

A proline-rich biostimulant enhances grapevine (*Vitis vinifera* L.) water tolerance to reduced irrigation volumes

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Abstract

In organic viticulture, tools that prevent climate change-induced losses are urgently needed. Summer stresses diminish photosynthetic gains and lead to reduced biomass accumulation and crop formation. This study seeks to assess the use of an organic biostimulant consisting of proline-rich yeast derivatives (SYD) on pot-grown grapevines cv. Chardonnay as a priming strategy to enhance vine water status. Well-watered vines (WW-C) were compared to those vines with reduced irrigation at 80% of daily evapotranspiration for 43 days, with (WS1-T) and without (WS1-C) foliar SYD applications. Additionally, a group of vines received only 40% of daily evapotranspiration along with foliar SYD applications (WS2-T).

The soil moisture content clearly mirrored the three irrigation levels (WW, WS1, and WS2). However, WS1-T vines maintained a higher midday leaf water potential than WS1-C. WS1-T and WW-C vines exhibited similar stomatal conductance and assimilation rates, while WS1-C showed lower stomatal conductance than WS1-T. On DOY 201, WS1-T leaves accumulated nearly twice the proline concentration compared to WS1-C. Meanwhile, WS2-T had stomatal conductance and leaf assimilation rates comparable to WS1-C vines. At harvest, the yield of WS1-T vines was similar to WW-C (1.75 vs. 1.82 kg), but grapes showed higher soluble solids concentrations and lower average titratable acidity while no differences were observed between WS1-C and WS2-T vines in terms of titratable acidity.

The application of proline rich SYD therefore improved the grapevine physiological and productive performance, effectively compensating for modulations in irrigation and represents a powerful tool for the adaptation of organic vineyard to climate change pressures.

Keywords: Biostimulants; water stress; organic viticulture; physiology; priming

Introduction

In the current climate change scenario, viticulture faces increasing summer heat stress and water scarcity. In rainfed vineyards, drought and high temperatures shorten the phenological stages of growth, impairing plant growth, increasing crop evapotranspiration, yield and fruit composition, thereby reducing profitability (Bertamini et al., 2006; Buesa et al., 2021). Recent studies have investigated the role of biostimulants and foliar applications in enhancing crop tolerance to abiotic stress (Rouphael et al., 2017; Frioni et al., 2021; Monteiro et al., 2022) and the environmental impact associated with the use of biostimulants and synthetic fertilizers (Menegat et al., 2022; Calia et al., 2025). Proline plays a major role in the abiotic stress response of grapevines (Ozden et al., 2009) and grapevines have been demonstrated to accumulate significantly higher concentrations of proline when they are subjected to abiotic stress (Wei et al., 2022).

We hypothesize that the priming effect of proline could enhance the vine water status, prevent leaf photoinhibition, and enhance the gas exchange parameters at comparable irrigation levels. Therefore, the focus of our study was to observe whether the application of

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proline rich yeast derivatives enable grapevines to maintain adequate physiological functioning with reduced irrigation requirements due to a proline priming effect.

Material and Methods

The study was conducted on 16 three-year-old potted (35L) grapevines cv. Chardonnay divided randomly into 4 treatments- i) well-watered control (WW-C); ii) moderate water stress control (WS1-C); iii) moderate water stress treated (WS1-T); iv) severe water stress treated (WS2-T). The WW watered vines received irrigation equivalent to 100% evapotranspiration (ET); WS1 at 80% and WS2 only 40% of the vine daily evapotranspiration. The treated vines were subjected to five foliar applications of a proline rich biostimulant between BBCH 73-81 (Lorenz et al., 1995) at a dosage of 3.3g/L. The leaf gas exchange parameters (A_N , g_s , E) and soil moisture content were assessed on 7 distinct days of year (DOY) along with the midday leaf water potentials and sampling of leaves under liquid nitrogen. Leaf proline and hydrogen peroxide concentrations were quantified on the sampled leaves for DOY 201. The grapes were harvested once the grapes total soluble solids concentrations reached 20° Brix. Three representative bunches per vine were sampled to determine the bunch morphology and fruit composition.

Results and Discussion

The soil moisture content clearly mirrored the three irrigation levels (WW, WS1, and WS2) (Figure 1). Despite receiving the same amount of irrigation, WS1-T vines showed a higher midday leaf water potential than WS1-C vines, especially towards the end of the experiment (+0.22 MPa on Day of Year – 214). WW-C and WS1-T vines exhibited similar leaf A_N on most of the dates examined (0.24 vs. $0.25 \text{ mol m}^{-2} \text{ s}^{-1}$, and 14.9 vs. $15.3 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ on average from all measurements). WS2-T vines displayed leaf A_N and leaf g_s comparable to WS1-C and even surpassed WS1-C at the experiment's conclusion (0.17 vs. $0.20 \text{ mol m}^{-2} \text{ s}^{-1}$ and 11.7 vs. $11.5 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ on average). Correlations between leaf A_N and leaf g_s showed that WW-C and WS1-T exhibited higher leaf A_N than WS1-C and WS2-T when g_s exceeded $0.20 \text{ mol m}^{-2} \text{ s}^{-1}$.

On DOY 201, WS2-T and WS1-T had the highest leaf proline concentrations (22.6 and 17.8 $\mu\text{mol/g}$ respectively) while WS1-C showed significantly lower leaf proline concentrations (10.6 $\mu\text{mol/g}$), yet higher than WW-C (+6.72 $\mu\text{mol/g}$). At the same time, WS1-T and WS2-T had similar leaf H_2O_2 concentrations ($\sim 0.78 \text{ } \mu\text{mol/g}$), that were comparable to the unstressed WW-C vines (0.7 $\mu\text{mol/g}$) while the WS1-C vines showed a dramatic increase of leaf H_2O_2 accumulation to 1.06 $\mu\text{mol/g}$.

The WW-C and WS1-T vines showed the highest yields (1.82 vs 1.75 kg respectively). WS1-T and WS2-T showed significantly higher grape total soluble solid concentrations than WW-C, with increases of +1.89° Brix and +2.06° Brix, respectively, while WS1-C showed intermediate values. In addition, WS1-T showed a *lower average titratable acidity than WW-C* (6.62 vs. 7.7 g/L in WW-C), while no differences were observed between WS1-C and WS2-T vines in terms of titratable acidity (6.15 g/L).

In conclusion, the application of biostimulants such as specific yeast derivatives in organic vineyards can be a valuable tool to enhance leaf gas exchange and productive performance under rainfed conditions or limited irrigation availability.

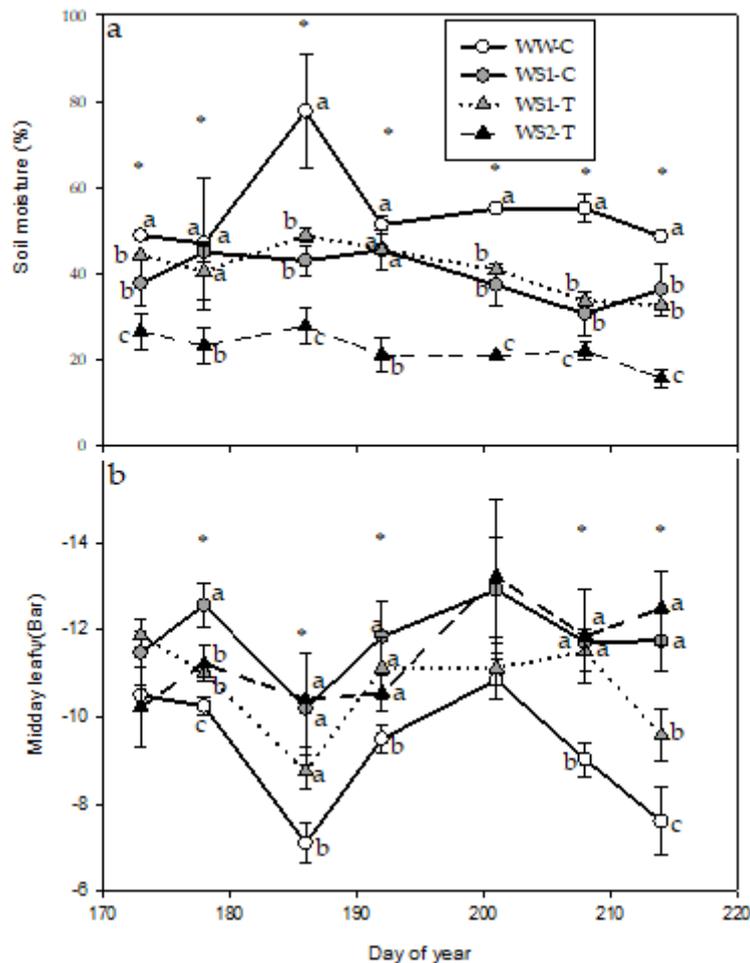


Figure 1: Seasonal evolution of soil moisture (%) ; panel a) and midday leaf water potential (Ψ ; panel b) in grapevines cv. Chardonnay subjected to multiple applications of proline-rich specific yeast derivatives (T) and control vines (C) in accordance with different irrigation levels from Day Of Year 171 (BBCH 73, groat-sized berries) to harvest (BBCH89, full ripening), based on measured daily evapotranspiration (ET): WW = 100% ET; WS1 = 80% ET; WS2 = 40% ET. Asterisks indicate significant difference between treatments within date per $p < 0.05$. Different letters indicate significant difference between treatments within date per $p = 0.05$ (Tukey's HSD test). Bars represent standard errors ($n = 4$)

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