynthesis of G3SH in grapevine and in 372 the synthesis of GST’s, VvGST1, VvGST3, and VvGST4, which 373 are expressed under stress conditions in leaves and berry skin. 374 Both VvGST1 and VvGST4 were also observed to be related in 375 the transport of anthocyanin into vacuole of grape cells. A 376 higher expression of those genes in red fruit varieties (for 377 anthocyanin transportation) could explain the higher concentra- 378 tion of thiol precursors found in our red fruit varieties. 379 Nicoli et al. observed higher concentrations of G3SH 380 when comparing 23 red (0.82 μmol/kg) and 15 white (0.29 381 μmol/kg) resistant varieties.

3.3. Effect of the Water Deficit on the Fruit 382 Composition at a Physiological Ripe Stage. 3.3.1. Method- 383 ology for Sampling. In the present study, the effects of WD on 384 new fungi-resistant genotypes were characterized on the basis of 385 leaf predawn water potential (Table 1), of berry primary 386 metabolites (Table 2, Figure 2) and thiol precursors (Figure 1, 387 Figure 3, Table S2). The difficulty in deciphering water balance 388 variations (accumulation and losses) and actual biosynthesis 389 highlights the importance (i) to analyze berry metabolites 390 content and concentration and (ii) to properly determine the 391 sampling/harvest date as a function of the physiological 392 development instead of the technological maturity. Yet, 393 analyzing results based solely on concentration values, sampled 394 in different physiological stages, can lead to analytical bias and 395 consequently opposite conclusions as observed previously. 396 Therefore, in the present study, to avoid any analytical bias, all 397 samples were harvested at the same physiological stage (at 398 the arrest of phloem unloading in the fruit, i.e., berry V_{max} 399 in Figure 2). The reduction in soluble sugars content ranged from 400 7,31,45,46

- Figure 2. Soluble sugars (SS × 10^{-3}), organic acids (OA × 10^{-3}), yeast 401 assimilable nitrogen (YAN × 10^{-8}) means ± standard deviations in mol 402 per berry, for Syrah and 6 resistant genotypes, per water deficit class (M, 403 H, and S indicate moderate, high, and severe water deficit classes), 404 Gruissan - France, 2021. Different letters with the same color, within 405 genotype, indicate a statistical difference (LSD, p-value < 0.05); ns indicates 406 nonsignificance.

- Figure 3. Thiols precursors (G3SH, Cys3SH, and CysGly3SH) 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.
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. 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.

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 ratio of total thiol precursors to C eq per unit of fruit (Figure 4 and Table S4). Indeed, in the berry, considering the NS-C pool, sugars and organic acids are the main metabolic C sink with secondary metabolites showing a low C sink strength, representing 1–2% of NS-C. The ratio between thiol precursors and soluble sugars varied from 5.9 to 43.6 $10^{−3}$ 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, water deficit had no significant effect on the ratio of total thiol precursors per 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.

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.
(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, root-stock, 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)

## REFERENCES

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