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Enhancing vineyard resilience: evaluating sustainable practices in the Douro demarcated region

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Abstract. In Mediterranean agriculture, sustainability and productivity are seriously threatened by climate change and water scarcity. This situation is exacerbated by poor management practices such as excessive use of agrochemicals, overgrazing, and monoculture. The Douro Demarcated Region (DDR) is an emblematic region, classified as a World Heritage Site by UNESCO in 2001. Viticulture is the main agricultural activity in DDR, widely known to produce Port wine. So far, new approaches have been developed to redesign Mediterranean agroecosystems with greater resilience and productivity, focusing on the development of sustainable agricultural production systems through the combined use of biotechnological tools and environmentally respectful agronomic practices, enhancing soil functions and health by employing bioinoculants, remediation techniques, cultivation systems, and climate-adapted crops in each studied region. The present study aims at assessing the impact of bioinoculants' application and cover cropping on grapevine growth and water stress management in DDR vineyards. A trial was conducted in a commercial vineyard, where treatments with bioinoculants (plant growth promoting bacteria and/or arbuscular mycorrhizal fungi) and cover cropping (sown with hydrogel), were applied. Exposed leaf area and predawn leaf water potential were measured to assess treatment impacts on grapevines. The results indicate that bioinoculants' application in cover cropping seeded with hydrogel promote grapevine leaf expansion, increasing vegetative biomass, and enhancing nutrient uptake. Additionally, cover cropping contributed to greater soil water availability, reducing plant water stress during dry periods. These findings underscore the potential of these sustainable practices to improve vine health and increase resilience to adverse climatic conditions in the Douro region.

1. Introduction

The Douro Demarcated Region (DDR) is a historically significant region, renowned for its winemaking tradition, particularly in the production of Port wine. However, like many Mediterranean regions, DDR is facing significant challenges posed by climate change, particularly water scarcity, which poses serious threats to the sustainability and productivity of its vineyards. Vineyards are characterised by their schistous soils and steep terraces, which, although ideal for grape growing, present considerable challenges for water management [1, 2].

These challenges have been exacerbated by climate change, which has increased the frequency and intensity of drought periods, making the use of effective water management practices crucial for sustaining vineyard productivity and resilience and preserving grape quality [3]. Traditional viticultural practices often rely on the extensive use of agrochemicals and monoculture, which have become increasingly unsustainable in the face of shifting climatic conditions. These practices can degrade soil health, reduce biodiversity, and ultimately diminish the long-term viability of vineyards [4].

To address these issues, recent research has focused on developing sustainable agricultural practices aimed at enhancing the resilience of vineyards, particularly in the face of extreme climatic conditions. Sustainable agricultural production systems can integrate biotechnological tools and environmentally friendly agronomic practices in order to enhance soil functions and health, thereby reducing the impact of water stress on grapevines [5, 6]. Among these strategies are the application of bioinoculants and water retainers as well as the cultivation of climate-adapted cover crops. Bioinoculants, such as plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF), have shown great potential in enhancing nutrient uptake and stimulating plant growth, while cover crops can protect soil from erosion and improve water infiltration [7-10]. Furthermore, incorporating water retainers, such as hydrogels, into the soil has proven to be an effective strategy for increasing soil water holding capacity while minimising water loss through percolation [11]. These sustainable practices have shown to promote grapevine vegetative growth and biomass, and nutrient uptake, which are critical for maintaining grapevine health and productivity under adverse climatic conditions. By evaluating these practices, this research contributes to the growing body of knowledge on sustainable viticulture and offers potential solutions for improving the resilience of vineyards in the face of climate change in the DDR. The present study aims to evaluate the impact of bioinoculants application, cover cropping and the use of hydrogels on grapevine vegetative growth and water status in DDR.

2. Material and Methods

2.1. Plant material and growth conditions

This study was conducted in an experimental field trial established in 2018 at 'Quinta dos Aciprestes' (São João da Pesqueira, Portugal), comprising grapevines cv. Touriga Nacional. The experimental plot is installed in a schistous soil with a sandy-loam texture, a south solar exposure, an altitude of around 350 m and a slope of 2 to 5%. Grapevines were planted in a single upward cordon system in a one-line terrace and spaced 1 m apart. Fertilisation and pest and disease control followed the integrated pest management system standards.

2.2. Experimental design

2.2.1. Trial I - Bioinoculants application

To test the effect of microbial inoculants on grapevines' water status and vegetative growth two bacterial strains (B1 - *Pseudomonas fluorescens* and B2 - *Arthrobacter nicotivorans*) and an arbuscular mycorrhizal fungus (AMF, INOQAgri; Germany) were inoculated at the time of plantation, in 2018, and in May 2022. Five different treatments were applied: PGPR-1 (B1), PGPR-2 (B2), PGPR-1 + PGPR-2 (B1+B2), Fungi (FR) and the mixture of B1, B2 and F treatments (Mix). These treatments were applied to the soil near the trunk of the grapevines and each treatment consisted of 5 replicates.

2.2.2. Trial II - Combined use of bioinoculants, cover cropping and hydrogel

This trial aimed to assess the effect of sowing cover crops and applying hydrogel in the interrow, on non-inoculated and inoculated grapevines' vegetative growth and physiology. In November 2022, two treatments were applied in the interrow of the experimental plot consisting of in: natural cover (spontaneous plants, not sown, **C**), sowing commercial seeds (REVIN®) combined with hydrogel (POLYTER®) – **T1**. Sowing was done in November 2022.

2.3. Predawn Leaf Water Potential (Ψpd)

Predawn leaf water potential (Ψpd) was measured in 2023, at veraison and at harvest time, to assess the water status of grapevines in both trials..Healthy and fully developed leaves were randomly selected from grapevines in each treatment. Measurements were taken using a Scholander pressure chamber (PMS, Albany, USA), a method established by [12]. The measurements were conducted at dawn, more specifically 1-2 hours before sunrise, at veraison stage (26th July) and harvest time (31st August). This timing is critical as it ensures that the stomata are still closed, allowing for determination of the maximum amount of water retained by the plant before transpiration begins. This method provides valuable insights into the grapevine's capacity to absorb and retain water during the night, a critical factor for maintaining its overall health and maximising productivity.

Predawn leaf water potential data were analysed and discussed according to a scale established by [13] (**Figure 1**).

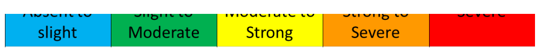

Figure 1. Leaf Water Potential scale [13].

2.4. Exposed Leaf Area (ELA)

The exposed leaf area was calculated by quantifying the leaf area available for direct solar radiation exposure, an important factor in grapevine photosynthesis and overall vigour. Likewise, ELA was measured at veraison stage (26th July) and harvest (31st August) and on the same plants used for Ψpd assessment. The methodology followed the approach described by [14], where a tape measure was used to record the height and width of the grapevine's canopy. This measurement provides a clear indication of the grapevine's vegetative growth, as it reflects the size of the leaf area exposed to sunlight. The data obtained from these measurements were crucial for analysing the relationship between grapevine water status and vegetative growth under the different treatment conditions in both trials.

2.5. Statistical analysis

Data analysis was performed using GraphPad Prism software (version 9.0). The results correspond to the mean ± standard error (SE). In Trial I, differences between treatments were tested with a one-way ANOVA followed by Tukey's post-hoc test with a confidence level of 95 % ($p < 0.05$). In Trial II, differences between treatments were carried out with a *t-student* test with a confidence level of 95 % ($p < 0.05$).

3. Results

In 2023, two trials were conducted in the DDR to evaluate the impact of microbial inoculants (Trial I), and cover cropping and hydrogel application (Trial II) on grapevine growth and physiology, specifically focusing on Ψpd and ELA.

3.1. Trial I - Effect of bioinoculation on grapevine water status and development

As shown in **Figure 2A**, there are no significant differences in Ψpd among the majority of the inoculation treatments. However, according to the water stress scale established by Deloire *et al.* (2011), grapevines inoculated with the Mix treatment showed slight to moderate water stress [13]. At harvest, the differences in Ψpd became more pronounced compared to veraison, reflecting the increasing water stress as the growing season progressed. In particular, Mix and B2 treatments showed significantly lower Ψpd values (i.e. increasing water stress) if compared to the other treatments (**Figure 2B**).

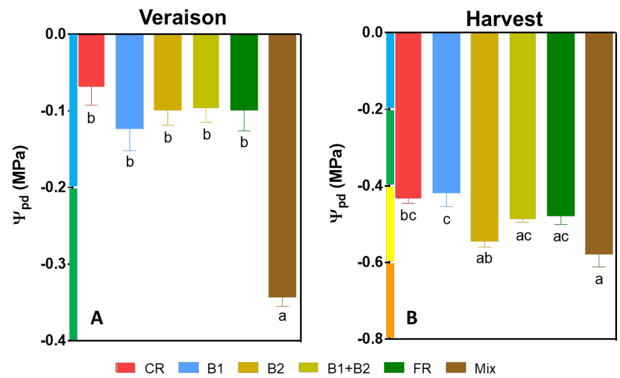

Figure 2. Predawn leaf water potential measured at veraison (A) and harvest (B) in non-inoculated grapevines (control, CR) and inoculated with different microorganisms. Different letters indicate significant statistical differences between treatments at $p < 0.05$.

ELA was significantly decreased in B1 and B2 treatments at both sampling times (Veraison and Harvest) when compared with Mix treatment ($p < 0.05$) (**Figure 3**).

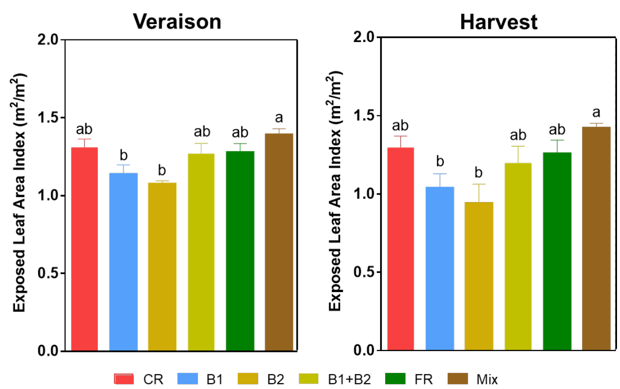

Figure 3. Exposed leaf area measured at veraison and harvest in non-inoculated grapevines (control, CR) and inoculated with different microorganisms. Different letters indicate significant statistical differences between treatments at $p < 0.05$.

3.2. Trial II - Effect of cover cropping and hydrogels on non-inoculated and inoculated grapevines' water status and development

Regarding the Trial II, the establishment of cover crops and application of hydrogel in the interrow (T1) in general decreased significantly the Ψpd of inoculated grapevines at veraison, being the reduction more pronounced in FR and Mix treatments (**Figure A**). At harvest, grapevines grown with natural cover in the interrow showed moderate water stress. In general, Ψpd values of C treatment were more negative than those found in T1 (sown cover crop + hydrogel), with exception to CR and B1 treatments (**Figure 4B**). In grapevines inoculated with B2, FR, Mix it was observed a decrease in Ψpd ($p < 0.05$) in T1 (sown cover crops + hydrogel) when compared to the same treatments carried out in natural cover (**C**).

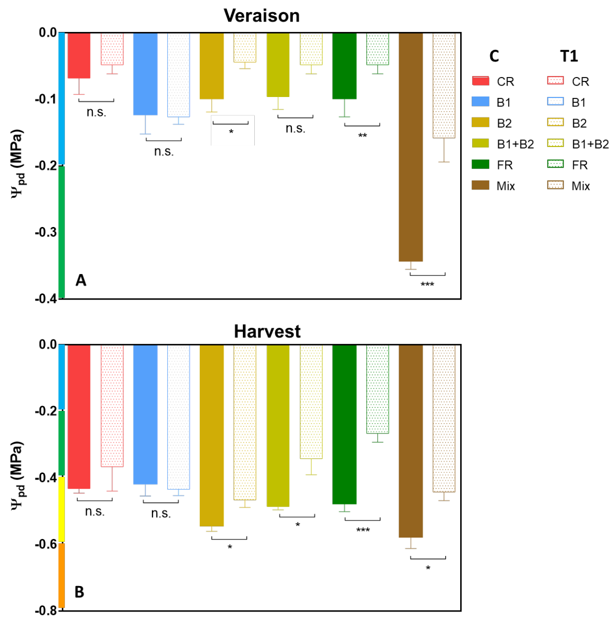

Figure 4. Predawn leaf water potential measured at veraison (A) and harvest time (B) in non-inoculated and inoculated grapevines grown in soil with natural cover crop (C) and with commercial seeds combined with hydrogel (T1). Mean differences between C and T1 treatments according to the *t-test* have been denoted as * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).

The application of cover crops and hydrogel (T1) did not influence ELA measurements of non-inoculated and Mix inoculated grapevines. However, significant increases were observed in the other inoculation schemes (B1, B2, B1+B2, FR) (**Figure 5**). At harvest (**Figure 5B**), the increases were not apparent, however, there was a noticeable trend where bioinoculants applied to soil with sown cover crops and hydrogel tended to improve ELA.

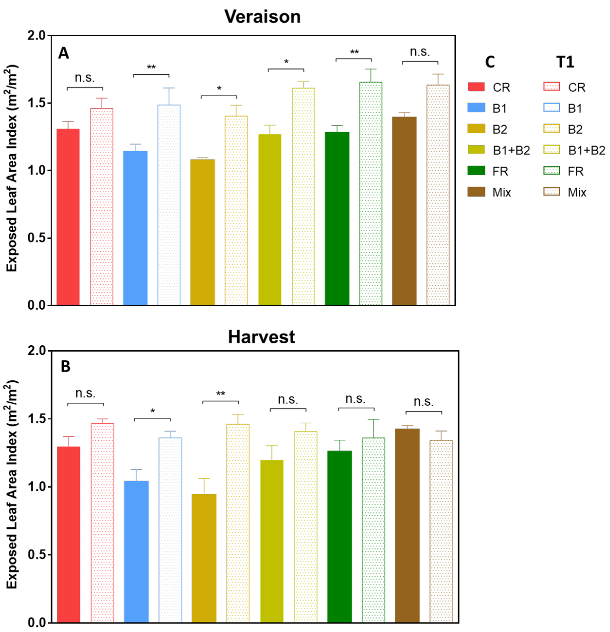

Figure 5. Exposed leaf area measured at veraison (A) and harvest time (B) in non-inoculated and inoculated grapevines grown in soil with natural cover crop (C) and with commercial seeds combined with hydrogel (T1). Mean differences between C and T1 treatments according to the *t-test* have been denoted as * ($p < 0.05$), ** ($p < 0.01$).

4. Discussion

The present study aimed to evaluate the impact of bioinoculation and the application in the interrow of cover crops and a hydrogel on grapevine growth and physiology, focusing on water status and ELA in the DDR. Predawn leaf water potential is a critical indicator of grapevine water status, representing the level of water stress experienced by the plant. Lower (more negative) Ψpd values indicate higher water stress, which can significantly impact grapevine health and grape quality. This study aimed at analysing the effects of different treatments on Ψpd during veraison and harvest time stages in two separate trials.

In Trial I, bioinoculation generally did not induce significant changes in Ψpd during the early stages of grapevines' growth, except in the case of Mix treatment, which induced a lower Ψpd (-0.35 MPa) (**Figure 2A**). However, at harvest, both Mix and B2 treatments exhibited significantly lower Ψpd (-0.55 MPa and -0.6 MPa, respectively) when compared to CR treatment, suggesting that these bioinoculants induced water stress (**Figure 2B**). Deloire *et al.* (2011) demonstrated that increased water stress results in reduced water potential, especially as the growing season progresses [15]. Despite the findings observed in our work, previous studies conducted by Nadeem *et al.* (2014) and Marasco *et al.* (2012) have shown that specific microbial consortia can improve drought tolerance by enhancing water uptake efficiency [16, 17]. Rouphael *et al.* (2015) also reported improvements in water retention and grapevines' growth throughout the season following bioinoculation [18]. Saravanakumar et al. (2011) demonstrated that the inoculation of *Pseudomonas fluorescens* enhanced plant tolerance by increasing catalase and peroxidase activity and promoting proline accumulation under water stress conditions [19]. Additionally, beneficial microbes can improve stress tolerance by increasing the accumulation of osmolytes in plant cells, maintaining turgor, and contributing to stress resilience [20]. These processes are mediated by phytohormone metabolism, including the biosynthesis of auxins, cytokinins, abscisic acid, gibberellins, and a reduction in ethylene [21-23].

The delayed effect seen in our study, where significant differences appeared only at harvest, may be due to the specific vineyard conditions or microbial strain combinations used. These results suggest that while bioinoculants combined with cover crops may enhance resilience to water stress (in Trial II), the timing of their effects and the environmental context are key factors in determining their success. This highlights the importance of carefully selecting specific strategies to manage water stress in grapevines, which is critical for optimising growth and yield under different environmental conditions. Therefore, the selection of microbial strains, as well as the timing and frequency of inoculation, is crucial for maximising the benefits of microbial inoculants.

In Trial II, the combination of cover crops with hydrogel consistently reduced Ψpd when compared to natural cover crop, indicating a decrease in water stress. These results are supported by Choukr-Allah *et al.* (2016), who demonstrated that hydrogels improve soil moisture retention, thereby mitigating water stress in crops under drought conditions [24]. The capacity of hydrogel to enhance soil water-holding capacity could explain the better performance of grapevines in this treatment at both veraison and harvest measurements.

Interestingly, while sown cover crops combined with hydrogel showed improved water status, previous studies indicated that cover crops could sometimes compete with grapevines for water, especially in dry conditions [25, 26]. However, our study suggests that the addition of a hydrogel mitigates this competitive effect, enhancing the plants' water status. This finding is in agreement with Altieri *et al.* (2020), who also observed that combining cover crops with water management technologies can optimise water use efficiency in vineyards [27].

ELA index measures the surface area of grapevine leaves that is exposed to sunlight, expressed in m^2/m^2. This metric is crucial as it reflects the plant's capacity for photosynthesis, which directly influences grape development and overall yield and quality. In both trials, ELA was significantly reduced in certain bioinoculant treatments, particularly B1 and B2, during veraison and harvest. These findings align with the work of Medrano *et al.* (2015), who found that reductions in ELA can be indicative of water stress [28]. However, in Trial II, grapevines inoculated with B1 and B2 combined with sown cover crops and hydrogel presented higher ELA at veraison, indicating better grapevine vigour under these conditions. This result mirrors the findings of Coniberti *et al.* (2013), who observed improved canopy development in drought-stressed vineyards treated with bioinoculants and soil amendments [29].

The different responses observed on ELA measurements at both sampling times suggests that the interaction between microbial inoculants, cover crops, and hydrogels is complex and may depend on the specific stage of grapevine development. In this way, while hydrogel treatments seemed to improve ELA early in the growing season, their impact diminished at harvest, potentially due to the depletion of soil moisture reserves, in accordance with the research conducted by Montesinos *et al.* (2021) [30].

Summing up, the findings from both trials suggest that bioinoculants and cover cropping can vary significantly depending on the specific treatments and environmental conditions, particularly on grapevine water status, vigour and growth. This underscores the importance of site-specific management practices, as highlighted in a previous study, which emphasised the need for tailored viticultural practices to optimise grapevine performance under varying climatic conditions [31].

In this way, the discrepancy of the results between our work and previous reported studies may be attributed to the specific environmental conditions of the DDR or even particular grapevine varieties used, suggesting that local context plays a crucial role in determining the effectiveness of cover crops.

On the other hand, since this study was conducted over a single year, it is important to extend the research over multiple years and assess additional parameters. This would allow for a deeper understanding of how these treatments affect grape composition and wine quality, providing more comprehensive insights into their long-term implications.

5. Conclusions

The present study provided valuable insights into the impact of bioinoculants, cover crops and hydrogel applications on grapevine water status and development in the DDR.

In **Trial I**, bioinoculation showed no significant effects on grapevine water status during the early stages, although an increased water stress was observed in the Mix and B2 treatments at harvest. This suggests that bioinoculants, while potentially beneficial, may induce water stress under certain conditions, highlighting the need for careful selection and timing of microbial treatments. In contrast, **Trial II** demonstrated that the combination of sown cover crop and hydrogel application significantly improved grapevine water status by reducing Ψpd and supporting grapevine vigour, particularly at veraison. The combination with hydrogels appeared to mitigate water stress.

The variability in ELA across treatments, particularly the enhanced ELA observed in hydrogel-treated grapevines, underscores the complexity of interactions between microbial inoculants, cover crops, and hydrogels. While these treatments showed positive early-season effects, their impact diminished by harvest, indicating that long-term water management strategies are critical for sustained benefits, either in environmental or economic point of view.

In conclusion, bioinoculants and hydrogels show promise in enhancing grapevine performance; however, their effectiveness may be highly dependent on local conditions, the timing of application, and vineyard management practices. To gain a comprehensive understanding of their long-term impacts, future research should be conducted over multiple growing seasons, with a focus on additional factors such as grape composition and wine quality.

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