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Extreme viticulture:
from a cultural landscape to an economic and environmental sustainability

11-14 May 2022, Vila Real (UTAD)

Book of Proceedings

EDITED BY: Alberto Baptista and Catarina Cepêda
Universidade de Trás-os-Montes e Alto Douro (UTAD)
Centre for Transdisciplinary Development Studies (CETRAD)

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Silicon application effect on berry quality of Touriga Franca variety in the Douro Demarcated Region

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Abstract. The warmer and drier climate will challenge Portuguese viticulture negatively impacting the economy, particularly for renowned winemaking regions such as the Douro Demarcated Region. In the context of climate change, the production of grapes and the sustainability of the vineyard are vulnerable to the effects of high radiation, heat and drought during the summer period. These conditions affect negatively the vines at the phenological, physiological and biochemical levels. The shortage of water resources makes irrigation practically unsustainable from an economic and environmental point of view, being crucial to implement alternative and eco-friendly measures for a better balance between vines and the environment, helping to mitigate the effects of climate change. In this work, we applied a suspension of silicon (2.5 %) in a commercial vineyard located in the Douro Superior sub-region (Santa Comba da Vilariça) to understand its effects on increasing tolerance to water stress in Touriga-Franca cv. grapevines. The experiments were divided into three treatments: i) positive control - with a deficit irrigation (25% of ETc), ii) negative control - without irrigation and iii) silicon - SiO_2, applied in pré *veraison*. After the statistical analysis, the results showed that at *veraison* silicon treated plants presented fruits with higher significant values of total phenols, pH and brix°, when compared with both irrigated and non-irrigated ones. At maturation, the silicon treated plants showed fruits with higher anthocyanins, alcohol, pH and brix° content than the negative control. Accordingly, we can suggest that silicon foliar treatment could be an alternative to deficit irrigation strategies to mitigate water stress without negative impact on berry quality.

Introduction

The Douro Valley is the first formally demarcated wine region of the world (Lourenço-Gomes,Pinto & Rebelo, 2015). In a climate change scenario, an increase in temperature, radiation and water deficit is predicted to increase its vulnerability to production losses and threaten berries/wine quality as well as the vines' sustainability in Mediterranean-type climate regions (Fraga et al., 2014). A reshaping of the main Portuguese winemaking regions is likely to occur in the upcoming decades, therefore, it is essential to emphasize the need for the appropriate cultural practices for the adaptation of climate change in order to maintain wine typicity and styles. Several studies in the Douro Demarcated Region (DDR), reveal that grapevine physiology, agronomic and genetic attributes were modified by particular microclimate conditions (Carvalho et al., 2018; Dinis et al., 2018a), especially by low water availability and high light/temperature levels.

Severe summer stress negatively affects the vineyards, provoking a significant decline in photosynthetic productivity, mostly due to stomatal limitations, leading to changes in hormonal contents (Dinis et al., 2018b), yield, berry weight and sugar levels, as well as low aroma components resulting in wines with high alcohol and low acidity (Jones et al.,2005; Dinis et al.,2020)

The impact of anthropogenic actions along with climate variability in the Mediterranean wine regions can be devastating. Therefore, to maintain the sector's sustainability, several short and long-term strategies have been studied to minimize the negative impacts of climate change. One summer stress mitigation practice is investing in deficit irrigation strategies to sustain yield while preserving or even improving berry features. However, given the high natural limitations in water resources, water capitation and distribution systems on a large scale involve high costs and are environmentally unsustainable. Therefore, it is crucial to develop mitigation alternatives, not only in economic terms but also in terms of grape quality and environmental sustainability (Bernardo et al., 2018).

The exogenous application of minerals can be used as one of the mitigation techniques in order to reduce the use of water and preserve or improve the wine quality. Once applied as an aqueous suspension, silicon dioxide (SiO_2) particles forms a physical or mechanical barrier (as precipitated amorphous silica) in the cell walls. It also seems to modulate plant metabolism altering physical activities, especially by plants that are under water stress conditions. According to other studies, tomato plants under saline stress treated with silicon (Si) showed a 40% increase in water content, and the water use efficiency was 17% higher than those plants without Si treatment (Romero-Aranda, Jurado & Cuartero 2006). Moreover, under water stress conditions, the presence of Si may result in better potassium (K+) uptake by plants (Pavlovic et al., 2021). The potassium (K+) influences the pH of most wines and, consequently, their chemical and microbiological stability and the perception of wine flavour (Mpelasoka, 2003). Finally, Si showed a positive antioxidant activity and stomatal response, increasing the tolerance of vine plants grown in toxic boron and saline soil (Soylemezoglu et al., 2009).

In this study, the foliar silicon application will be used in order to test its effectiveness as a mitigation strategy against summer stress in a vineyard located in the Douro Region. To test this hypothesis, we evaluated several berry quality traits (i.e. phenolics, acidity parameters, total soluble solids, and antioxidant activity) throughout the growing season (*veraison* and maturation stages). The ultimate goal is to assess if the foliar silicon treatment could be a sustainable alternative to standard irrigation practices.

Methods and sources

Experimental design

The experiment was carried out in a commercial vineyard in the Douro Demarcated Region (Douro Superior sub-region, NE Portugal) in Santa Comba da Vilariça (41º20'48"N 7º35103'48"W), the hottest and driest one of the Douro Region (Santos et al., 2019) during the summer of 2021. The variety used was Touriga Franca, (*Vitis vinifera* L.) characterized by its regular grape quality potential and moderate adaptability to warm and dry climates. The vines were exposed to two levels of soil water availability over the growing season: no irrigation and ii) deficit irrigation (25% of ETc). At the beginning of summer (pre-*veraison* stage, on 1st July 2021), a single foliar application (2.5%) of Si (Humigel Plus A formulated with 15% of SiO_2, Tecniferti S.A, Leira, Portugal) was carried out on non-irrigated vines. Three treatments were then established: i) positive control (C +) - with deficit irrigation; ii) negative control (C -) - without irrigation, and iii) silicon-treated vines (Si).

A total of 300 berries per treatment were randomly collected from different positions in the clusters and vines on two different dates: one month after pulverization (22nd July, *veraison* stage) and two months after pulverization (24th August, maturation stage). Whole fruits (n=300/treatment/variety and stage) were frozen in triplicate (n = 100/triplicate) in liquid nitrogen and stored at −80°C, posteriorly lyophilized for 96h and converted to a fine dried powder. The experimental procedures for fruit quality performed were:

I) Total anthocyanin content

Total anthocyanins were quantified according to the differential pH method (Lee et al., 2005) and expressed as mg malvidin-3-O-glucoside equivalents (MVE) per gram of extract (mg MVE/g dry weight, DW). All the absorbances were determined using a microplate scanning spectrophotometer (SPECTROstar Nano, BMG Labtech GmbH).

II) Brixº

The Brixº was measured in triplicate on 30 fruits per treatment using an ATAGO digital refractometer (CO., LTD. Tokyo, Japan) and recorded as "degrees Brix" (Brixº) which is equivalent to a percentage (%). The Brix scale or degrees Brix is numerically equal to the percentage of sugar and other dissolved solids in the solution (Ball, 2006).

III) Acidity parameters and pH

The L-malic acid and tartaric acids were measured by an enzymatic method with an automated clinical chemistry analyzer (Miura One, TDI, Spain) (Escribano-Viana et al., 2019; Franquès et al., 2018). The quantification of total acidity was performed using Fourier transform infrared spectrometry (FTIR).

IV) Phytochemical analysis

Phenolic compounds were extracted as described by Mendes Lemos *et al.* 2020. The total phenolic contents were determined by the Folin–Ciocalteu method at 725 nm as described previously (Rodrigues et al., 2015). It was expressed as mg gallic acid equivalents per gram of extract (mg GAE/g DW). The aluminium chloride ($AlCl_3$) complex method at 510 nm, as described by Rodrigues et al. (2015), was used for the quantification of the total flavonoids content of extracts. It was expressed as mg of catechin equivalents per gram of extract (mg CAE/g DW. The total tannins content was determined at 280 nm in UV-adapted microplates according to the methyl cellulose precipitable assay (Dambergs *et al*., 2012). The concentration of tannins was obtained by subtracting the absorbance of methyl cellulose treated samples and using epicatechin as standard. All measurements were performed in triplicate, and the results were expressed as mg ECE/g DW.

V) Statistical analysis

Berry data analysis was performed using the SPSS 20.0 software (SPSS Software, Chicago, IL, USA). After testing for analysis of variance (ANOVA) assumptions, statistical differences among treatments within each developmental stage were evaluated by one-way factorial ANOVA, followed by the post hoc Tukey test. Different lowercase letters represent significant differences (P < 0.05) between treatments.

Results and Discussion

It is known that regions with severe summer conditions can induce a significant decline in photosynthetic productivity (Dinis et al., 2018) yield, berry weight, sugar levels and aromas, resulting in wines with high alcohol content and low acidity (Jones et al., 2005; Dinis et al., 2020). The analysis of phenolic compounds, levels of alcohol, total acidity, tartaric acid, pH, malic acid, tartaric acid and Brix° were tested on Touriga Franca cv. berries to verify the effect of Si treatment on its quality.

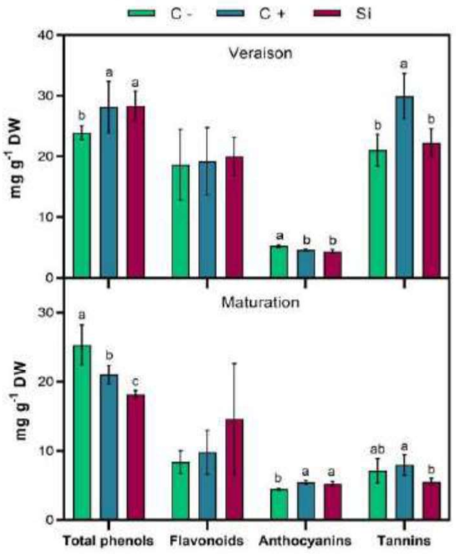

Figure 1. Total phenols, flavonoids, anthocyanins and tannins content in the berries of the negative control (without irrigation, C-), positive control (irrigation 25 %, C+) and silicon-treated (Si) vines of Touriga Franca in two developmental stages (*veraison* and maturation) during the 2021 growing season. Data are mean of six replicates ± standard deviation. Different lowercase letters indicate significant differences (P < 0.05) between treatments (C-, C+, and Si). Absence of letters indicates no statistical difference.

Figure 1 shows the effects of Si application on the content of several secondary metabolites, namely total phenols, flavonoids, anthocyanins and tannins. At *veraison*, berries from C+ and Si-treated vines showed significantly higher total phenols content than unirrigated vines, suggesting that Si treatment could display similar berry quality impacts to those observed under deficit irrigation practices (C+). Though no statistical differences were observed between treatments on flavonoids accumulation throughout maturation, anthocyanins levels were decreased in the berries from Si and C+ vines compared to unirrigated vines at *veraison*. This effect was reversed at maturation, whereas C- berries presented lower anthocyanins content than Si and C+. The highest tannins content was observed in the berries from deficit irrigated vines at *veraison* (29.91 mg g^{-1} DW) and maturation (7,95 mg g^{-1} DW) .

Grape berries have a high variability of phenolic compounds typically linked to the flavour (acidity, bitterness and astringency) and color of wines (Ribéreau-Gayon, 2006) that could be preserved with pre-harvest silicon application under adverse environmental conditions. For instance, in a two-year experiment in commercial vineyards, Gomes et al. (2019) found that 4 and 8% silicon application in Sauvignon Blanc grapevines over the growing cycle improved yield and the phytochemical characteristics of berries. This effect was also observed in commercial apple orchards, which presented high anthocyanins content in fruits from silicon-treated plants (Karagiannis et al.,2021). Despite the reduced content of total phenols and tannins at maturation, our results show increased anthocyanins accumulation at this stage. Due to their relevance in shaping the colour of red wines, the use of silicon can then have positive effects on anthocyanins and the maintenance and/or evolution of berry maturation. Nevertheless, further experiments over consecutive growing seasons should be conducted to elucidate the role of silicon in alleviating summer stress conditions in grapevines and underlying physiological mechanisms.

Laanne (2018) recently reported that seedless grapevines sprayed with silicon-based treatments showed higher growth, yield, and berry quality than the control one. Moreover, fruit weight, total sugars and % of total reducing acidity also increased in berries from Si treated plants, demonstrating the positive effects of using silicon-based foliar sprays (Al-Wasfy, 2014). Accordingly, our results show that berries from silicon treated vines presented higher values of Brix° degree (Table 1) compared to C- and C+ throughout ripening (15.16 % at *veraison* and 22.86% at maturation), highlighting the positive correlation between the sugar levels and anthocyanins present in the fruit, possibly induced by earlier sucrose accumulation in berries (Dai et al., 2014). In contrast, no statistical difference was found regarding malic acid content at both developmental stages. Regarding alcohol content, there were no significant differences between treatments at *veraison*, while at maturation, it was observed an increase of up to 1% of alcohol content in the berries of Si treated vines.

When looking at table 1, the results for malic acid did not show significant differences between treatments at *veraison* and maturation. In contrast, the tartaric acid analysis showed a difference between the treatments. In *veraison*, Si (4.39 g L^{-1}) and C+ (4.96 g L^{-1}) are statistically equal but lower than C- (5.86 g L^{-1}). So, we can suggest that silicon had the same effect as an irrigation treatment at veraison, but there was no difference between treatments at the maturation stage. There was a significant decrease in malic and tartaric acid concentrations in the maturation stage. The reduction in these parameters throughout ripening is common because the organic acid content is supposed to decrease during grape maturation, especially the levels of malic acid (Orduna, 2010; Adams, 2006; Coombe, 1987). Typically in regions with high temperatures, a decrease in titratable acidity and an increase in pH can result, both largely due to a decrease in malic acid concentration (Orduna, 2010; Buttrose et al., 1971).

Table 1. Levels of Alcohol (%), total acidity (g L^{-1} tartaric acid), pH, malic acid (g L^{-1}), tartaric acid (g L^{-1}) and Brix° in the berries of the negative control (without irrigation, C-), positive control (irrigation 25 %, C+) and silicon-treated (Si) vines of Touriga-Franca in two developmental stages (*veraison* and maturation) during the 2021 growing season. Data are mean of six replicates. Different lowercase letters indicate significant differences (P < 0.05) between treatments (C-, C+, and Si). Absence of letters indicates no statistical difference.

	C-	C+	Si
Veraison			
Alcohol (%)	7.22	7.43	7.32
Total acidity (g L^{-1})	11.78a	11.07a	9.88 b
pH	2.94b	2.98a	3.04a
Malic acid (g L^{-1})	4.52	4.36	4.39
Tartaric acid (g L^{-1})	5.86a	4.96b	4.39b
Brix°	13.70b	13.16b	15.16a
Maturation			
Alcohol (%)	11.86b	11.81b	12.94a
Total acidity (g L^{-1})	4.75a	4.18b	4.43b
pH	3.63	3.55	3.58
Malic acid (g L^{-1})	1.52	1.42	1.50
Tartaric acid (g L^{-1})	2.73	2.78	2.78
Brix°	21.1b	20.93b	22.86a

In the pH assessments, the Si treatment was quite significant at *veraison* (Table 1). The difference in pH between *veraison* and maturation stages in the vines treated with Si was smaller than in the other treatments (C- and C+). In addition, treatments with Si and C + were statistically equal and superior to the negative control, suggesting that Si presents a similar performance to the positive control during *veraison*.

However, at maturation stage all treatments were statistically similar. The pH is a critical determinant of the wine quality, being one of the most important parameters (Conde et al., 2007). Thus, especially for regions with extreme climates such as the Douro Superior, it is essential to use new tools, especially sustainable ones, on the biotic and abiotic effects on the vine and that bring positive benefits without altering the potential quality of berries.

Conclusions

In this study, pre-*veraison* silicon application in Touriga Franca grapevines revealed preliminary insights regarding the use of this treatment in alleviating summer stress conditions and preserving berry quality traits, particularly anthocyanins and total soluble solids. The results suggest that silicon foliar application could sustain berry quality standards, particularly in regions with arid climates. However, more research will be needed to deeply understand its effects on grapevine physiology and berry ripening. In summary, this study points out that silicon thin-film application in leaves could be an alternative strategy for cost-saving water in grapevine production in Mediterranean-type climate conditions.

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