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OmicBots – An innovative and intelligent multi-omics platform facing wine sector challenges

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Abstract. To address emerging competition and challenges, wine producers globally rely on Precision Viticulture (PV) solutions to improve vineyard management. However, traditional PV methods depend on multispectral sensor data which often lack in grapevine physiological insights. The Omicbots project aims to develop an automated platform that combines low-cost robotic sensors with artificial intelligence and systems biology (in silico) to better understand how the physiology and metabolism of grapevines are influenced by the interaction of Genotype, Environment, and Management (GxExM). By integrating multi-omics approaches with smart sensing technology, this project intends to bridge the gap between vineyard mapping and plant phenomics attributes. As a key partner in this project and a leader in the grape-wine industry, ADVID / CoLAB VINES & WINES team has established experimental plots in two distinct locations within the Douro Region. Data was collected from these plots, for two years, involving two grapevine varieties and varying irrigation and sunlight exposure conditions. Leaf and grape samples were collected and used for transcriptional analyses, focusing on genes associated with different grapevine biosynthetic pathways. This study showed that *UFGT, PAL1,* and *CHS3* genes respond differently through growth stages and irrigation levels, highlighting their sensitivity to water stress and the need for optimal irrigation for grapevine productivity. *RCA, LBCY2*, and *CHLG3* in leaves indicate adaptive responses to water availability, with varying sensitivity among genes. Sunlight exposure significantly promotes gene expression, particularly for light-sensitive genes like *PAL1* and *CHS* genes.

1. Introduction

Precision Viticulture (PV) has become an essential tool for wine producers worldwide, especially in regions facing significant environmental challenges and increased global competition. Portugal, for instance, is a leading wine producer in terms of quantity, quality, and diversity, reaching a trade record of 928 million euros in 2023. To maintain and enhance this position, the wine industry increasingly depends on advanced PV techniques. Traditionally, PV has relied on data-driven approaches, leveraging advanced sensor technologies to monitor vineyards [1]. However, while these methods are effective in mapping vineyard variability, they often fall in providing a comprehensive understanding of grapevine physiology [2]. This gap limits their ability to accurately predict the impacts of the complex interaction between Genotype, Environment, and Management (GxExM) on grapevine health and wine quality. The emerging paradigm in PV pursue these limitations by shifting from entirely data-driven approaches to a more automatic understanding of the physiological processes underlying grapevine responses to their environment [3]. In this context, advanced omics technologies, including phenomics, genomics, metabolomics, and transcriptomics, provide insights into how different GxExM interactions influence plant function [2]. By integrating these omics datasets, researchers can advance towards creating a "smart" and "personalized" PV solution that not only captures vineyard variability but also understands it in terms of plant health and productivity. While omics technologies have advanced significantly in laboratory settings, translating these advances into practical, field-based applications remains a considerable challenge. The complexity of the data generated by multi-omics studies, coupled with the

difficulty in modelling the intricate GxExM interactions, has limited the adoption of these approaches in vineyard management. To address these challenges, OmicBots project proposes a novel integration of omics technologies with cutting-edge systems biology and artificial intelligence (AI) to create a more precise and physiologically informed PV solution. This project relies on the principle that understanding grapevine physiology at a molecular level, under different environmental and management settings, is fundamental to moving PV forward. Furthermore, OmicBots intends to bridge the gap between laboratory-based omics research and in-situ agricultural applications by developing an in-silico model of grapevine physiology that incorporates multi-omics data and create a digital-twin platform that facilitates the bi-directional transfer of information between the lab and the vineyard. In this context, the study aims to supply this platform with transcriptional data on genes linked to various grapevine biosynthetic pathways.

2. Material and methods

2.1. Characterization of the Vineyard Regions and Sample Collection

Samples were collected from two experimental plots, considering different grapevine varieties (using both leaf and grape samples) as well as varying irrigation and sunlight exposure conditions. One trial was carried out in Quinta dos Aciprestes (latitude 41°12′31.0"N, longitude 7°25'57.6"W) (Fig. 1), located in the sub-region Cima Corgo in the Douro Demarcated Region (DDR) between 100 m and 350 m of altitude, alongside the Douro River. This vineyard belongs to Real Companhia Velha wine company and is planted in steep slopes gradient levels between 30 to 50%. Quinta dos Aciprestes' climate is characterized by high aridity index due to high temperatures during the summer period leading to a rapid thermal concentration and significant water deficit. During the 2022 and 2023 growing seasons, grapes and leaves from the grapevine variety "Touriga Nacional" - Vitis International Variety Catalogue (VIVC) -12594" were collected from an experimental trial that included three irrigation treatments: non-irrigated plants, serving as the control (ETo; R0); plants irrigated at 30 % of crop evapotranspiration (ETc; R30); and plants irrigated at 60% (ETc; R60). The experimental set-up comprised 3 blocks corresponding to the different irrigation treatments. Each block was composed by two rows of plants comprising 6 grapevines (two separate groups). Leaves and grapes were collected from the 3 blocks × 2 experimental units x 6 plants and the analysis was performed on both years, at two different phenological stages: Véraison (V) and at the end of maturation (H).The other trial was established in Quinta de Vale de Cavalos (latitude 41°07′ N, longitude 7°28′ W and 500 m of altitude) (Fig.1), owned by Sociedade Vinícola Terras de Valdigem - Poças Vinhos wine company, and it is located in the sub-region Douro Superior in DDR. This sub- region presents Mediterranean-like climate with warm and dry summers with pronounced hydric stress typically observed post-flowering. During the 2022 and 2023 growing seasons, grapes were collected from grapevine 'Moscatel Galego Branco' (VIVC 8031) within a non- irrigated, commercial parcel encompassed by sections shaded by nets that were installed above and to the sides of the grapevine rows. The experiment was conducted in both shaded (D) and unshaded (S) sections of the parcel. Grape collection was performed on a single date at the end of maturation in 2022 (H) and on two dates, at different phenological stages: Véraison (V) and at the end of maturation (H) in 2023. Grapes were collected from the selected grapevines from shaded (D) and unshaded areas (S).

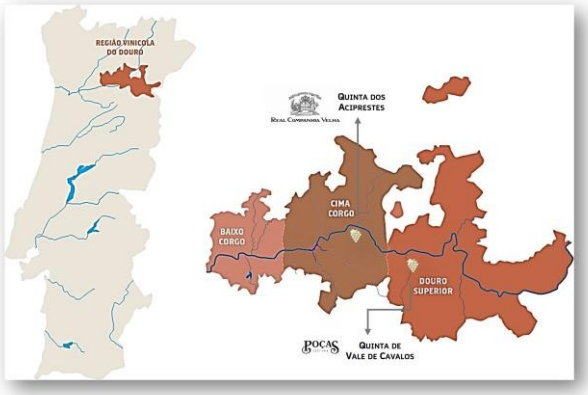

Figure 1. Location of the experimental plots across DDR. Each point represents an individual vineyard from which grapes and leaves were collected.

2.2. RNA extraction and qPCR analysis of the different biosynthetic pathway's genes

Grapes and leaves were sampled and ground with a mortar and pestle in liquid nitrogen. The powder was stored at −80 °C for posterior use. Total RNA was extracted from 300 mg of ground tissue (leaves and /or grapes) following the classical method described by [4] Briefly, extraction was performed in buffer containing 100 mM Tris-HCl (pH 8), 2 M NaCl, 25 mM ethylenediaminetetraacetic acid, 2% (w/v) cetyltrimethylammonium bromide, 2% (w/v) polyvinylpyrrolidone and 0.04 M Dithiothreitol (DTT). RNA purification was performed with the GRS Total RNA kit– Plant (Grisp Research Solutions) and samples were treated with DNAse to remove any contaminating DNA. One µg of mRNA was converted to cDNA by reverse transcription with a Xpert cDNA Synthesis Kit and oligo (dT) primers (Grisp Research Solutions). Quantitative real-time PCR (qPCR) was performed in a Thermo Scientific PikoReal Real-Time PCR System (SPL0961) on 96-well plates with Xpert Fast SYBR Master Mix (Grisp Research Solutions). Briefly, for each sample (biological replicate), qPCR reactions were performed in triplicate (technical replicates) using 10 µL MasterMix, 300 nM of each primer, 1 µL of cDNA and nuclease-free H_2O to a final volume of 20 µL. The following cycler conditions were used: 15 min at 95 °C and 45 cycles of 15 s at 95 °C, 30 s at 55 °C and 30 s at 72 °C, as described previously [5,6]. Fluorescence was measured at the end of each amplification cycle. The sequences of

gene-specific primers used were retrieved from previously published studies, as detailed in Table 1. The specificity of PCR reactions was checked through dissociation curves at the end of each qPCR reaction, by heating the amplicons from 65 to 95 °C. Relative fold changes in gene expression were calculated based on the threshold cycle (Cq) using the $2^{-\Delta\Delta Cq}$ method [7] and normalized with the *VvGAPDH* reference gene [4,8].

Table 1. Genes and primer sets used for real time RT-PCR.

Gene	Pathway		Primer 5'-3'	Reference
VvGAPDH	HK	fwd	TTCCGTGTTCCTACTGTTG	[8]
		rev	CCTCTGACTCCTCCTTGAT	
VvCHLG	Chlorophyll	fwd	GCGTACGGTGGCAGACTGAATATC	[9]
		rev	AGCAGAGGCCATGATACGTTGC	
VvLBCY2	Carotnoids	fwd	TGGAACAGCTGGAATGGTCCAC	[9]
		rev	TGCTGCTAGAGTCCTTGCTACC	
UFGT	Phenylpropanoid/ Anthocyanins	fwd	CCTCATGCAGTCTTCTCCTTCTTC	[10]
			ACACCGTCGGAGATATCATAGGACT	
		rev	T	
CHS	Phenylpropanoid	fwd	CCGACGAAGTTCACACTGATTCAAG	[10]
		rev	GATAGTCAGCCTGGTAGACACAGT	
PAL1	Phenylpropanoid	fwd	CCGAACCGAATCAAGGACTG	[11]
		rev	GTTCCAGCCACTGAGACAATC	
RCA	Photosynthesis	fwd	GCTCTTGGAGATGCGAACGT	[12]
		rev	*GGGCTGCCTTGCCATAAA*	

3. Results and discussion

3.1. Expression of Target Genes in response to differential water management

Gene expression levels were assessed for *UFGT, PAL1* and *CHS3* in grape samples considering two years and two different phenological stages, Véraison (V) and at the end of maturation (H), as previously described in Material and Methods section. In 2022, the expression levels between V and H for grapes of "Touriga Nacional" and for the three irrigation treatments (Fig.2a) of *UFGT* gene shows the most significant change in expression between V and H, with the highest expression in non- irrigated vines. *PAL1* gene present the highest expression in the R30 condition, indicating it might be more sensitive to water level. On its turn, *CHS3* gene showed moderate changes in expression, with the lowest fold change expression in R30 condition.

Surprisingly, in 2023 (Fig. 2b), *UFGT* showed a significant reduction in its expression from V to H, especially in the control group (0% ETc). Irrigation mitigated this reduction, with higher irrigation (60% ETc) resulting in the least negative fold change, suggesting that *UFGT* gene expression is more stable under higher irrigation levels. On its turns *PAL1* gene exhibits the most significcant decrease in expression at 0% ETc, indicating a strong downregulation from V to H without irrigation. Interestingly, at 30% ETc, the expression is nearly stable, suggesting this irrigation level might help maintain *PAL1* expression across phenological stages. However, at 60% ETc, there is still a decrease, but it is less severe than in the control group. *CHS3* gene expression declines between V and H across all irrigation treatments. The most substantial decrease occurs at 60% ETc, followed by 30% ETc, suggesting that increased irrigation levels might contribute to further downregulation of *CHS3* through water irrigation conditions.

Comparing years at V stage (Fig. 3a), *UFGT* and *PAL1* genes exhibited contrasting patterns under increased irrigation, with *UFGT* gene decreasing significantly at 60% ETc, while *PAL1* gene increased severely. *CHS3* gene showed a moderate response to higher irrigation levels.

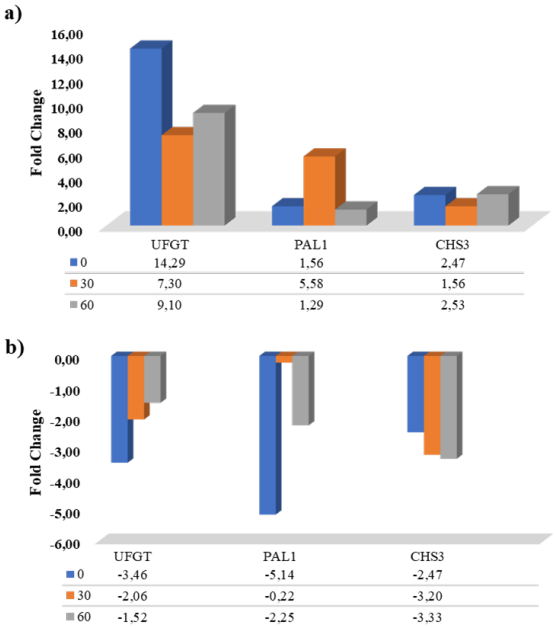

Figure 2. Expression levels of *UFGT*, *PAL1*, and *CHS3* genes between the V and H phenological stages in 2022 (a) and 2023 (b) under three different irrigation treatments: 0% (blue bars), 30% (orange bars), and 60% (gray bars) of grapevine evapotranspiration.

Concerning H (Fig. 3b) *UFGT* and *PAL1* genes showed a consistent negative fold change, indicating reduced expression levels in 2023 compared to 2022. *PAL1* gene showed a stronger negative response to higher irrigation levels. *CHS3* gene behaved differently, with a slight increase in expression under moderate irrigation, suggesting a potential threshold beyond which its expression declines again.

An overall interpretation suggests that genes belonging anthocyanin biosynthesis pathway respond uniquely to different irrigation levels that also may be related to the specific roles these genes play within the pathway. Nevertheless, these data must be complemented with climate information and occasional vineyard practices to optimize irrigation practices towards grapevine health and productivity.

The same analysis was performed for leaves samples of "Touriga Nacional" grapevine variety. In this case, gene expression level was assessed for *RCA*, *LBCY,* and *CHLG3* genes considering also the two years and two different phenological stages, V and H. In 2022, the expression of all three genes (*RCA*, *LBCY2*, and *CHLG3*) (Fig.4a) showed water irrigation treatment-dependent variability in response to the conditions being tested (H vs V). For instance, *RCA* gene was consistently downregulated across ETcS tested, while *LBCY2* and *CHLG3* initially showed upregulation (at ET_0) but then transition to downregulation at Etc 30 and ETc 60. The largest change was observed for RCA gene at Etc 30 (- 1.82), suggesting that this gene is particularly sensitive or responsive to this condition.

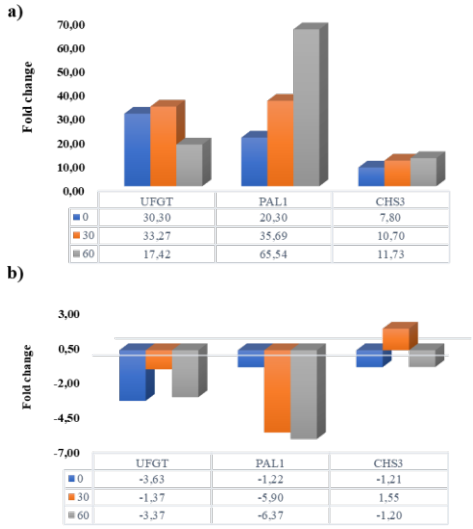

Figure 3. Expression levels of *UFGT*, *PAL1*, and *CHS3* genes between 2023 and 2022 at the V (a) and H (b) phenological stages under three different irrigation treatments: 0% (blue bars), 30% (orange bars), and 60% (gray bars) of grapevine evapotranspiration (ETc).

For 2023 (Fig. 4b), all three genes show overall upregulation across the conditions. The upregulation of *RCA* gene is gradually expressed over the conditions, with fold changes increasing slightly from 0.69 to 0.85. Initially, *LBCY2* gene has a strong upregulation, which decreases steadily at ETc 30 e ETc 60, yet remains significantly above 1, indicating continued upregulation. *CHLG3* gene shows strong upregulation at ET_0, a marked decrease at ETc 30, and then a slight recovery at ETc 60, indicating a potentially sensitive or highly regulated response to the conditions. These results suggest that while all three genes are upregulated in response to the conditions, the extent and stability of this upregulation vary. In particular, *LBCY2* and *CHLG3* genes, showed strong initial upregulation, but *CHLG3* undergoes significant fluctuation, which might indicate different regulatory mechanisms or sensitivities to environmental or experimental conditions. A comparative analysis between years for V (Fig. 5a), shows that *RCA* gene is consistently downregulated across all conditions in 2023 compared to 2022, with the level of downregulation increasing thought the ETcS. *LBCY2* gene initially shows downregulation at ET_0 but then undergoes a reversal, leading to significant upregulation at ETc 30 and ETc 60. *CHLG3* is consistently upregulated in 2023 compared to 2022, with the degree of upregulation increasing the different irrigation systems.

The expression patterns suggest that *RCA* gene might be negatively impacted under the conditions in 2023 relative to 2022, while *LBCY2* and *CHLG3* genes show a trend toward increasing expression levels, particularly at ETc 30 and ETc 60. This may indicate that *RCA* gene responds differently or is more sensitive to the environmental or experimental changes between the two years, whereas *LBCY2* and *CHLG3* appear to adapt with increased expression.

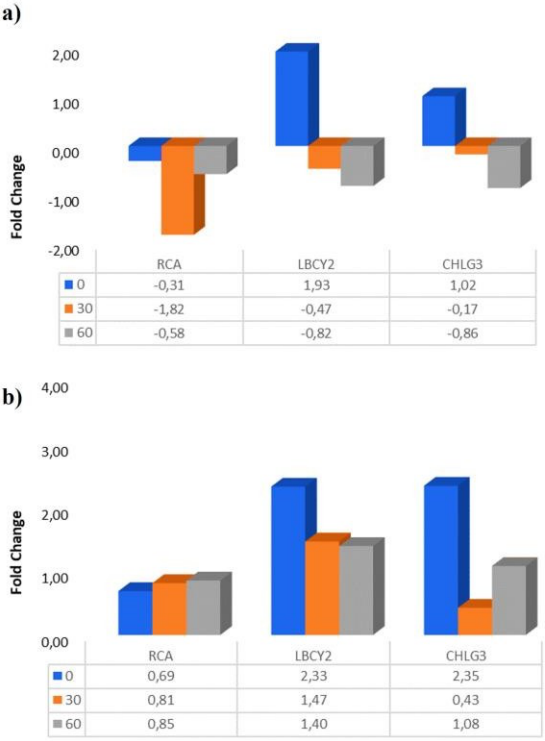

Figure 4. Fold change in gene expression for *RCA, LBCY2*, and *CHLG3* genes at the V (a) and H (b) phenological stages under three different irrigation treatments: 0% (blue bars), 30% (orange bars), and 60% (gray bars) of grapevine evapotranspiration (ETc).

Concerning H (Fig. 5b), the expression patterns suggest that *RCA* gene shows a fluctuating behavior, moving from downregulation at ET_0 to upregulation at under moderate irrigation, followed by a marked downregulation ETc 60. *LBCY2* gene shows a consistent and increasing upregulation across all irrigation conditions, with the most expressive upregulation observed at ETc 60. *CHLG3* gene responds differently to the different irrigation conditions starting with slight upregulation, followed by downregulation at ETc 30, and ending with significant upregulation at ETc 60. *RCA* behaves differently from *LBCY2* and *CHLG3*, showing a contrasting pattern of downregulation as they become upregulated. This suggests that different regulatory mechanisms may be responsible, potentially due to the biological pathways these genes are

involved in, the environmental conditions they are exposed to, or even variations in vineyard practices.

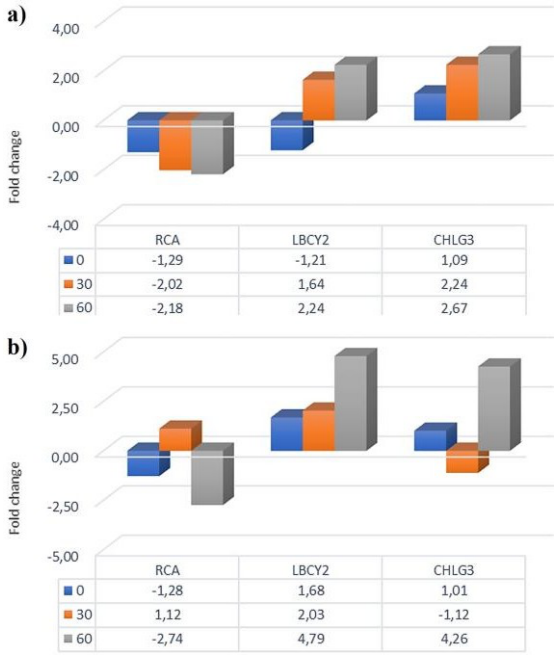

Figure 5. Fold change in gene expression for *RCA, LBCY2,* and *CHLG3* genes between 2023 and 2022 at the V (a) and H (b) phenological stages under three different irrigation treatments: 0% (blue bars), 30% (orange bars), and 60% (gray bars) of grapevine evapotranspiration (ETc).

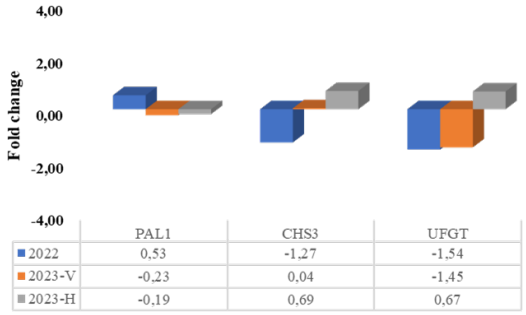

Figure 6. Expression levels of *UFGT*, *PAL1*, and *CHS3* genes between H (2022) and, V and H (2023) phenological stages under different sunlight exposure conditions, shaded (D) and unshaded (S).

3.2. Expression of Target Genes in response to different sunlight exposure conditions

The gene expression level was assessed for *UFGT, PAL1* and *CHS* genes for grapes samples considering two years and two different sunlight exposure conditions. Expression level was assessed for V in 2022, and V and H in 2023. In 20223, two different environmental conditions, shaded (D) and unshaded (S) were analyzed (data not shown). Nevertheless, the comparison between S and D conditions in 2023 reveals that sunlight stimulates gene expression, particularly for *PAL1* and *CHS* genes, while dark conditions either maintain or reduce gene expression. This suggests that these genes may be light-sensitive which is according to the fact that they belong to the flavonoid biosynthesis pathway. In contrast, dark conditions appear to either repress or maintain their expression [13].

A comparative analysis between years (Fig.6) *PAL1* gene shows an initial upregulation in 2022, followed by a downregulation in both 2023 periods. *CHS3* shows significant recovery from downregulation in 2022, transitioning to upregulation by 2023-H. *UFGT* gene, like *CHS3*, undergoes a major recovery from strong downregulation in 2022 to upregulation in 2023-H.

4. Conclusion

The results highlight the unique regulatory mechanisms of genes involved in anthocyanin biosynthesis and photosynthesis-related, chlorophyll and carotenoid pathways in response to differential water management. *UFGT, PAL1*, and *CHS3* respond distinctly across phenological stages and irrigation levels, indicating their sensitivity to water stress and the importance of optimal irrigation for maintaining gene stability and grapevine productivity. Similarly, *RCA, LBCY2*, and *CHLG3* in leaves suggest adaptive responses to varying water availability, with some genes being more sensitive to environmental changes than others.

Regarding sunlight exposure conditions its evident that sunlight exposure plays a crucial role in stimulating gene expression, especially for light-sensitive genes like *PAL1* and *CHS* [13]. These genes are essential for flavonoid biosynthesis, a process influenced by light confirmed by the dark conditions that tend to suppress or maintain gene expression, indicating that certain genes in the flavonoid pathway are less active without sunlight. Gene expression patterns vary between years, suggesting that environmental factors, including sunlight, influence the expression of the genes.

OmicBots represents a significant advancement in the field of PV by integrating cutting-edge technologies with a deep understanding of grapevine physiology, the project intends to revolutionize vineyard management practices, ensuring that wine producers can continue to produce high-quality, profitable, and eco-friendly wines in the face of emerging challenges. This approach is particularly crucial in regions like the Douro Valley, where climate change poses significant threats to traditional viticultural practices. The integration of multi-omics data with systems biology and AI not only enhances the accuracy of vineyard management but also aligns with broader sustainability goals. In conclusion, the comprehensive dataset generated within this study offers valuable insights into grapevine responses to GxExM interactions, which is being used to develop a new plant screening platform. Moreover, ongoing efforts are focused on including additional datasets, such as metabolomic analyses, water potential data, environmental and climate variability factors, and vineyard management practices, to further support and validate the outcomes. This integrated approach will provide a more thorough explanation of the results, ensuring a more accurate platform. This platform will allow researchers and vineyard managers to simulate and predict how grapevines will respond to different GxExM scenarios, enabling more targeted and effective management practices.

5. Acknowledgements

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