WATER DEFICIT IMPACTS GRAPE DEVELOPMENT WITHOUT DRAMATICALLY CHANGING THIOL PRECURSOR LEVELS

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Abstract:

Context and purpose of the study - The use of new fungus disease-tolerant grapevine varieties is a long-term and promising solution to reduce chemical input in viticulture. However, little is known about the effects of water deficit (WD) on the thiol aromatic potential of new varieties coming up from breeding programs. Varietal thiols such as 3-sulfanylhexan-ol (3SH), 4-methyl-4-sulfanylpentan-2-one (4MSP) and their derivatives are powerful aromatic compounds present in wines coming from odorless precursors in grapes, and could contribute to the wine typicity of such varieties. This study aimed to characterize the fruit response of 6 new INRAE fungus disease-tolerant varieties and the Syrah control in (i) thiol precursor levels and (ii) main primary metabolites accumulation.

Material and methods - Field grown vines of 3 white (Floréal, 3159B and G5) and 3 red (Artaban, 3176N and G14) fungus disease-tolerant varieties and Syrah were individually monitored during the 2021 season. A gradient of WD was applied and followed by weekly measures of predawn water potentials. Grapes were sampled at the physiological ripe stage (phloem unloading arrest into berries). Primary metabolites (glucose, fructose, tartrate, malate and yeast assimilable nitrogen) were assessed by HPLC and enzymatic methods. Thiol precursors as *S*-conjugate to glutathione (G3SH and G4MSP), to cysteine (Cys3SH and Cys4MSP) and to dipeptides (CysGly3SH and γ-GluCys3SH) were analyzed by LC-MS/MS using Stable Isotope Dilution Assay quantification approach.

Results - Despite genotypes showing low differences on primary metabolites concentrations, a great variability on thiol precursors levels was reported. Among all precursors only G3SH, Cys3SH and CysGly3SH were identified and quantified in all genotypes. For all genotypes, G3SH contents accounted for 75% - 100% of the thiol aromatic potential, while Cys3SH and CysGly3SH accounted for a maximum of 16% and 13%, respectively. Regardless of WD level, the concentrations of G3SH ranged from 31 µg/kg to 132 µg/kg in white varieties (G5 and Floréal respectively) and from 68 µg/kg to 466 µg/kg in red varieties (Syrah and 3176N respectively). Minor effects of WD were observed on soluble sugars, organic acids, YAN and thiol precursor concentrations expressed as µg/kg (average concentrations). However, severe WD strongly reduced all metabolites production per unit of fruit and per plant. The most impacted genotype by severe WD was Floréal, which showed reductions of 70% in primary metabolites and thiol precursors quantity per plant. The least impacted genotype regarding primary metabolites quantity per plant was 3176N (-20%) while Artaban showed the lowest reductions in thiol precursors per plant (-25%). These results showed that a severe WD ultimately reduce the production of metabolites per unit area of cultivation or plant without significantly improving the concentration of compounds of interest in the grape, potentially causing significant economic losses.

Keywords: water deficit, thiol precursors, resistant varieties, YAN.

1. Introduction

Viticulture today faces the dual challenge of reducing chemical inputs and adapting plant material to future climatic conditions. The introduction of fungal disease-tolerant varieties appears to be a long-term solution. However, little is known about how these new varieties modulate the accumulation of compounds of interest, as thiol precursors under water deficit (WD).

Indeed, water availability plays a major role on yield and fruit composition. Despite decreasing berry volume (Mirás-Avalos and Intrigliolo 2017), moderate WD might be beneficial in the accumulation of secondary metabolites, such as anthocyanins and polyphenols (Rienth et al. 2021). Nonetheless, its effects on the aromatic potential are less clear and relative to each compound and its respective molecular group (Alem et al. 2019). Previous studies reported that high WD was detrimental to the accumulation of thiol precursors in grapes of Sauvignon Blanc (Cataldo et al. 2021, Peyrot des Gachons et al. 2005). Whereas Kobayashi et al. (2011) observed an up-regulation of both G3SH and Cys3SH precursors in grape leaves and berries of Koshu, Chardonnay and Merlot, under WD. In addition, thiol precursor levels are also known to be highly dependent on grapevine genotype (Nicolini et al. 2020, Peña-Gallego et al. 2012). Thus, this study aimed to characterize berry primary metabolites and thiol precursors levels in response to WD in 6 new INRAE fungus disease-tolerant varieties and the Syrah control.

2. Material and methods

Plant material and growing conditions

Plant materials - Experiments were conducted at INRAE experimental unit of Pech Rouge (southern France; 43°14N, 3°14E) with adult and grafted field-grown vines, during the 2021 season. The genotypes studied consisted of 3 white berry (Floréal, 3159B and G5) and 3 red berry (Artaban, 3176N and G14) fungus disease-tolerant varieties and Syrah as *V. vinifera* standard. A total of 30 plants per genotype were individually monitored through the season where weekly predawn water potentials from flowering to harvest were performed. The accumulated Ψb between veraison and final sampling date (Acc- Ψb) was calculated and vines were regrouped in three WD classes: moderate WD "M" (-0.3 MPa > accΨb ≥ -0.6 MPa), high WD "H" (-0.6 MPa > accΨb ≥ -0.8 MPa) and severe WD "S" (accΨb < -0.8 MPa).

Sampling and berry metabolites analysis – Berries of all genotypes were sampled at phloem unloading arrest into berries, which corresponds to the physiologically ripe stage (Bigard et al. 2019). The kinetic of cluster growth was monitored through image analysis, carried out in 6 plants per variety. A total of 200 berries per plant were randomly sampled and weighted to assess individual berry weight and their juice was extracted. Soluble sugars "SS", glucose and fructose, and organic acids "AO", tartaric (H2T) and malic (H2M) acids were analyzed by HPLC. Amino acids and NH_4^+ concentrations were assessed by colorimetric method and by enzymatic method, respectively. Yeast assimilable nitrogen (YAN) was calculated by the sum of amino acids and NH_4^+ concentrations. In a sample of 50 berries per plant, thiol precursors as *S*-conjugate to glutathione (G3SH and G4MSP), to cysteine (Cys3SH and Cys4MSP) and to dipeptides (CysGly3SH and γ-GluCys3SH) were analyzed by LC-MS/MS using Stable Isotope Dilution Assay quantification approach through direct injection of grape must (Dournes et al. 2022).

Statistical analysis – All results were presented in mol per volume, berry or plant. All variables were analyzed with the non-parametric test Kruskal-Wallis (0.05 significance level) with genotype and water deficit level as factors. Corrections for multiple comparisons were performed with Bonferroni adjustment. Graphical processing and statistical tests were performed using R Studio® software. Image analysis was done using ImageJ® software.

3. Results and discussion

3.1. *Genotypic variations of the composition of the fruits at physiological ripe stage*

At the physiological ripe stage, the fresh berry weight varied from 1.0 g to 1.6 g, respectively for Syrah and 3176-N. 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, Floréal, 3159-B and G5) to M and S. The negative effect of WD on berry size has been broadly reported (Mirás-Avalos and Araujo 2021), and is related to an impaired cell expansion due to a reduced water flow (Ojeda et al. 2001). SS 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. The OA concentration (H2M + H2T) varied from 52 mmol/L (in G14 and G5), to 70 mmol/L (in Syrah and Floréal), which represents a range of total acidity from 71.9 meq/L to 93.7 meq/L. Slightly lower OA concentrations were observed when comparing with values found by Bigard et al. (2018) but the proportion of H2M to H2T was found to be similar, varying from 0.18 to 0.39, in 3159B and G5, respectively. YAN concentration values in musts ranged from 0.4 mmol/L (37 mg/L) to 2.1 mmol/L (183 mg/L) in G14 and Floréal, respectively. All varieties (except Floréal) showed YAN values below 140 mgN/L, suggesting that a specific nitrogen supply, in grape must, would be necessary to successfully complete alcoholic fermentation.

Among all precursors only G3SH, Cys3SH and CysGly3SH were identified and quantified. For all genotypes, G3SH contents accounted for 75% - 100% of the thiol aromatic potential, while Cys3SH and CysGly3SH accounted for a maximum of 16% and 13%, respectively. The concentrations of G3SH ranged from 0.09 µmol/kg to 0.29 µmol/kg in white varieties (G5 and Floréal respectively) and from 0.17 µmol/kg to 1.11 µmol/kg in red varieties (Syrah and 3176N respectively). The Cys3SH and CysGly3SH precursors 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. To our knowledge, this is the first time where one CysGly3SH has been identified and quantified in disease-resistant varieties. These values are in accordance with previous studies conducted with *V. vinifera* varieties, interspecific hybrids and other fungi-resistant genotypes (Nicolini et al. 2020, Nicolle et al. 2022, Peña-Gallego et al. 2012).

3.2. *Effect of water deficit on the fruit composition at physiological ripe stage*

In the present study all samples were harvested at the same physiological stage (arrest of phloem unloading in the fruit), in order to avoid analytical bias linked to water balance variations and actual biosynthesis of metabolites. Therefore, WD effects were analyzed based on the quantity of molecules per berry (mol per berry) and per plant as previously described for other metabolites (Alem et al. 2021a, 2021b, De Royer Dupré et al. 2014, Ojeda et al. 2002)**.** Water deficit negatively affected the accumulation of all primary metabolites for all genotypes, with a significant decrease between M and S treatments for soluble sugars, organic acids and YAN content per berry (p-value < 0.05, Figure 2). The highest reductions in primary metabolites per berry were reported in G14, which showed -51%, -43% and -72% in SS, AO and YAN contents in mol per berry, respectively. While Floréal showed the lowest reduction in SS (-32%), Artaban in AO (-27%), and 3176N in YAN (-20%) (Figure 1). In addition, 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). Altogether, these results show that WD negatively affects synthesis of thiol precursors in grapes, contrary to results observed previously (Kobayashi et al. 2011).

Besides reducing berry size and metabolite content per berry, WD reduces total plant yield, leading to further losses in the total production of metabolites per plant and cultivation unit area. Indeed, the total production of SS, AO and YAN per plant were strongly decreased by WD (p-value < 0.05) in 3176N, 3159B, Floréal and Syrah. Yet, the G3SH per plant was negatively affected by WD in 5 of the 6 hybrids studied (3176N, Artaban, G14, Floréal, 3159B) and Syrah. Floréal was the most affected genotype, showing reductions up to 70% in primary metabolites and thiol precursors quantity per plant, which translate in losses of 1,043 kg, 44 kg, 1 kg and 487 µg per hectare in SS, OA, YAN and thiol precursors, respectively. The least impacted genotype regarding primary metabolites quantity per plant was 3176N (-20%) while Artaban showed the lowest reductions in thiol precursors per plant (-25%). These results show that severe WD can ultimately reduce the production of metabolites per unit area of cultivation without significantly improving the concentration of compounds of interest in the grape, potentially causing significant economic losses.

4. Conclusions

Small differences regarding soluble sugars and organic acids concentrations among genotypes was reported, but a great variability among varieties regarding their levels on YAN and thiol precursors was found. From those, 3176N was identified with very high levels of thiol precursors, showing a strong aromatic potential. 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 suggests that high and severe WD cause a significant economic loss for the producer.

5. Acknowledgments

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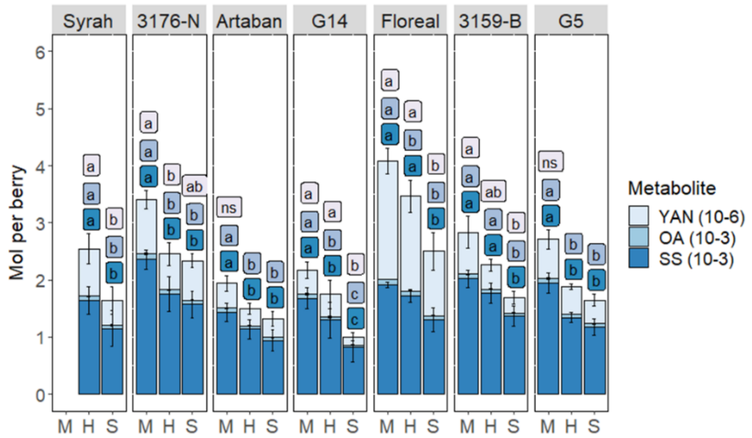

Figure 1: Soluble sugars (SS x 10-3), organic acids (OA x 10-3), yeast assimilable nitrogen (YAN x 10-6) means ± standard deviations in mol per berry, for Syrah and 6 resistant genotypes, per water deficit class (M, H and S indicate moderate, high and severe water deficit classes), Gruissan – France, 2021. Different letters with the same colour, within genotype, indicate statistical difference (LSD, p-value < 0.05), ns indicate non-significance.

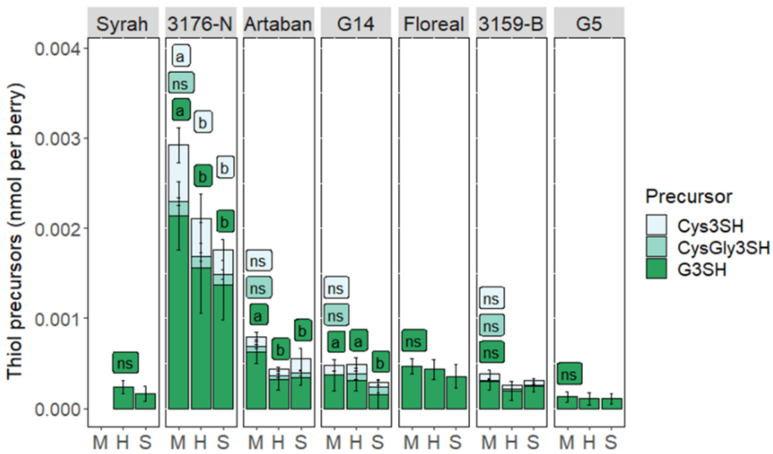

Figure 2: Thiol precursors (G3SH, Cys3SH and CysGly3SH) mean in content per berry (nmol/berry) for Syrah and 6 resistant genotypes, per water deficit class (M, H and S indicate moderate, high and severe water deficit classes), Gruissan – France, 2021. Different letters with the same colour indicate statistical difference (LSD, p-value < 0.05), ns indicate non-significance.