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A holistic approach to using biostimulants on the red grapevine variety, Mouhtaro - from grape to wine

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One of the main effects of applying biostimulants to grapevines is an increase in berry phenolic compound content. In the current study, grapes treated at veraison with abscisic acid (ABA), benzothiadiazole (BTH) and chitosan (CHT) were vinified. Treated grapes, compared to untreated grapes (the control), resulted in wines with a significantly higher concentration of anthocyanins and phenolic content. Furthermore, in a descriptive sensory analysis, wines made from treated grapes were found to have significantly more intense fruity notes and aroma and better overall mouthfeel quality.

Study context

The phenolic profile of red grape berries is a key quality factor and several techniques have been applied to increase the accumulation of those compounds in berries[1]. In the last decade, research on the topic has focused mainly on innovative agronomical techniques, such as the application of bioelicitors in order to enhance phenolic content[2]. Plants interact with diverse microbial communities inside the roots and in the rhizosphere,phyllosphere and carposphere[3]. Plant biosynthetic products, like secondary metabolites, affect this interaction; thus, the exogenous application of resistance elicitors and phytohormones trigger the secondary metabolism and promote the biosynthesis of specific compounds that can alter the structure of the associated microbial communities[4 5].

Material and Methods

In an open field experiment (Muses Valley, Askri, Viotia, Greece), biostimulants were applied to *Vitis vinifera* L. Mouhtaro grape vines at three time points during veraison (400 mg/L ABA, 0.3 mM, BTH and 0.3 % CHT, all in aqueous solutions). Grapes were harvested at optimum sugar maturity to produce red wine. Conventional analysis for total phenolic content and anthocyanins was carried out on the harvested grapes. Standard wine analyses recommended by OIV were used on grape and wine (pH, alcoholic content, total acidity). Additionally, total phenolic index and total anthocyanins and tannins by precipitation with a methyl cellulose assay were measured in wines. More details can be found in Miliordos *et al.* (2021)[6].

Microbial analysis

The carposhere biofilm of the epiphytic microbial communities were washed with sterile PBS and detached with 4 cycles of 2 min pulse sonication at 120 kHz followed by vortex. Total genomic DNA was extracted from biofilm pellets with the DNeasy Power soil DNA isolation kit following the manufacturer's protocol (MoBio Laboratories, Carlsbad, CA, USA). Prokaryotic and fungal communities were analysed by amplicon sequencing analysis with Illumina HiSeq 2x250bp. The V4 region of the 16S rRNA gene was analysed for prokaryotes (515f - 806r primer pair) and the ITS2 region for fungi (fITS7 - ITS4 primer pair). Bioinformatic analysis was performed with DADA2 and α- & β- diversity analysis with phyloseq R packages v. 1.38.0. Final differences in the abundance of microbial members of the prokaryotic and fungal community between the treatments

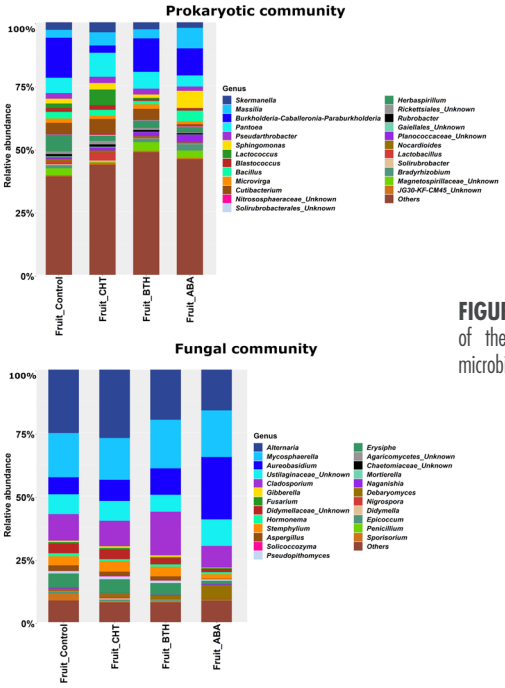

FIGURE 1. Relative abundance of the dominant carposphere microbiome.

(Control, CHT, BTH, ABA) was tested using linear discriminant analysis (LDA) and effect size analysis (LEfSe). More details can be found in Miliordos *et al.* (2021)[6].

Sensory analysis

Sensory trials were carried out using 12 trained assessors (equal representation of the two genders) with the majority having professional experience in the wine industry at least two years of experience in red wine tasting. Samples were served in ISO standard glasses 3591 and coded with a randomised three-digit number[7]. The evaluation consisted in describing eight descriptors quantitatively using a scale of 1 to 10 (lowest to highest intensity) for the following characteristics: Colour, Overall Aroma, Strawberry, Sour Cherry, Blackberries, Vegetal Aroma, Spiciness, Vanilla, Red Flowers and Caramel.

Effect of the biostimulants

Differences in the microbial communities were observed after the application of the plant activators (Figure 1).

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. The ABA treatment increased the relative abundance of native yeasts and decreased that of putative fungal pathogens, while CHT treated grapes demonstrated an increase in lactic acid bacteria.

- Regarding the prokaryotic community, the ABA treatment resulted in a higher proportion of *Massilia* and an unassigned genus of Planococcaceae and *Staphylococcus,* while the CHT treatment resulted in a higher proportion of *Cutibacterium*, *Lactococcus* and *Lactobacillus*.
- *Oenococcus* was only present in the carposphere of the ABA treated berries, but in low relative abundance: 0.08 % of the total community.
- With respect to the fungal community, the ABA treatment had the strongest effect, resulting in an increased relative abundance of *Aureobasidium* and a decreased abundance of *Cladosporium* and *Stemphylium*.
- The *Saccharomyces* was only present in the carposphere of the ABA treated berries, but in low relative abundance: 0.36 % of the total community.

A decreasing trend in mean berry weight after the application of all treatments was observed (Figure 2), with the CHT-treated berries having a significantly lower value compared to the other treatments. The pH tended to be slightly decreased compared to the control, with a lower value observed for CHT. On the other hand, all biostimulants increased the berry anthocyanins concentration in comparison with the control. Furthermore, the ABA-treated berries showed the highest total phenolic index value.

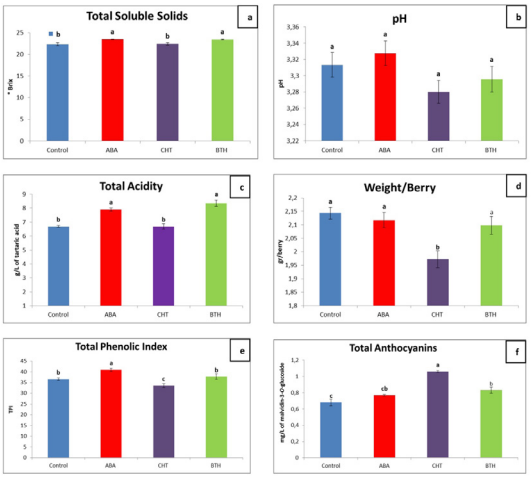

FIGURE 2. Grape berry and basic must analysis. Results from One–Way Analysis of Variance (ANOVA) with Tukey's test.

The treatments did not affect the conventional wine parameters (Figure 3), while it was possible to differentiate the phenolic composition of the produced wines (Figure 4). Tannins and anthocyanin concentrations, as well as colour intensity, were mainly increased by ABA and BTH.

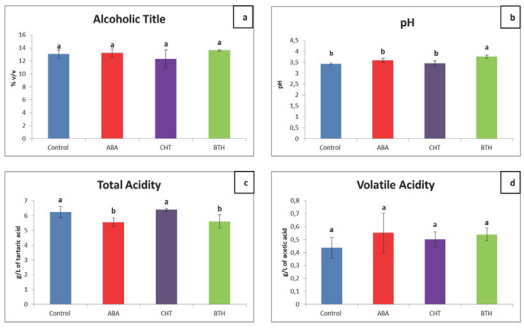

FIGURE 3. Results of the conventional wine analysis parameters. (alcohol strength, pH, total acidity and volatile acidity). Results from One–Way Analysis of Variance (ANOVA) with Tukey's test.

The sensory analysis demonstrated that wines from treated grapes tended to have higher colour and overall aroma intensity, sour cherry notes and less intense vegetal aromas (Figure 5).

Conclusions

Biostimulant treatment in vineyards at veraison influenced the microbial communities that inhabit the surface of berries and stimulate the accumulation of phenolic compounds, including anthocyanins. As a result, the wines produced from these grapes showed different chemical and sensory profiles. The exogenous application of biostimulants could be used to induce the accumulation of secondary metabolites of oenological interest in order to produce wines with different styles and make a shift towards sustainable viticulture. ■

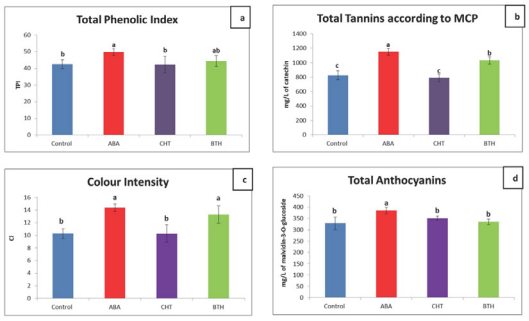

FIGURE 4. Results from One–Way Analysis of Variance (ANOVA) with Tukey's test of phenolic and colour characteristics of the produced wines.

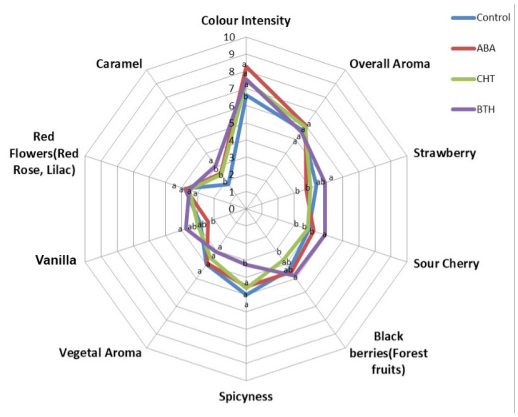

FIGURE 5. Sensory profile (web chart) of the produced wines from the treated berries, assessed by a group of professional tasters from the Laboratory of Enology and Alcoholic Beverages of the Agricultural University of Athens. Wines were judged using predefined quality attributes on a scale of 1 (absent) to 10 (high). Different letters in the same series indicate significant difference between means based on a t-test ($p < 0.05$).

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