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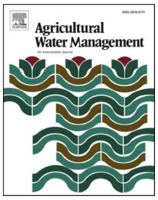

Molecular and hydraulic responses of grapevine to water status and phenology under long-term differential irrigation treatments

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ABSTRACT

Understanding seasonal interactions among hydraulic, chemical and molecular signalling under water deficit is crucial for improving vineyard irrigation strategies under climate change and increasing water scarcity. We aimed to test how irrigation strategies and phenology affect the hydraulic adjustment mechanisms of eight field-grown grapevine varieties under different long-term irrigation regimes: Full-irrigated (100 % ETc), deficit-irrigation (50 % Full-irrigated), and non-irrigated. Leaf pre-dawn water potential, leaf hydraulic conductivity (K_{leaf}), stomatal conductance, aquaporin gene expression and hormone content, were assessed at four key phenological stages, from pea-size to post-harvest. Stomatal closure was the earliest water-stress response across genotypes, while hormonal balance differentiated responsive from non-responsive genotypes regarding chemical signalling. K_{leaf} was primarily driven by phenology, peaking at early stages and declining thereafter. Aquaporin gene expression was both genotype- and phenology-dependent, with high activity during early stages followed by progressive down-regulation, aligned with changes in stomatal conductance and K_{leaf} and reflecting each variety iso-/anisohydric behaviour. This pattern confirms the role of aquaporins in grapevine hydraulic adjustments and stomatal regulation under decreasing water potentials. Aquaporins and K_{leaf} showed positive responses to water availability at post-harvest, indicating differential watering benefits among genotypes. Our results emphasize the need for field-phenotyping studies to fine-tune water management strategies in viticulture.

1. Introduction

Grapevine (*Vitis vinifera* L.) is considered a drought-avoiding species (Zamorano et al., 2021), well-adapted to semi-arid Mediterranean climates. However, recent climate changes have intensified drought impacts on yield and quality due to increased atmospheric demand and altered precipitation patterns (Chaves et al., 2002, 2010; Gambetta et al., 2020). Consequently, in drought-prone regions undergoing climatic uncertainty, deficit irrigation is becoming more prevalent in vineyards. This practice is contributing to the sustainability of the wine sector by overriding the negative effects of water stress and heat waves on yield and wine quality. However, optimizing irrigation is a prerequisite to balance productivity and quality (Zarrouk et al., 2016a), while reducing competition for the increasingly scarce natural resource that is water.

However, the implementation of irrigation should also consider the

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post-harvest period, which is particularly important for fine root growth and the replenishment of carbohydrate reserves that will sustain the new vegetative growth in the subsequent growing season until the start of the following season's photosynthesis activity (Holzapfel et al., 2006).

The high variability in responses among varieties under different levels of water availability should also be considered (Chaves et al., 2010). Differences between grapevine varieties regarding stomatal closure are well documented and highlight that, in certain climatic situations, some varieties tend to be more reactive to external factors, closing their stomata (isohydric), whilst others tend to be less reactive (anisohydric) (Chaves et al., 2010; Lovisolo et al., 2010; Costa et al., 2012). A recent work by Jacinto et al. (2023) showed a wide dispersion of δ^{13}C among 172 grapevine varieties in response to water deficits, indicating a high variability in stomatal regulation and water use efficiency among genotypes. Additionally, a study by Levin et al. (2020) showed that the distinct stomatal responses of 17 grapevine genotypes depended on the severity of the imposed water stress, suggesting an adjustment of stomatal responses, such that they may operate as anisohydric at high water status and as isohydric at low water status. Carvalho et al. (2016) further demonstrated that grapevine varieties displayed differential physiological responses to combined heat, drought, and light stresses, with some varieties showing higher capacity for heat dissipation. It appears thus that the response mechanisms to water stress in grapevine are extremely intricate, and still need further research (Zarrouk et al., 2016a). On the other hand, extended drought periods late in the season, as experienced in recent years due to climate change, were reported to shorten the dormancy cycle in grapevine (Shellie et al., 2018). This suggests the importance of post-harvest irrigation to ensure adequate storage of photoassimilates in woody perennial structures such as trunks and roots and, thus, the maintenance of crop production and the longevity of the plants.

Grapevine presents efficient water deficit adaptation mechanisms to transfer water from roots to growing shoots, involving effective stomatal control of transpiration (Chaves et al., 2010), xylem embolism avoidance system (Lovisolo et al., 2002; Vandeleur et al., 2009), hydraulic vulnerability segmentation (McElrone et al., 2021), as well as osmotic adjustment ability (Chaves et al., 2010; Labarga et al., 2023). An interplay between hydraulic and chemical signalling governs grapevine responses to water stress (Dayer et al., 2019), enabling an hydraulic adjustment in grapevine at the level of roots and leaves under water stress is well documented (Lovisolo et al., 2010; Xiong and Nadal, 2020). Studies by Tombesi et al. (2015) suggested that stomatal conductance (g_s) is mostly regulated by hydraulic signals at the early phases of water stress, while abscisic acid (ABA) acts as a keeper of the long-term maintenance of stomatal closure under prolonged water stress. However, in drought acclimated grapevines, several reports evidenced an absence of xylem embolism before stomatal closure and turgor loss point (Hochberg et al., 2017b; Skelton et al., 2017; Scoffoni et al., 2018), suggesting that grapevines close their stomata as a primary response before more drastic events can affect plant hydraulics and that other signalling (e.g., chemical, or/and molecular) regulate stomatal conductance decrease. Moreover, Pou et al. (2013) observed that leaf hydraulic conductivity (K_{leaf}) is also affected by air vapor pressure deficit (VPD), reinforcing the role of abiotic effects on this complex cascade mechanism. Altogether, an interplay between hydraulic and chemical signalling seems to govern grapevine's responses to water stress more than a single control, either hydraulic or chemical (Dayer et al., 2019).

The key role of ABA as chemical signalling mediating stomatal regulation and photosynthetic assimilation is well documented in grapevine (reviewed by Lovisolo et al., 2010; Gambetta et al., 2020; Marusig and Tombesi, 2020). Nevertheless, several studies showed the low sensitivity of anisohydric varieties to ABA increase (Coupel-Ledru et al., 2017; Dayer et al., 2020). In this regard, it has been considered that isohydric stomatal control is driven by ABA, whereas anisohydric regulation is, at least initially, governed by water potential (Brodribb and McAdam, 2013; Tramontini et al., 2014).

On the other hand, auxins are suggested to be an important node in the crosstalk network among different hormones for water stress signalling response (Wang et al., 2019; Marusig et al., 2020), while new insights showed the interplay of auxin response factors (ARFs) in plant water stress responses (Verma et al., 2022) with influence on ABA level (Korver et al., 2018). Besides, the increase of ABA biosynthesis was shown to negatively regulate the transport and biosynthesis of auxin, both at transcriptional and post-translation levels, in a crosstalk network under osmotic stress (Rowe et al., 2016). More recently, auxin signalling has been identified as miRNA targets and may regulate their expression under drought stress or ABA treatment (Liang et al., 2024). Although the specific role of auxins in grapevines subjected to water stress is not fully understood, they are involved in the complex network of hormonal and physiological responses that need to be considered alongside ABA.

During the processes of plants' response to water stress, aquaporins (AQPs) are deeply involved in the non-vascular transport of water through inter- and intracellular pathways (Maurel et al., 2015) within the plant. Under water stress, they contribute to rapid and reversible regulation of cells hydraulic conductance in several organs by adjusting the membrane water permeability (Vandeleur et al., 2009; Pou et al., 2013; Sabir et al., 2021), playing an essential role in the adaptation to water stress by maintaining water and ion homeostasis. Aquaporins are water channels responsible for the cell and organelle membrane regulation of the movement of water and small molecules, such as gases (CO_2), solutes and other substances (Kaldenhoff and Fischer, 2006; Moshelion et al., 2015; Vitali et al., 2016; Opazo et al., 2020; Sabir et al., 2021; Labarga et al., 2023). Functioning as plant water channels, without modifying the flux direction, AQPs can greatly increase the water movement across membranes (Vitali et al., 2016). They are classified into five subfamilies, where the plasma membrane intrinsic proteins (PIPs) and the tonoplast intrinsic proteins (TIPs) are the most studied for their putative roles in leaf hydraulic dynamics and stomatal conductance in grapevine under water stress (Pou et al., 2013; Zarrouk et al., 2016b; Wong et al., 2018; Opazo et al., 2020; Labarga et al., 2023). They are expressed in several plant tissues, and their expression and activity may be affected by environmental conditions (Lovisolo et al., 2010). Labarga et al. (2023) reported that, under water stress conditions, AQP expression is higher in roots than in leaves. Vandeleur et al. (2009) studied the expression of *VvPIP1;1* and *VvPIP2;2*, the two PIP aquaporins with higher expression in roots, and observed that they are expressed regardless of cultivar and soil water. Besides this evidence, further studies are needed to unravel the connection between AQPs and the hydraulic conductance in plants and its effect on plant transpiration (Vitali et al., 2016; Opazo et al., 2020; Labarga et al., 2023).

Understanding the physiology of different grapevine varieties and studying their adaptability to extreme water deficit scenarios is paramount in viticulture and oenology, as this knowledge allows winegrowers to select the varieties better suited to endure the foreseeable climate scenarios. To optimize deficit irrigation management and identify the most suitable genotypes, it is crucial to understand the physiological and molecular mechanisms governing water relations and movement at both the cellular/tissue level and across whole plants within different grapevine genotypes. In this context, the current study aims to investigate the effects of grapevine water status and phenology on stomatal and hydraulic adjustments, as well as the expression of aquaporins in leaves of eight grapevine genotypes, selected according to their iso- or anisohydric behaviour and growing under different levels of water availability.

2. Material and methods

2.1. Plant material and experimental site

The study was conducted in the 2021 growing season at a private Ampelographic Collection of Herdade do Esporão S.A., located in the

Alentejo region, Portugal (38.380098, −7.560724). The region has a Mediterranean climate, characterized by hot and dry summers, classified as 'Csa' under the Koppen-Geiger Climate Classification (IPMA, 2021). The historical records from WorldClim 2.1 (Fick and Hijmans, 2017) show an average annual temperature of 16.1 °C and an average annual precipitation of 572 mm. The 10-hectare commercial vineyard was planted in 2011, with vines grafted onto 1103 Paulsen rootstock, spaced 1.5 m x 3.0 m apart. The vineyard has a north-south orientation, with vines trained on a vertical shoot-positioned system, spur-pruned on a bilateral Royat cordon system. All vines were pruned evenly, with 14–16 buds per vine.

The vineyard was drip-irrigated, with irrigation lines in the centre of the row, consisting of pressure-compensating 2.4 L h^{-1} emitters spaced 1 m apart. The inter-row was managed with resident and planted vegetation, mowed at the beginning of May and left on the ground as mulch. The soil in this area is classified as a Eutric Cambisol, with an ApBw1Bw2C profile; derived from granite and has a sand content of 75–80 %. The soil depth is 0.9 m and the estimated total available water is 105 mm, based on field capacity and permanent wilting point measurements and calculated according to Ramos et al. (2016). The soil has a pH ranging from 7.0 to 7.6, low organic matter (1.15 %) content and high phosphorus (P_2O_5, 21 ppm) and potassium (K_2O, 203 ppm) contents.

Since 2018, all varieties have been subjected to three irrigation treatments: full irrigation (FI, providing 100 % crop evapotranspiration (ETc)); deficit irrigation (DI, providing 50 % of FI); and non-irrigated (NI, rainfed). The FI treatment involved weekly irrigation (I), with the irrigation amount calculated as $I = ETo \times K_{cb} \times 1.1$, where ETo represents the reference evapotranspiration calculated for the week using the Penman–Monteith FAO 56 method (Allen et al., 1998) and K_{cb} is the estimated crop coefficient derived from the formula $K_{cb} = 1.44 \times NDVI - 0.1$ (Campos et al., 2010), where NDVI is the field average Normalized Difference Vegetation Index, measured along the season using Sentinel-2 image data. The use of the factor of 1.1 accounts for soil water evaporation (Table 1).

The genotypes investigated in the present study were selected according to previously assessed berry juice and phloem sap $\delta^{13}C$ differences between FI and NI plants (Jacinto et al., 2023). The varieties Alicante Bouschet, Tinta Caiada and Trincadeira were selected for their high $\delta^{13}C$ differences between FI and NI plants. Petit Verdot, was selected for its low $\delta^{13}C$ differences between FI and NI plants. Castelão, Syrah, Touriga Nacional and Vinhão were chosen for their intermediate results.

Each variety is planted in a row consisting of approximately 110 plants, divided by three irrigation treatments. For sampling purposes, the first and last five plants of each row were excluded. Additionally, buffer zones of five plants were established at the transition areas between the treatments, and these plants were not used for measurements. Plant monitoring was done at four phenological stages: pea-size (PS, BBCH 75), veraison (VER, BBCH 83), full-maturation (FM, BBCH 89) and post-harvest (Post-H, BBCH 91).

Unless stated otherwise, all variables were monitored at the four phenological stages. Plant sampling/measurements were done in five plants per genotype and treatment, using at least two leaves per plant. Measurements/sampling were taken from leaves that were well-exposed to sunlight, healthy, fully expanded and photosynthetically active. Leaves were selected from the third to fifth node of one of the main central shoots of the plant.

Table 1
Total irrigation amount applied in each irrigation treatment during the 2021 growing season.

Irrigation treatment	Irrigation depth (mm)
FI	246
DI	123
NI	0

2.2. Physiological parameters

2.2.1. Leaf water potential

Leaf water potential was monitored along the abovementioned phenological stages, by measuring predawn leaf water potentials (Ψ_{pd}, MPa), at 5 am, using a Scholander-type pressure chamber (Manofrigido, S.A., Lisboa, Portugal). Leaves were excised using sharp scissors and immediately sealed in a plastic bag containing a damp paper towel to prevent water loss and wrapped with aluminium foil to prevent transpiration before Ψ_{pd} determinations.

2.2.2. Stomatal conductance

Stomatal conductance (g_s, mmol m^{-2} s^{-1}) measurements were performed with a steady state Porometer (LI-1600, LI-COR Inc, Lincoln, NE, USA). Measurements were made on sunny, cloud-free days between 11 am and 12 pm.

2.2.3. Hydraulic conductance

Hydraulic conductance (K_h, kg s^{-1} MPa^{-1}) was calculated as the ratio between the flow through each segment and the corresponding hydrostatic pressure gradient was measured in whole petiole segments following Sperry et al. (1988), with a high precision flow meter, XYL'EM (Embolism Meter, Bronkhorst, Montigny-les-Cormeilles, France), as described in Zarrouk et al. (2016b).

2.3. Hormone extraction and analysis

Hormonal analysis was conducted at the veraison stage, as this phenophase corresponded to the seasonal peak values of various hydraulic parameters in almost all varieties. Four varieties, Petit Verdot, Syrah, Touriga Nacional and Trincadeira, were chosen for their representative hydraulic behaviour, reflecting the diversity observed among the eight varieties studied. Grapevine leaves were carefully harvested at 11 am and immediately frozen in liquid nitrogen to be transported to the lab. Leaf samplings were conducted at veraison and consisted of two leaves per grapevine, from ten plants per genotype and treatment. Three independent leaf subsamples were ground separately under liquid nitrogen, with a mortar and pestle. The samples were then promptly frozen dried, lyophilized and sent for hormonal analysis quantification.

Plant hormones indoleacetic acid (IAA) and abscisic acid (ABA) were identified and quantified by ultra-high performance liquid chromatography–mass spectrometry (UHPLC-MS) using a Thermo Scientific™ Q Extractive™ Hybrid Quadrupole-Orbitrap mass spectrometer at Institute for Plant Molecular and Cell Biology (IBMCP) Spain, following the protocol by Benítez García et al. (2020). The hormones were quantified (ng g DW^{-1}) based on internal deuterated standards by construction of calibration curves.

2.4. Pigment quantification

Chlorophyll-*a*, chlorophyll-*b*, total anthocyanins, β-carotenes and carotenoids were extracted from three independent subsamples of frozen leaves. Leaf extracts (100 mg) were incubated in 1 mL of acetone mixed with Tris-HCl 100 mM buffer (pH 7.5) in a 80:20 (v: v) and then centrifuged at 12,000 *g* for 15 min. The supernatants were collected for quantification. Absorbance was measured at 537, 647, and 663 nm for chlorophylls, anthocyanins and carotenoids, and at 505 and 453 nm for β-carotenes. To obtain pigment concentrations, the equations described by Sims and Gamon (2002) were used for chlorophyll-*a*, chlorophyll-*b*, total anthocyanins and carotenoids, and for β-carotenes the equation described by Nagata and Yamashita (1992) was used. The values obtained were converted to mg g^{-1} FW (Richardson et al., 2002).

2.5. RNA extraction and cDNA synthesis

Grapevine leaves were carefully harvested from the five apical nodes, always at 11 am to avoid diurnal variation of AQPs gene expression (Shelden et al., 2017), immediately frozen in liquid nitrogen, and transported to the lab. Samples were kept at −80 °C until processing. Three independent samples from at least five leaves were ground with a mortar and pestle, previously and continuously cooled with liquid nitrogen. Total RNA was extracted using the Spectrum™ Plant Total RNA kit (Sigma-Aldrich, St. Louis, MO, USA). Nucleic acid concentration was quantified spectrophotometrically using Take3 plate and the software Gen5 v1.09 in a Synergy HT (Bio-Tek Instruments, Winooski, VT, USA). The quality of the RNA extracted was evaluated through the ratios A_{260}/A_{280} and A_{260}/A_{230}, and the RNA integrity was assessed through 1.5 % agarose-gel electrophoresis under denaturing conditions.

RNA samples were treated with RQ1 RNase-Free DNase (Promega, Madison, WI). cDNA was synthetized from 1 μg of total RNA using oligo $(dT)_{20}$ in a 20 μL reaction, with RevertAid Reverse Transcriptase (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. cDNA was tested to assess gDNA contamination in PCRs, using intron spanning primers that yield a 229 bp amplicon in cDNA and a 547 amplicon in gDNA. Amplicon sizes were compared in 2 % agarose gels together with the molecular weight marker $1Kb^{+}$ (Thermo Fisher Scientific, Waltham, MA, USA), and no gDNA contamination was detected. cDNA was then stored at −20 °C.

2.6. Real-time quantitative PCR

The expression of 10 aquaporins was assessed: *VviPIP1;1*, *VviPIP1;2*, *VviPIP2;1*, *VviPIP2;2*, *VviTIP1;1*, *VviTIP2;1*, *VviTIP2;2*, *VviNIP2;1*, *VviNIP6;1* and *VviXIP1*. The respective primers were obtained from previous studies and their sequences, amplicon size, and respective references can be found in Table S1. Two genes were used as reference, *Actin 2* (ACT) and *Vitis vinifera translation initiation factor 3 subunit G* (TIF) (Coito et al., 2012). Real-time qPCR reactions were made in 96-well clear plates (Bio-Rad, Hercules, CA, USA), using CFX-connect Real Time PCR (Bio-Rad, Hercules, CA, USA) with three biological replicates x two technical replicates. 1 μL of cDNA diluted 50-fold, 0.5 μM of each specific primer, and the Master MixSsoFast EVA Green (Bio-Rad, Hercules, CA, USA), according to the manufacturer's recommendations. The total reaction volume of 20 μL was completed with Milli-Q Millipore Ultrapure Water. Amplification of PCR products was monitored via intercalation of the Eva-Green present in the master mix. The program applied was as follows: initial polymerase activation, 95 °C, 3 min, then 40 cycles at 94 °C for 10 s (denaturation), 60 °C for 20 s (annealing), and 72 °C for 15 s (extension), followed by a melting curve analysis to confirm the accurate amplification of target gene fragments and the absence of primer dimers. The PCR products were run on 2 % agarose gels to verify that there was only one amplicon of the expected size. PCRs with each primer pair were also performed in triplicate on samples lacking a cDNA template (no template controls). To generate a baseline-subtracted plot of the logarithmic increase in the fluorescence signal (ΔRn) versus the cycle number, baseline data were collected between cycles 5 and 17. All amplification plots were analysed with an Rn threshold of 0.2 at the beginning of the region of exponential amplification to obtain Cq (quantification cycle), and the data obtained were exported into an MS Excel workbook (Microsoft Inc., Albuquerque, New Mexico, USA) for analysis. Quantification of the relative gene expression was done with the ΔΔCq method and expressed as mean and standard error of three biological replicates and two technical replicates.

2.7. Statistical analysis

The experimental design consisted of a single factor analysis for each genotype, for which the factor was the irrigation treatment, with three levels: FI, DI, and NI. An exploratory and descriptive analysis was made of all physiological and molecular measurements, followed by a one-way ANOVA analysis in the cases where the response variable residuals were normally distributed, and a Kruskal-Wallis test if normality was not encountered. The differences between treatments of each physiological variable were calculated for each genotype. FI to DI, FI to NI, and DI to NI differences were tested through Student's *t*-test for a significance level of a 0.05, with the exception of the cases when normality was not encountered, for which the FI to DI, FI to NI, and DI to NI differences were tested through a Dunn's test, for a significance level of a = 0.05. All statistical analyses were made using R Statistical software version 4.3.1 (R Core Team, 2022). Regarding the aquaporins gene expression values were transformed into $\log_2$ and tested through ANOVA in software R v4.2.3. When the p-value of the ANOVA was lower than 0.05, a Tukey test was performed, and statistically significant differences were accepted for a *p*-value lower than 0.05. GraphPad Prism 10 for Windows (GraphPad Software, San Diego, CA, USA) was used to create the heatmap for aquaporin's gene expression data, and R Statistical software version 4.3.1 (R Core Team, 2022) was used for the other figures creation.

3. Results

3.1. Meteorological data

Total precipitation, air temperature and maximal VPD are shown in Fig. 1. Results point to mild mean temperatures (T_{mean}) along the growing season, with low total precipitation and moderate VPD. Overall, meteorological conditions during the 2021 season could be considered mild for the Alentejo Region, particularly concerning the incidence of high temperatures and heat waves (IPMA, 2025). Air temperatures were relatively low compared with those considered average for the region (Jacinto et al., 2023), with only 4 days with maximum temperatures above 40 °C.VPD was high throughout the season, with peaks of 7 kPa at the end of the maturation period (14/8/2021 and 15/8/2021, one week after FM sampling) (Table S2). Precipitation was almost absent during the growing period, but an abundant precipitation event (100 mm) occurred after harvest. This event substantially influenced Post-H soil moisture levels and was considered equivalent to a high-volume irrigation event, enabling a detailed analysis of grapevine recovery responses following post-harvest irrigation.

3.2. Grapevine water status

Predawn leaf water potentials (Ψ_{pd}) (Fig. 2) differed significantly among irrigation treatments at the four phenological stages in all genotypes. Grapevines subjected to FI experienced no stress conditions during the season, while plants subjected to DI suffered mild stress. In contrast, plants kept under NI showed signs of moderate stress, with Ψ_{pd} lower than −0.6 MPa (Carbonneau, 1998; Deloire et al., 2004).

At the first sampling date, PS, the differences between FI and DI were only significant for Alicante Bouschet. In contrast, NI was significantly lower than DI and FI in all genotypes, except Petit Verdot, for which no differences among treatments were observed. At VER and FM, Ψ_{pd} decreased further in DI and NI, with significant differences between both water stress treatments. Nonetheless, differences between FI and DI were observed only in Alicante Bouschet and Syrah at VER and Tinta Caiada at FM (Fig. 2). In general, the lowest Ψ_{pd} values were found at FM, with some exceptions: e.g., in Alicante Bouschet and Tinta Caiada the lowest Ψ_{pd} values were observed in VER in NI.

After the abundant rainfall that occurred before the Post-H sampling, a general increase in Ψ_{pd} was observed for all genotypes and treatments. This event raised the soil water to field capacity and Ψ_{pd} to above −0,05 MPa, regardless of the irrigation treatment and genotype.

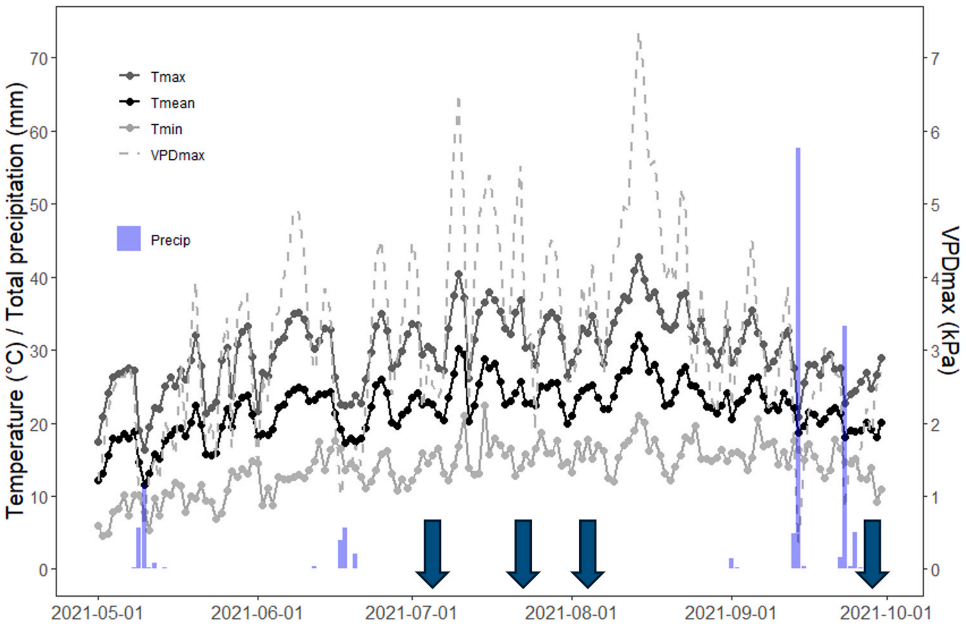


Fig. 1. Meteorological data (1st May to 1st October 2021) obtained from an automatic weather station located near the experimental site. The solid lines represent daily average values of minimum (Tmin, light grey), mean (Tmean, black) and maximum (Tmax, dark grey) air temperatures (°C). The light grey dashed line corresponds to daily maximum vapor pressure deficit (VPDmax, kPa). Blue bars correspond to daily total precipitation (Precip, mm). The dark blue arrows indicate the four sampling moments: Pea-size (PS, BBCH 75), Veraison (VER, BBCH 83), Full-maturation (FM, BBCH 89) and Post-harvest (Post-H, BBCH 91).

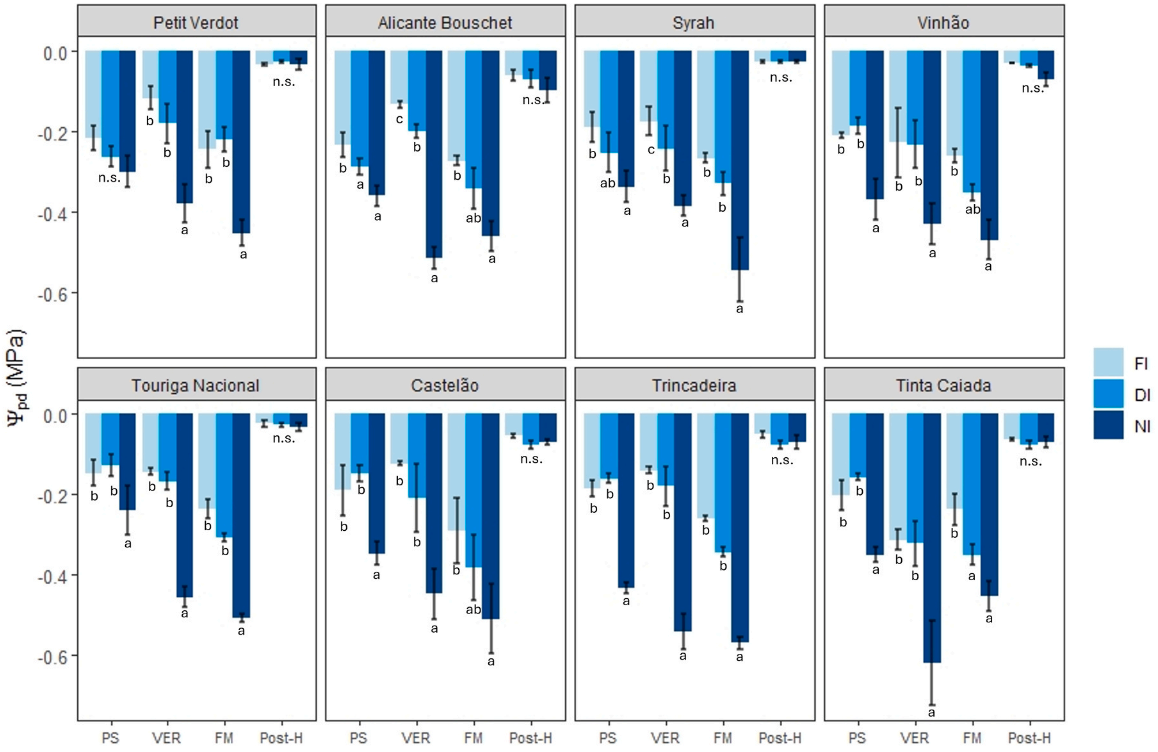


Fig. 2. Predawn leaf water potential (Ψ_{pd}, MPa) in Full-irrigated (FI), Deficit irrigated (DI) and Non-irrigated (NI) plants of the eight studied genotypes, Petit Verdot, Alicante Bouschet, Syrah, Vinhão, Touriga Nacional, Castelão, Trincadeira and Tinta Caiada, at the four phenological stages: Pea-size (PS), Veraison (VER), Full-maturation (FM) and Post-harvest (Post-H). Values are averages of five plants (measurements in two leaves per plant) and error bars indicate standard errors (n = 5). Different letters indicate significant differences between treatments according to Student's *t-test*, for $p < 0.05$; n.s. indicates no significant differences between treatments.

3.3. Stomatal conductance

Stomatal conductance (g_s) values were highest at VER, regardless of the irrigation treatment (Fig. 3), for all genotypes except Touriga Nacional, which peaked at PS, and Petit Verdot, which peaked at FM. After VER, g_s values decreased 1.5–2-fold in all genotypes except Petit Verdot and Tinta Caiada, that showed relatively stable g_s values during the growing season. In general, FI and DI showed higher g_s when

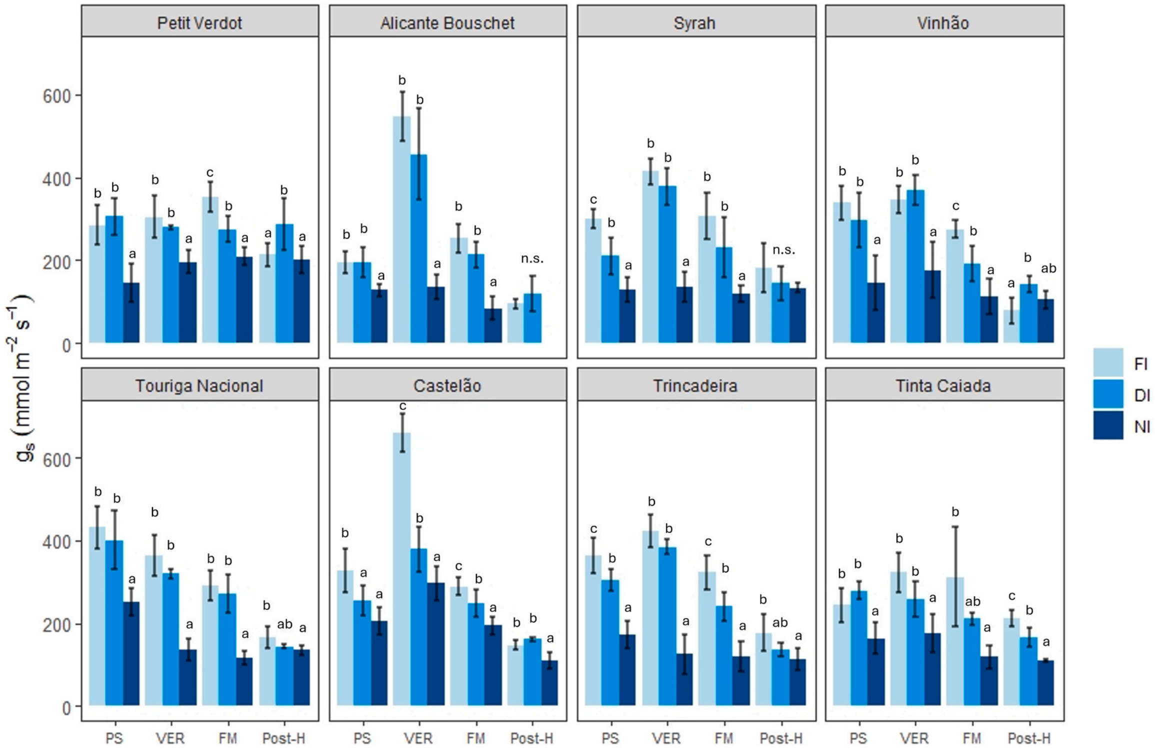


Fig. 3. Stomatal conductance (g_s, mmol m^{-2} s^{-1}) in Full-irrigated (FI), Deficit irrigated (DI) and Non-irrigated (NI) plants of the eight studied genotypes, Petit Verdot, Alicante Bouschet, Syrah, Vinhão, Touriga Nacional, Castelão, Trincadeira and Tinta Caiada, at the four phenological stages: Pea-size (PS), Veraison (VER), Full-maturation (FM) and Post-harvest (Post-H). Values are averages of five plants (measurements in two leaves per plant) and error bars indicate standard errors (n = 5). Different letters indicate significant differences between treatments according to Student's *t-test*, for $p < 0.05$; n.s. indicates no significant differences between treatments.

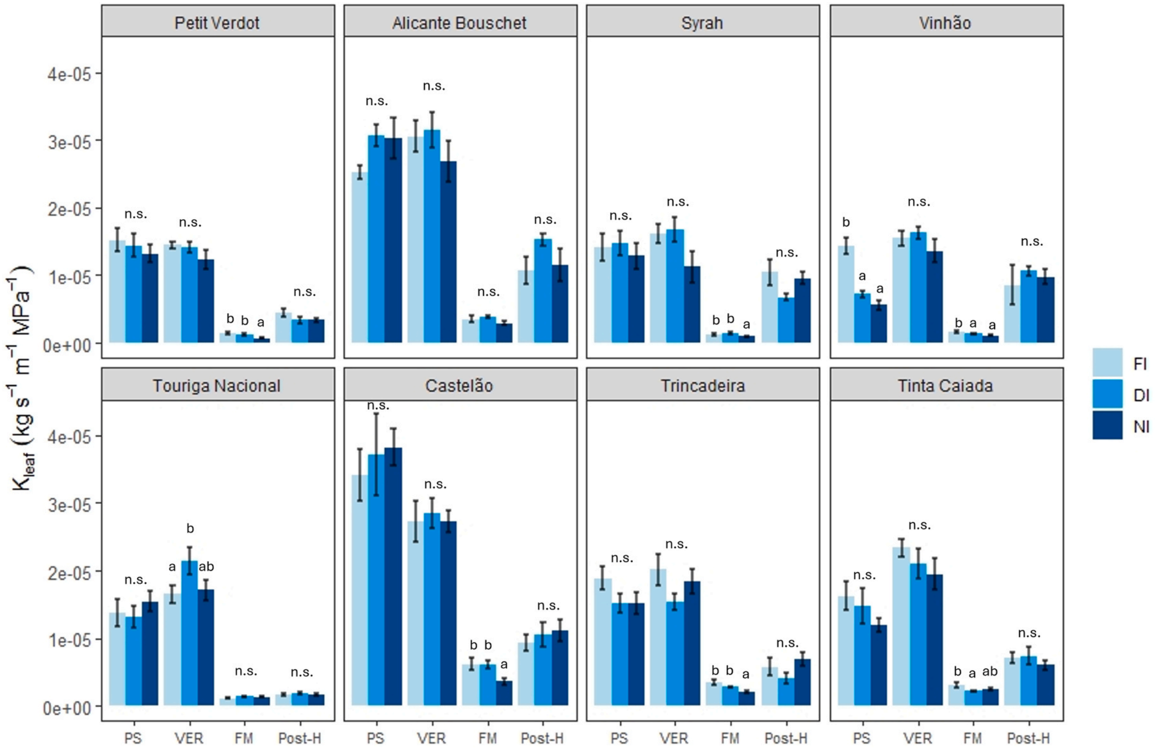


Fig. 4. Hydraulic conductivity (K_{leaf}, kg s^{-1} m^{-1} MPa^{-1}) in Full-irrigated (FI), Deficit irrigated (DI) and Non-irrigated (NI) plants of the eight studied genotypes, Petit Verdot, Alicante Bouschet, Syrah, Vinhão, Touriga Nacional, Castelão, Trincadeira and Tinta Caiada, at four phenological stages: Pea-size (PS), Veraison (VER), Full-maturation (FM) and Post-harvest (Post-H). Values are averages of ten plants and error bars indicate standard errors (n = 10). Different letters indicate significant differences between treatments according to Student's *t-test*, for $p < 0.05$; n.s. indicates no significant differences between treatments.

compared to NI from PS to FM.

At PS, significant differences between FI and DI were only observed in Syrah, Castelão, and Trincadeira, while at VER only Castelão maintained significantly lower g_s values under DI conditions. At FM, differences in g_s among treatments were accentuated in almost all genotypes, with significantly higher values in FI, lowest in NI and intermediate in DI (exceptions: Alicante Bouschet, Syrah and Touriga Nacional).

Interestingly, the tendency of g_s to decrease at the end of the growing season was maintained despite the significant rainfall event observed around Post-H. Nonetheless, differences among treatments were reduced, with NI plants showing g_s values close to FI and DI in several genotypes (Fig. 3), indicating a likely recovery of water status after stress in non-irrigated plants.

3.4. Hydraulic conductance

Results showed that K_{leaf} is a function of grapevine genotype and phenology (Fig. 4). The highest K_{leaf} values were observed at PS and VER, significantly decreasing thereafter (*circa* 80 %) in all genotypes and irrigation treatments (Fig. 4). Significant differences among treatments were observed mostly at FM, in which NI showed the lowest K_{leaf} in Petit Verdot, Syrah, Castelão and Trincadeira. The highest K_{leaf} values were observed in Alicante Bouschet and Castelão at the early phenological stages.

Interestingly, the abundant precipitation observed before Post-H led to statistically significant increases in K_{leaf} at Post-H (all genotypes and irrigation treatments), in all varieties except Touriga Nacional (Fig. 4), indicating a likely flux recovery due to late season increase in soil water availability. Nonetheless, no differences among treatments were observed.

3.5. Hormones: abscisic acid and auxin

Abscisic Acid ([ABA]) and the auxin Indoleacetic Acid ([IAA]) contents were measured at VER in leaves of four selected genotypes (Fig. 5A-B). The highest [ABA] was observed in Touriga Nacional and the lowest in Petit Verdot. [ABA] increased in water-stressed plants, particularly in NI, except for Touriga Nacional, where no significant differences were observed among treatments. Syrah was the only variety for which significant differences in [IAA] were observed between treatments, with a decrease in DI and NI, when compared to FI.

Interestingly, the [ABA]/[IAA] ratio showed two different trends among genotypes, a responsive trend in Syrah and Trincadeira, with an increase of [ABA]/[IAA] under water deficit conditions, and an unresponsive trend in Petit Verdot and Touriga Nacional, for which the [ABA]/[IAA] ratio suffered no changes (Fig. 5c).

3.6. Leaf pigments

Leaf pigments changed with genotype and phenology (Table S3). The highest chlorophyll-*a* values were observed at PS and the lowest at Post-H for almost all genotypes and irrigation treatments. The lowest chlorophyll-*b* values were also observed at Post-H for all genotypes and irrigation treatments. Petit Verdot presented relatively stable values of total anthocyanins, total carotenes and β-carotene during the growing cycle.

At PS, no significant differences were observed in chlorophyll and anthocyanin concentrations, but differences in β-carotene were observed between FI and NI in Vinhão, and between FI and DI in Trincadeira. At VER, Petit Verdot and Alicante Bouschet showed the highest chlorophyll-*a* and chlorophyll-*b* in DI, while no differences were observed between FI and NI. In contrast, Castelão showed the lowest chlorophyll (*a* and *b*) and carotenoid content in NI. This tendency was maintained at FM in Alicante Bouschet. However, in Castelão the tendency was reversed with the highest chlorophylls and carotenoids content observed in NI. At Post-H, a general decrease in all pigments was observed in all genotypes, except Alicante Bouschet, in which a significant increase in anthocyanins was observed in FI and DI.

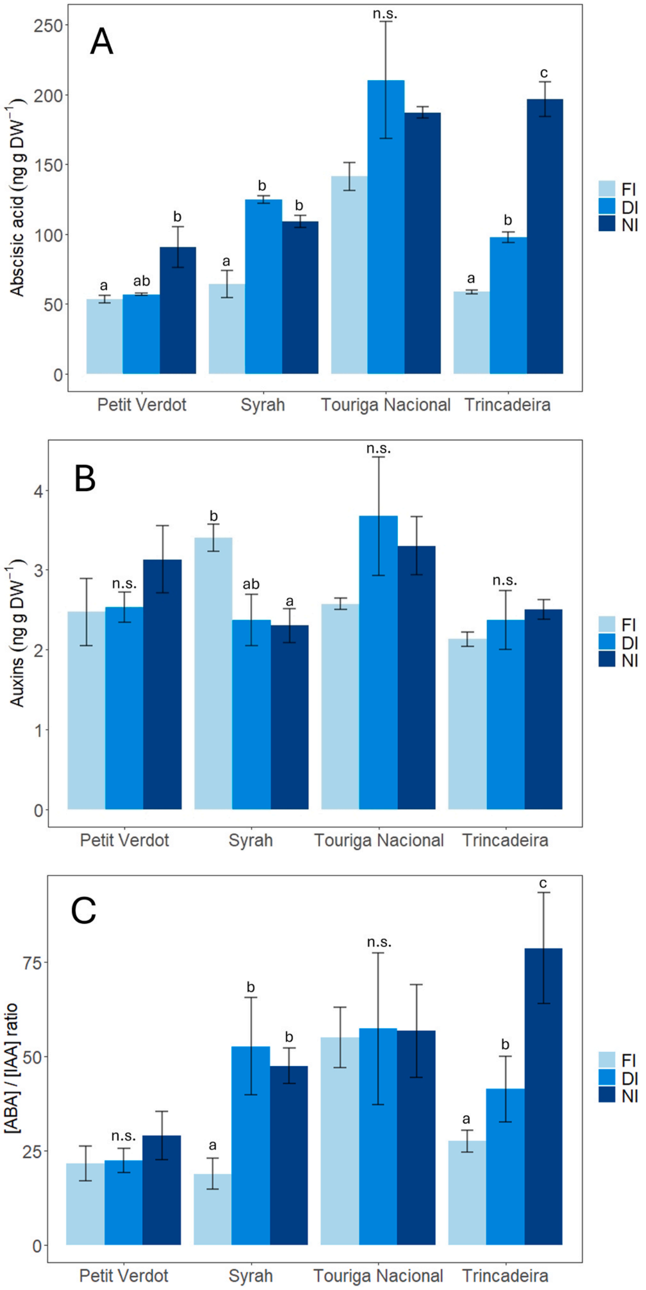
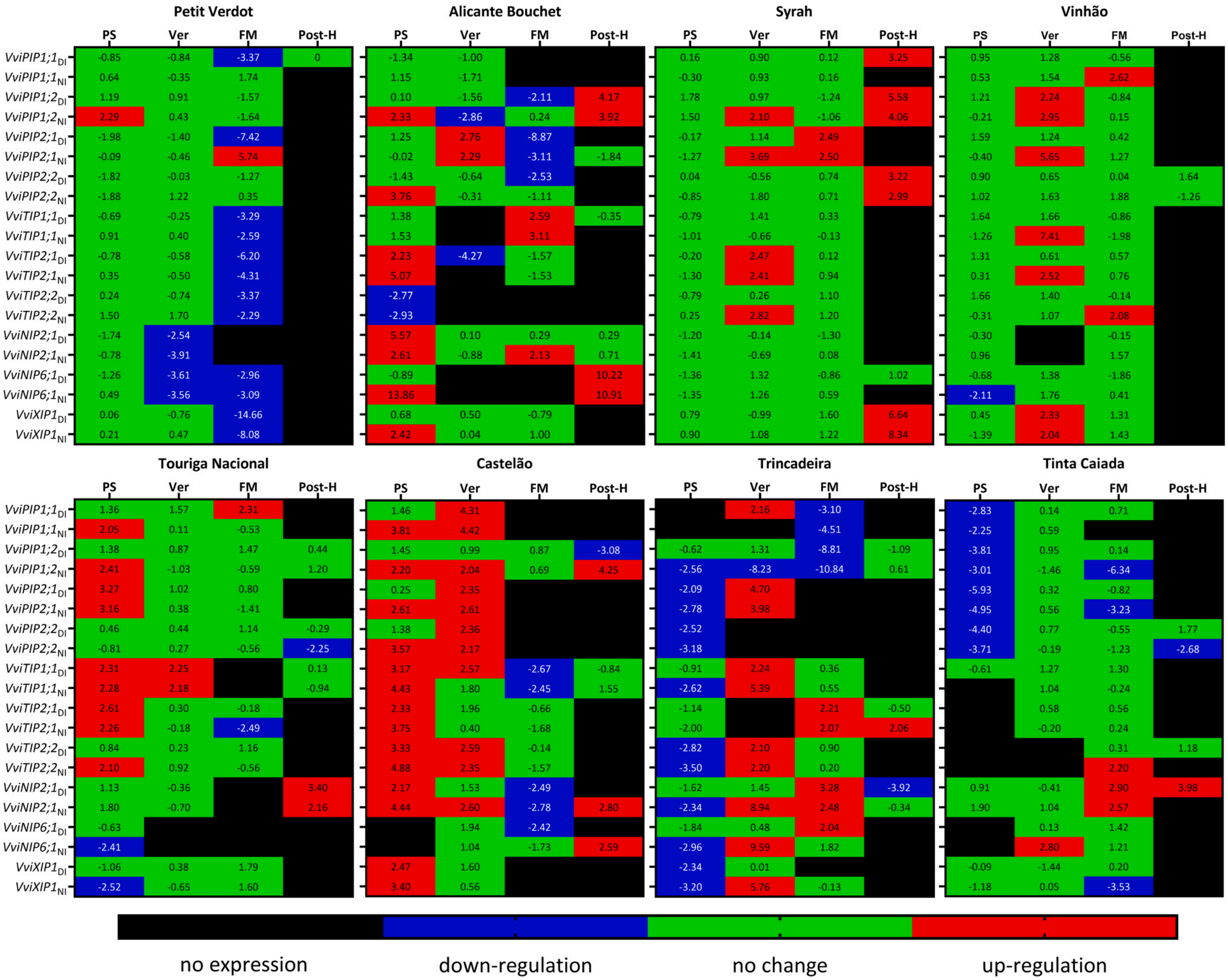


Fig. 5. Hormone concentration in leaves of four studied genotypes, Petit Verdot, Syrah, Touriga Nacional and Trincadeira, measured at Veraison (VER). A) Abscisic acid concentration ([ABA]); B) Indoleacetic acid concentration ([IAA]); C) Abscisic acid to Auxin ratio ([ABA] / [IAA]). Values are averages of threesamples. Error bars indicate standard errors. Different letters indicate significant differences between treatments according to Duncan's test, $p < 0.05$; n.s. indicates no significant differences between treatments.

Interestingly, Touriga Nacional did not show differences among irrigation treatments during the growth cycle. On the contrary, Alicante Bouschet was the genotype with more differences among irrigation treatments.

3.7. Aquaporin gene expression

Almost all studied AQPs were differentially expressed at PS and VER in all genotypes and water treatments (Fig. 6). In PS, there were two



Petit Verdot

	PS	Ver	FM	Post-H
VviPIP1;1$_{DI}$	-0.85	-0.84	-3.37	0
VviPIP1;1$_{NI}$	0.64	-0.35	1.74	
VviPIP1;2$_{DI}$	1.19	0.91	-1.57	
VviPIP1;2$_{NI}$	2.29	0.43	-1.64	
VviPIP2;1$_{DI}$	-1.98	-1.40	-7.42	
VviPIP2;1$_{NI}$	-0.09	-0.46	5.74	
VviPIP2;2$_{DI}$	-1.82	-0.03	-1.27	
VviPIP2;2$_{NI}$	-1.88	1.22	0.35	
VviTIP1;1$_{DI}$	-0.69	-0.25	-3.29	
VviTIP1;1$_{NI}$	0.91	0.40	-2.59	
VviTIP2;1$_{DI}$	-0.78	-0.58	-6.20	
VviTIP2;1$_{NI}$	0.35	-0.50	-4.31	
VviTIP2;2$_{DI}$	0.24	-0.74	-3.37	
VviTIP2;2$_{NI}$	1.50	1.70	-2.29	
VviNIP2;1$_{DI}$	-1.74	-2.54		
VviNIP2;1$_{NI}$	-0.78	-3.91		
VviNIP6;1$_{DI}$	-1.26	-3.61	-2.96	
VviNIP6;1$_{NI}$	0.49	-3.56	-3.09	
VviXIP1$_{DI}$	0.06	-0.76	-14.66	
VviXIP1$_{NI}$	0.21	0.47	-8.08	

Alicante Bouchet

	PS	Ver	FM	Post-H
VviPIP1;1$_{DI}$	-1.34	-1.00		
VviPIP1;1$_{NI}$	1.15	-1.71		
VviPIP1;2$_{DI}$	0.10	-1.56	-2.11	4.17
VviPIP1;2$_{NI}$	2.33	-2.86	0.24	3.92
VviPIP2;1$_{DI}$	1.25	2.76	-8.87	
VviPIP2;1$_{NI}$	-0.02	2.29	-3.11	-1.84
VviPIP2;2$_{DI}$	-1.43	-0.64	-2.53	
VviPIP2;2$_{NI}$	3.76	-0.31	-1.11	
VviTIP1;1$_{DI}$	1.38		2.59	-0.35
VviTIP1;1$_{NI}$	1.53		3.11	
VviTIP2;1$_{DI}$	2.23	-4.27	-1.57	
VviTIP2;1$_{NI}$	5.07		-1.53	
VviTIP2;2$_{DI}$	-2.77			
VviTIP2;2$_{NI}$	-2.93			
VviNIP2;1$_{DI}$	5.57	0.10	0.29	0.29
VviNIP2;1$_{NI}$	2.61	-0.88	2.13	0.71
VviNIP6;1$_{DI}$	-0.89			10.22
VviNIP6;1$_{NI}$	13.86			10.91
VviXIP1$_{DI}$	0.68	0.50	-0.79	
VviXIP1$_{NI}$	2.42	0.04	1.00	

Syrah

	PS	Ver	FM	Post-H
VviPIP1;1$_{DI}$	0.16	0.90	0.12	3.25
VviPIP1;1$_{NI}$	-0.30	0.93	0.16	
VviPIP1;2$_{DI}$	1.78	0.97	-1.24	5.58
VviPIP1;2$_{NI}$	1.50	2.10	-1.06	4.06
VviPIP2;1$_{DI}$	-0.17	1.14	2.49	
VviPIP2;1$_{NI}$	-1.27	3.69	2.50	
VviPIP2;2$_{DI}$	0.04	-0.56	0.74	3.22
VviPIP2;2$_{NI}$	-0.85	1.80	0.71	2.99
VviTIP1;1$_{DI}$	-0.79	1.41	0.33	
VviTIP1;1$_{NI}$	-1.01	-0.66	-0.13	
VviTIP2;1$_{DI}$	-0.20	2.47	0.12	
VviTIP2;1$_{NI}$	-1.30	2.41	0.94	
VviTIP2;2$_{DI}$	-0.79	0.26	1.10	
VviTIP2;2$_{NI}$	0.25	2.82	1.20	
VviNIP2;1$_{DI}$	-1.20	-0.14	-1.30	
VviNIP2;1$_{NI}$	-1.41	-0.69	0.08	
VviNIP6;1$_{DI}$	-1.36	1.32	-0.86	1.02
VviNIP6;1$_{NI}$	-1.35	1.26	0.59	
VviXIP1$_{DI}$	0.79	-0.99	1.60	6.64
VviXIP1$_{NI}$	0.90	1.08	1.22	8.34

Vinhão

	PS	Ver	FM	Post-H
VviPIP1;1$_{DI}$	0.95	1.28	-0.56	
VviPIP1;1$_{NI}$	0.53	1.54	2.62	
VviPIP1;2$_{DI}$	1.21	2.24	-0.84	
VviPIP1;2$_{NI}$	-0.21	2.95	0.15	
VviPIP2;1$_{DI}$	1.59	1.24	0.42	
VviPIP2;1$_{NI}$	-0.40	5.65	1.27	
VviPIP2;2$_{DI}$	0.90	0.65	0.04	1.64
VviPIP2;2$_{NI}$	1.02	1.63	1.88	-1.26
VviTIP1;1$_{DI}$	1.64	1.66	-0.86	
VviTIP1;1$_{NI}$	-1.26	7.41	-1.98	
VviTIP2;1$_{DI}$	1.31	0.61	0.57	
VviTIP2;1$_{NI}$	0.31	2.52	0.76	
VviTIP2;2$_{DI}$	1.66	1.40	-0.14	
VviTIP2;2$_{NI}$	-0.31	1.07	2.08	
VviNIP2;1$_{DI}$	-0.30		-0.15	
VviNIP2;1$_{NI}$	0.96		1.57	
VviNIP6;1$_{DI}$	-0.68	1.38	-1.86	
VviNIP6;1$_{NI}$	-2.11	1.76	0.41	
VviXIP1$_{DI}$	0.45	2.33	1.31	
VviXIP1$_{NI}$	-1.39	2.04	1.43	

Touriga Nacional

	PS	Ver	FM	Post-H
VviPIP1;1$_{DI}$	1.36	1.57	2.31	
VviPIP1;1$_{NI}$	2.05	0.11	-0.53	
VviPIP1;2$_{DI}$	1.38	0.87	1.47	0.44
VviPIP1;2$_{NI}$	2.41	-1.03	-0.59	1.20
VviPIP2;1$_{DI}$	3.27	1.02	0.80	
VviPIP2;1$_{NI}$	3.16	0.38	-1.41	
VviPIP2;2$_{DI}$	0.46	0.44	1.14	-0.29
VviPIP2;2$_{NI}$	-0.81	0.27	-0.56	-2.25
VviTIP1;1$_{DI}$	2.31	2.25		0.13
VviTIP1;1$_{NI}$	2.28	2.18		-0.94
VviTIP2;1$_{DI}$	2.61	0.30	-0.18	
VviTIP2;1$_{NI}$	2.26	-0.18	-2.49	
VviTIP2;2$_{DI}$	0.84	0.23	1.16	
VviTIP2;2$_{NI}$	2.10	0.92	-0.56	
VviNIP2;1$_{DI}$	1.13	-0.36		3.40
VviNIP2;1$_{NI}$	1.80	-0.70		2.16
VviNIP6;1$_{DI}$	-0.63			
VviNIP6;1$_{NI}$	-2.41			
VviXIP1$_{DI}$	-1.06	0.38	1.79	
VviXIP1$_{NI}$	-2.52	-0.65	1.60	

Castelão

	PS	Ver	FM	Post-H
VviPIP1;1$_{DI}$	1.46	4.31		
VviPIP1;1$_{NI}$	3.81	4.42		
VviPIP1;2$_{DI}$	1.45	0.99	0.87	-3.08
VviPIP1;2$_{NI}$	2.20	2.04	0.69	4.25
VviPIP2;1$_{DI}$	0.25	2.35		
VviPIP2;1$_{NI}$	2.61	2.61		
VviPIP2;2$_{DI}$	1.38	2.36		
VviPIP2;2$_{NI}$	3.57	2.17		
VviTIP1;1$_{DI}$	3.17	2.57	-2.67	-0.84
VviTIP1;1$_{NI}$	4.43	1.80	-2.45	1.55
VviTIP2;1$_{DI}$	2.33	1.96	-0.66	
VviTIP2;1$_{NI}$	3.75	0.40	-1.68	
VviTIP2;2$_{DI}$	3.33	2.59	-0.14	
VviTIP2;2$_{NI}$	4.88	2.35	-1.57	
VviNIP2;1$_{DI}$	2.17	1.53	-2.49	
VviNIP2;1$_{NI}$	4.44	2.60	-2.78	2.80
VviNIP6;1$_{DI}$		1.94	-2.42	
VviNIP6;1$_{NI}$		1.04	-1.73	2.59
VviXIP1$_{DI}$	2.47	1.60		
VviXIP1$_{NI}$	3.40	0.56		

Trincadeira

	PS	Ver	FM	Post-H
VviPIP1;1$_{DI}$		2.16	-3.10	
VviPIP1;1$_{NI}$			-4.51	
VviPIP1;2$_{DI}$	-0.62	1.31	-8.81	-1.09
VviPIP1;2$_{NI}$	-2.56	-8.23	-10.84	0.61
VviPIP2;1$_{DI}$	-2.09	4.70		
VviPIP2;1$_{NI}$	-2.78	3.98		
VviPIP2;2$_{DI}$	-2.52			
VviPIP2;2$_{NI}$	-3.18			
VviTIP1;1$_{DI}$	-0.91	2.24	0.36	
VviTIP1;1$_{NI}$	-2.62	5.39	0.55	
VviTIP2;1$_{DI}$	-1.14		2.21	-0.50
VviTIP2;1$_{NI}$	-2.00		2.07	2.06
VviTIP2;2$_{DI}$	-2.82	2.10	0.90	
VviTIP2;2$_{NI}$	-3.50	2.20	0.20	
VviNIP2;1$_{DI}$	-1.62	1.45	3.28	-3.92
VviNIP2;1$_{NI}$	-2.34	8.94	2.48	-0.34
VviNIP6;1$_{DI}$	-1.84	0.48	2.04	
VviNIP6;1$_{NI}$	-2.96	9.59	1.82	
VviXIP1$_{DI}$	-2.34	0.01		
VviXIP1$_{NI}$	-3.20	5.76	-0.13	

Tinta Caiada

	PS	Ver	FM	Post-H
VviPIP1;1$_{DI}$	-2.83	0.14	0.71	
VviPIP1;1$_{NI}$	-2.25	0.59		
VviPIP1;2$_{DI}$	-3.81	0.95	0.14	
VviPIP1;2$_{NI}$	-3.01	-1.46	-6.34	
VviPIP2;1$_{DI}$	-5.93	0.32	-0.82	
VviPIP2;1$_{NI}$	-4.95	0.56	-3.23	
VviPIP2;2$_{DI}$	-4.40	0.77	-0.55	1.77
VviPIP2;2$_{NI}$	-3.71	-0.19	-1.23	-2.68
VviTIP1;1$_{DI}$	-0.61	1.27	1.30	
VviTIP1;1$_{NI}$		1.04	-0.24	
VviTIP2;1$_{DI}$		0.58	0.56	
VviTIP2;1$_{NI}$		-0.20	0.24	
VviTIP2;2$_{DI}$			0.31	1.18
VviTIP2;2$_{NI}$			2.20	
VviNIP2;1$_{DI}$	0.91	-0.41	2.90	3.98
VviNIP2;1$_{NI}$	1.90	1.04	2.57	
VviNIP6;1$_{DI}$		0.13	1.42	
VviNIP6;1$_{NI}$		2.80	1.21	
VviXIP1$_{DI}$	-0.09	-1.44	0.20	
VviXIP1$_{NI}$	-1.18	0.05	-3.53	

Fig. 6. Relative gene expression of different aquaporins isoforms: *VvPIP1;1, VvPIP1;2,VvPIP2;1, VvPIP2;2, VvTIP1;1, VvTIP2;1, VvTIP2;2, VvNIP2;1, VvNIP6;1* and *VvXIP1* ($\log_2$(fold change)), in leaves of Full-irrigated (FI), Deficit irrigated (DI) and Non-irrigated (NI) plants of the eight studied varieties, Petit Verdot, Alicante Bouschet, Syrah, Vinhão, Touriga Nacional, Castelão, Trincadeira and Tinta Caiada, at the four phenological stages: Pea-size (PS), Veraison (VER), Full-maturation (FM) and Post-harvest (Post-H). Relative values for the treatments DI and NI are expressed in comparison to FI. A significant variation to the control (FI) was only considered when $|\log_2(\text{gene expression ratio})| > 2$.

main trends of expression: the first, a general down-regulation or no changes of almost all AQPs, observed for Petit Verdot, Syrah, Vinhão, Trincadeira and Tinta Caiada; the second, an up-regulation, reported for, Alicante Bouschet, Touriga Nacional and Castelão.

A significant regulation by water stress was also observed at VER for all genotypes. In FM, several AQPs were down-regulated or not expressed in all genotypes except Syrah and Vinhão. In general, these two genotypes did not show significant changes in AQPs expression both at PS and FM (except *VviPIP2;1* in Syrah; *VviPIP1;1* and *VviTIP2;2* in Vinhão NI).

Most AQPs were not expressed at Post-H, except for *VviPIPs* and *VviNIPs* in Alicante Bouschet, Syrah, Castelão and Trincadeira and *VviTIPs* in Trincadeira and Tinta Caiada. Petit Verdot and Vinhão only showed expression of *VviPIP1;1* in DI and VviPIP2;2 in DI and NI, respectively. Interestingly, in Castelão *VviPIPs* were only up-regulated in NI, while *VviTIPs* were up-regulated in both DI and NI.

4. Discussion

Vitis vinifera's large genetic variability results in different water stress responses among varieties, which are reflected particularly in hydraulic patterns (stomatal conductance, hydraulic conductivity) and biochemical responses (hormonal, molecular) (Chaves et al., 1987, 2010; Flexas et al., 2010; Lovisolo et al., 2010; Hochberg et al., 2017b; Gambetta et al., 2020; Tamayo et al., 2023). The results of the current study contribute to improve knowledge on the physiological plasticity of eight red genotypes under different water status across key phenological stages, reinforcing the specificity of each variety's unique behaviour. This information may be used for an appropriated establishment of irrigation management, specially under the coming conditions of climate change.

Even though the studied varieties were chosen based on their δ^{13}C results (Jacinto et al., 2023), some varieties exhibited contrasting physiological responses in the present study. Considering that δ^{13}C in plants provides an estimation of carbon fixation over the entire period during which the carbon was formed (Brugnoli and Farquhar, 2000; Taskos et al., 2020), our data suggests that the 3-year span may have induced a cumulative water stress effect response on water stressed grapevines. This behaviour could be related to a borderline status of some varieties (near-iso/near-anisohydric), more prone to shift from a status to another depending on the soil water availability threshold (Vandeleur et al., 2009). Additionally, it may also be related to the slow response behaviour to water stress of these varieties, exhibiting differences only when exposed to long-term water shortages.

4.1. Irrigation strategy modulates stomatal behaviour

Decreases in grapevine stomatal conductance under long-term water stress have been reported by several authors (Winkel and Rambal, 1993; Tombesi et al., 2015; Pagay et al., 2016; Hochberg et al., 2017b; Lavoie-Lamoureux et al., 2017; Herrera et al., 2024). In the present study, all non-irrigated genotypes, except Castelão, maintained low and constant g_s since the beginning of the growth season. These results confirm our first hypothesis of acclimation of some genotypes to non-irrigated conditions and the existence of a cumulative long-term water stress impact on stomatal behaviour, leading to a more water saving response. This could indicate a priming effect in these genotypes' response to drought, enabling primed plants to use water more efficiently than non-primed ones (Tombesi et al., 2018; De Deus et al., 2023). However, further biochemical validation is required to confirm the mechanisms underlying this enhanced water-use efficiency. The absence of such behaviour in Castelão confirms its tendency to be near-anisohydric under the Alentejo's climatic conditions (Damásio et al., 2023). This is further confirmed by a decline in photosynthetic performance (lower leaf pigment concentration) of this genotype when subjected to rainfed conditions. However, it is worth noting that the consistent low g_s observed in non-irrigated genotypes may also indicate a seasonal drought response when water potential declines later during the season.

The dynamic trend of stomatal aperture during the growing season, with the highest stomatal conductance at veraison, observed in our study in almost all genotypes independently of the irrigation regime, can be associated with the plant's increased demand for carbon assimilation through photosynthesis to meet plant vegetative growth and support high carbon-demanding processes in berries, such as sugar and secondary metabolite accumulation (Ollat and Gaudillère, 2000; Conde et al., 2007; Rienth et al., 2021).

Interestingly, while stomatal activity peaks at VER in almost all studied genotypes, Touriga Nacional apparently peaked at PS, which may suggest a physiological phenology anticipation of the growth program and of water requirements in favour of the berry.

Castelão showed similar g_s in DI and NI at veraison, but closer Ψ_{pd} values between DI and FI, an indication of decoupling between stomatal aperture and plant water status, probably due to the high sensitivity of this variety to high VPD (values around 5,5 kPa at VER) when subjected to mild stress (De Deus et al., 2023). Previous studies indicate a relative insensitivity of grapevine stomata to VPD under well-watered conditions (Charrier et al., 2018; Dayer et al., 2020; Rogiers et al., 2012), but a high sensitivity when subjected to water stress.

The absence of differences in g_s between irrigation treatments at FM could be explained by a shift in the growth program from expansion to reserve accumulation, which may imply a reduction in carbon fixation through tight control of stomatal aperture.

4.2. Phenology modulates the leaf hydraulic behaviour

The highest K_{leaf} observed at early development stages (PS and VER) and the abrupt drop thereafter, for all genotypes and in irrigation treatments, corroborates the need for high waterflow to maintain rapid vegetative growth rates during the early stages and the shift to carbohydrate storage at later stages (Flexas et al., 2002; Lovisolo et al., 2010). A recent review by Scoffoni et al. (2023) pinpoints the role of increasing cell wall lignification during the leaf maturation cycle in reducing K_{leaf}, as a protection mechanism against cavitation, (Zhang et al., 2016, 2022; Scoffoni et al., 2017; Albuquerque et al., 2020). In this sense, our results may suggest that cell wall lignification during leaf maturation in grapevine is part of the plant intrinsic cycle growth program since the K_{leaf} decrease was observed in all studied genotypes, independently of the irrigation treatment. The K_{leaf} decrease at this late stage is also accompanied by a reduction in g_s independently of the water regime, suggesting that at later stages stomatal activity is mainly driven by hydraulic conductance signalling due to a predetermined growth program than by chemical signalling.

Variations in K_{leaf} observed along the different phenological stages were mostly driven by the genetic background of the variety and phenological stage rather than by the irrigation treatment. Seasonal hydraulic plasticity was recently described by Sorek et al. (2021) and was suggested to be a predetermined growth strategy rather than just a response to seasonal environmental changes. In general, the irrigation treatments did not significantly influence K_{leaf} (except at FM), corroborating the findings by Sorek et al. (2021), who suggested that drought has a limited ability to shape grapevine hydraulics compared with developmental changes. It is possible that water stress may have affected xylem anatomical properties of petioles, as lower water availability has been related to decreased size of grapevine vessels (Lovisolo and Schubert,1998; Lovisolo et al., 2002; Munitz et al., 2018), but without affecting hydraulic conductivity. Our results are also in agreement with previous works showing no evidence of improved xylem resistance in grapevine leaves (Hochberg et al.,2017a) in plants acclimated to drought. The different flux values among genotypes likely depend on the more anisohydric behaviour of some genotypes, such as Alicante Bouschet and Castelão. This is consistent with previous studies showing that anisohydric genotypes have a higher ability to maintain high K_{leaf} under water stress, compared to isohydric ones (Hochberg et al., 2017a). In this context, considering the significant stomatal closure in Alicante Bouschet, coupled with stable photosynthetic performance under stress, suggesting a more near-isohydric behaviour of this genotype, K_{leaf} results reinforce the need for multi-parameter assessment for grapevine drought response and genotype classification.

In our study, the decrease in stomatal conductance in water-stressed grapevines at the early stages of development was not accompanied by a reduction in leaf hydraulic conductivity, suggesting the maintenance of K_{leaf} through sustained root hydraulic capacity, likely mediated by AQPs or/and by root anatomy traits. Lovisolo and Schubert (1998), (2006) pointed out, root-to-shoot signalling involving ABA and hydraulic feedback that may modulate g_s. In our case, this might explain why g_s declined while K_{leaf} remained relatively unchanged. This compensation may enable water supply to the leaf to remain stable, even when g_s decreases in response to hormonal signals and may indicate a predominance of root hydraulic adjustment over leaf hydraulics in controlling stomatal activity, as previously observed by Zarrouk et al. (2016a). This highlights the complexity of hydraulic mechanisms' responses to drought in grapevine.

4.3. Hormonal role in hydraulic signalling: ABA versus auxins

Our study confirms that stomatal closure is the earliest grapevine response to water deficit of all studied genotypes as reported earlier in several species (Flexas et al., 2002; Lovisolo et al., 2010; Tombesi et al., 2014). This rapid response is regulated by a complex network of signalling hormonal pathways, in which the major and best-known player, abscisic acid (ABA), acts in concert with others, such as auxins (Nemhauser et al., 2006; Huang et al., 2008; Bharath et al., 2021). The balance between the two hormones may explain different stomatal plasticity among genotypes. The [ABA]/[IAA] ratio enables the identification of two main behaviours upon water stress: genotypes responsive to water stress intensity (Syrah and Trincadeira) and genotypes that are non-responsive (Petit Verdot and Touriga Nacional). In responsive genotypes, [ABA] was linked to water stress intensity, with higher [ABA] observed in plants with more negative Ψ_{pd} (NI), indicating a likely chemical control of water potential. On the other hand, the significant decrease in Ψ_{pd} in NI of non-responsive genotypes (Touriga Nacional and Petit Verdot) was not accompanied by an increase in [ABA] nor changes in the [ABA]/[IAA] ratio. This suggests the hydraulic involvement, i.e., water potential and/or hydraulic conductance, in these genotypes. In previous reports by Carvalho et al. (2016), Touriga Nacional stomatal closure was also independent of [ABA] and was related to protection from desiccation brought about by reactive oxygen species (ROS) signalling, namely through reduced glutathione. However, how the interaction between ABA and auxin integrates drought-induced ROS signals and ROS scavengers is not yet elucidated. In any case, the [ABA]/[IAA] ratio changes in grapevine genotypes under water stress may indicate differential oxidative responses among genotypes, which warrant further research. On the other hand, Dayer et al. (2020) attribute such a response to the down-regulation of several AQPs in the roots.

Overall, the present study indicates that the response of Petit Verdot and Touriga Nacional to water stress is primarily controlled by hydraulic mechanisms (stomatal regulation and water potential), but only beyond a certain stress threshold. Whether this response is dependent or not on hormonal signalling has been previously discussed in various species (Stoll et al., 2000; Romero et al., 2012; Brodribb and McAdam, 2013; Tramontini et al., 2014). This regulation has been linked to isohydric stomatal control, which is driven by ABA, whereas anisohydric regulation is, at least initially, governed by water potential as observed in the present study.

Our classification of Syrah as isohydric contrasts with previous statements dealing with the near-anisohydric behaviour of this variety (Schultz, 2003; Hochberg et al., 2013; Tramontini et al., 2014). Despite the controversy about the iso/anisohydric classification of grapevine genotypes (Villalobos-González et al., 2019; Gambetta et al., 2020), it is important to further discuss the divergence between our observations and other reports. Experiments reported by Schultz et al. (2003) were performed in southern France and southern Germany, with significant climatic differences from the Alentejo region in Portugal (characterized by significant high atmospheric demand during grapevine growing season, with diurnal VPD averages higher than 5 kPa in July and August). This higher VPD may have impacted the plastic adaptation of the Syrah clones cultivated in Portugal, inducing a higher control of its stomata. On the other hand, the studies by Hochberg et al. (2013) and Tramontini et al. (2014) were conducted using potted plants, which do not mimic field conditions, particularly in terms of the plant water relations (Passioura, 2006). In the field, plants have time and space to adapt to water stress, due to their high root volume and slow decrease in water potentials, being able to adjust their hydraulic properties as a function of soil dehydration and/or growth cycle (Sorek et al., 2021). In this regard, Zhang et al. (2022) illustrated the complex and variable responses of stomata to leaf-to-air VPD under high and low soil moisture, showing that even typically anisohydric genotypes may constrain g_s during periods of extremely low Ψ_{soil} and that the same individuals can switch from an isohydric-like behaviour when transpiration is low to an anisohydric-like behaviour (and *vice versa*). Noteworthy, while the tight stomatal control aligns with an isohydric response of Syrah, its ability to sustain photosynthetic activity suggests alternative drought tolerance mechanisms, warranting further investigation into ROS activity and antioxidant defences.

4.4. The role of aquaporins in the hydraulic balance

Previous studies have demonstrated that AQP gene expression is modulated by the circadian clock (Takase et al., 2010; Caldeira et al., 2014; Shelden et al., 2017). In this study, we provide evidence that AQP gene expression is also finely regulated across the phenological stages of the plant cycle. Our results reveal that AQP gene expression in leaves is both genotype- and phenology-dependent, showing high activity during early developmental stages, followed by progressive down-regulation, or silencing during ripening and later stages. Interestingly, this dynamic pattern of AQP expression aligns closely with stomatal conductance (g_s) and hydraulic conductivity (K_{leaf}) activities, reinforcing the hypothesis that aquaporins play a critical role in hydraulic adjustments in grapevine (Pou et al., 2013; Vitali et al., 2016; Wang et al., 2020; Sabir et al., 2021; Grunwald et al., 2022). These findings underscore the functional relationship between AQPs and the hydraulic responses necessary to meet the changing physiological demands of grapevines throughout their seasonal growth, paving the way for future research to unravel the precise roles of aquaporins in grapevine plasticity and their adaptive responses to environmental cues.

Two general main expression trends of AQPs were observed, giving rise to two distinct clusters depending on the up-regulation or down-regulation/no change of AQPs expression under DI and NI at the early stages of development. Interestingly, the genotypes classified in the up-regulated AQPs cluster show near-anisohydric behaviour while the genotypes in the down-regulated AQPs clusters are mainly near-isohydric. Previous studies have demonstrated that grapevine cultivars isohydric or anisohydric responses may be linked to aquaporin expression (Vandeleur et al., 2009). Also, studies by Shelden et al. (2017) showed greater AQP down-regulation in near-isohydric Grenache than in the near-anisohydric Chardonnay when subjected to water stress. AQPs have a role in the regulation of the opening and closure of stomata, by enabling plants to modify water permeability rapidly and reversibly (Chaumont and Tyerman, 2014) and their up-regulation could enable the maintenance of stomatal opening under continuous decrease of plant water potential, which is mainly characteristic of the anisohydric behaviour.

The most significant differences were observed in cell and vacuole membrane classes (PIPs and TIPs), but other aquaporins (NIP and XIP) also responded to water stress. This indicates the existence of intracellular regulatory mechanisms beyond those associated with membrane water flow facilitation, likely for the maintenance of cellular homeostasis and the metabolic functions of cell leaves, such as CO_2 fixation (Sabir et al., 2021), mediation of H_2O_2 uptake (Noronha et al., 2016, 2020), mineral element transport (Ma et al., 2004; Sabir et al., 2014, 2020; Coskun et al., 2019), among others (Sabir et al., 2021).

A remarkable regulation of aquaporins (AQPs) was observed in Castelão prior to maximal stomatal conductance (e.g., in PS). Specifically, in this genotype, *VviPIPs* were up-regulated only in the most water-stressed plants (NI), while all *VviTIPs* were up-regulated in both DI and NI plants. Previous studies have reported that the water permeability of *VviTIPs* is higher than that of *VviPIPs* (Sabir et al., 2021). These findings suggest that the observed up-regulation of *VviTIPs* in Castelão is closely associated with maintaining cellular water permeability under water stress whilst the up-regulation of *VviPIPs* in NI plants may be linked to additional drought-induced mechanisms such as oxidative stress regulation (Bienert et al., 2006). TIPs expression was previously associated with the promotion of anisohydric behaviour in an isohydric transgenic tomato variety (Sade et al., 2008), which could suggest the role of *VviTIPs* in enabling the anisohydric behaviour of Castelão. Anisohydric genotypes are known to sustain high stomatal conductance and photosynthetic activity even under decreasing soil water potential. However, this strategy often incurs trade-offs, including reduced ATP production and the accumulation of oxidative metabolites (Carvalho et al., 2014). These traits align with our observations, as Castelão showed increased stomatal conductance at veraison in NI, a behaviour contrasting with most other genotypes, despite significant water depletion. Similarly, Touriga Nacional, another anisohydric genotype, showed a similar pattern of *VviPIP* and *VviTIP* regulation as Castelão in PS, except in *VviPIP2;2*. The two other genotypes classified as near-anisohydric, Alicante Bouschet and Petit Verdot, with less pronounced changes in AQP expression, also exhibited up-regulation of several AQPs at early stages. We hypothesize that at the beginning of the season (PS), the cellular hydraulic adjustment (via AQPs) in near-anisohydric varieties contributes to the maintenance of stomatal opening even under decreasing water status. This could be a strategy employed by these varieties to ensure adequate photosynthetic performance. In contrast, near-isohydric varieties either do not involve aquaporins are (showing no change) or experience down-regulation of aquaporins because of lower water flux (leaf hydraulic conductance). Previous reports (Afzal et al., 2016) also indicate that under water stress conditions, aquaporins are not only responsible for minimizing water loss from plant tissues, but they also play a key role in facilitating CO_2 homeostasis. This upregulation of AQPs likely contributes to the capacity of these genotypes to maintain cellular hydraulic homeostasis, which may support leaf hydraulic integrity and enable continued gas exchange under water-limited conditions. Altogether, our data suggests a distinct adaptive strategy within this group, which is marked by low AQP involvement in hydraulic regulation. Unlike Castelão and Touriga Nacional, Alicante Bouschet and Petit Verdot maintained relatively stable stomatal conductance (g_s) across the season in non-irrigated plants. This stability likely, resulting from a long-term adaptation to water scarcity, reduces their reliance on AQPs for hydraulic regulation, reflecting structural and anatomical changes rather than being solely driven by short-term molecular signalling mechanisms, implying an alternative strategies for coping with water stress.

In near-isohydric genotypes, differential AQP regulation was also observed but the patterns were less consistent between DI and NI treatments. In general, *VviPIPs* and *VviTIPs* were down-regulated or unchanged at PS, corroborating previous works in Chasselas (Dayer et al., 2017). However, in several isohydric genotypes these AQPs were up-regulated at veraison. This variability underscores the complexity of AQP-mediated hydraulic responses, which are influenced by genotype, environmental conditions and stress threshold.

These findings suggest that AQPs are linked to the regulation or exacerbation of the iso/anisohydric strategy and may explain the discrepancies among reports in their classification in the literature. This supports previous studies suggesting the involvement of AQPs in the ability of grapevine genotypes to switch from a near-isohydric to a near-anisohydric behaviour (Vandeleur et al., 2009). The observed *VviPIPs* down-regulation at an early phenological stage together with [ABA] increase corroborate the hypothesis of Shatil-Cohen et al. (2011) that the inactivation of *VviPIP* AQPs is a result of increased ABA in xylem sap, exerting a feed-forward signal for stomata to close (Dayer et al., 2020). Our data pinpoints an activity decrease of AQPs after the onset of ripening (VER), where most AQPs were significantly down-regulated or inactivated at and after FM, independently of the genetic background of the variety or irrigation treatment. This decrease is concomitant with the significant drop in K_{leaf} and g_s. Grapevine reduces leaf hydraulic conductivity after VER, likely to prioritize water transport to the developing fruit (Greenspan et al., 1994, 1996; Knipfer et al., 2015). Our results also indicate that, in grapevine, hydraulic control is growth-program related and may also be related to AQPs activities. In this regard, the majority of the AQPs were not expressed at Post-H, which may be related to the lower protein synthesis at this stage as a result of the beginning of leaf senescence at the end of the annual plant cycle (Ohtsuka et al., 2018), with a shift of energy focus from reproduction towards reserve accumulation in the perennial structures of the plant. During leaf maturation, osmolyte accumulation leads to a significant reduction of the turgor loss point, a known marker for stomatal closure (Herrera et al., 2022). This accumulation may be caused by a reduction in AQPs activity at Post-H, since one of the AQPs functions is the maintenance of cytosolic osmolarity (Liu et al., 2006), which may be one of the explanations for the low g_s maintenance.

4.5. *Leaf hydraulic components are actively regulated at post-harvest in grapevine genotypes*

At Post-H, the soil was at field capacity due to the intensive rainfall, reducing the pressure of water retention in the plant (*circa* 0,05 MPa in all genotypes and treatments). However, at the same time, g_s reached its minimum in all genotypes, particularly in FI and DI, and AQPs activity almost ceased, with most genes showing minimum levels of expression, in most of the genotypes and treatments, indicating a delayed stomatal response to soil water availability and corroborating the loss of plasticity due to leaf ageing. Such a combination seems to be related to the absence of maintaining leaves and berries since the main physiological process occurring in the plant after harvest is the backflow of nutrients from leaves to the perennial structures. Interestingly, some *VviNIPs* isoforms and *VviXIPs,* mainly involved in mineral element transport (Wang et al., 2016; Sabir et al., 2021), were activated at Post-H, even up-regulated in some genotypes, independently of K_{leaf} recovery range.

The differential K_{leaf} increase among genotypes after the rain event suggests a differential benefit of late-season water availability among genotypes. Syrah and Alicante Bouschet had the highest K_{leaf} increase, along with a significant up-regulation of *VviPIPs*, suggesting the involvement of these AQPs in this hydraulic recovery, as previously observed by Pou et al. (2013). The up-regulation of AQPs at Post-H after rainfall could also be related to foliar water uptake as suggested by Vignesh and Palanisamy (2021). The involvement of *PIP2;1* in diurnal water uptake in response to increasing air relative humidity was demonstrated in the desert tree *T. ramosissima* (Yan et al., 2015). However, despite the considerable recovery of K_{leaf} in Vinhão at Post-H, the identification of the cellular mechanisms involved was not possible to infer, due to the lack of AQPs expression.

5. Conclusions

The present study contributes to the understanding of physiological plasticity in eight grapevine varieties across the growing season and under different water status. We demonstrate that hydraulic adjustment mechanisms in grapevine are highly dynamic throughout the season, emphasizing the complexity of water regulation strategies. Stomatal closure was the earliest response to water stress in all genotypes while, hydraulic conductivity (K_{leaf}) was variety-specific, and primarily driven by phenology rather than water status. Early in the season, K_{leaf} supports high water flow to sustain intense vegetative growth, shifting with time to align with carbohydrate storage demands. Additionally, non-irrigated plants kept low and stable stomatal conductance (g_s) from the beginning of the season, reinforcing their long-term acclimation to water-limited conditions and suggesting a cumulative impact of sustained water stress on stomatal behaviour. This could be related to a borderline status of some genotypes (near-iso/near-anisohydric), prone to shifts from one status to another, depending on the soil water availability threshold or/and related to slow responses to water stress, thus only revealing differences when exposed to long-term water shortage. The balance between [ABA] and [IAA] at veraison evidenced that some genotypes were responsive to water status levels, with high [ABA] associated with low Ψ_{pd}, suggesting a chemical control of water status, while others were irresponsive to water status levels, with decreases in g_s and Ψ_{pd} being independent of [ABA]. Aquaporins played a crucial role in hydraulic adjustments, and their gene expression was tightly regulated by phenology and closely aligned with g_s and K_{leaf}. This result identified two AQP expression trends in water-stressed plants at early developmental stages: a general up-regulation, indicative of near-anisohydric behaviour, and a down-regulation or stable expression, indicative of near-isohydric behaviour. The benefit of late-season water availability in some genotypes, highlighted by K_{leaf} and AQP expression, can have significant implications for post-harvest irrigation strategies. Overall, the highly dynamic interplay between AQP expression, stomatal conductance, hydraulic conductivity, and hormonal crosstalk in response to water stress underscores the need for further field phenotyping studies covering the full duration of the growing season to refine water management strategies in viticulture.

CRediT authorship contribution statement

Olfa Zarrouk: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Luísa C. Carvalho:** Writing – review & editing,

Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **João de Deus:** Writing – review & editing, Investigation. **Maria Victoria Alarcón:** Writing – review & editing, Investigation, Data curation. **José Silvestre:** Writing – review & editing, Funding acquisition. **Teresa Soares David:** Writing – review & editing, Investigation. **Miguel Damásio:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Julio Salguero:** Writing – review & editing, Investigation, Formal analysis. **Clara Pinto:** Writing – review & editing, Writing – original draft, Methodology, Investigation.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agwat.2025.109708.

Data availability

Data will be made available on request.

References

Afzal, Z., Howton, T.C., Sun, Y., Mukhtar, M.S., 2016. The roles of aquaporins in plant stress responses. J. Dev. Biol. 4, 9. https://doi.org/10.3390/jdb4010009.

Albuquerque, C., Scoffoni, C., Brodersen, C.R., Buckley, T.N., Sack, L., McElrone, A.J., 2020. Coordinated decline of leaf hydraulic and stomatal conductances under drought is not linked to leaf xylem embolism for different grapevine cultivars. J. Exp. Bot. 71, 7286–7300. https://doi.org/10.1093/jxb/eraa392.

Allen, R., Pereira, L., Raes, D., Smith, M., 1998. Crop evapotranspiration: Guidelines for computing crop water requirements – FAO Irrigation and Drainage Paper 56. FAO, Rome.

Benítez García, I., Dueñas Ledezma, A.K., Martínez Montaño, E., Salazar Leyva, J.A., Carrera, E., Osuna Ruiz, I., 2020. Identification and quantification of plant growth regulators and antioxidant compounds in aqueous extracts of Padina durvillaei and Ulva lactuca. Agronomy 10 (6), 866. https://doi.org/10.3390/agronomy10060866.

Bharath, P., Gahir, S., Raghavendra, A.S., 2021. Abscisic acid-induced stomatal closure: an important component of plant defense against abiotic and biotic stress. Front. Plant Sci. 12, 615114. https://doi.org/10.3389/fpls.2021.615114.

Bienert, G.P., Schjoerring, J.K., Jahn, T.P., 2006. Membrane transport of hydrogen peroxide. Biochim. Et. Biophys. Acta Biomembr. 1758, 994–1003. https://doi.org/10.1016/j.bbamem.2006.02.015.

Brodribb, T.J., McAdam, S.A.M., 2013. Abscisic acid mediates a divergence in the drought response of two conifers. Plant Physiol. 162, 1370–1377. https://doi.org/10.1104/pp.113.217877.

Brugnoli, E., Farquhar, G.D., 2000. Photosynthetic fractionation of carbon isotopes. In: Leegood, R.C., Sharkey, T.D., Von Caemmerer, S. (Eds.), Photosynthesis. Advances in Photosynthesis and Respiration. Springer Netherlands, Dordrecht, pp. 399–434.

Caldeira, C.F., Jeanguenin, L., Chaumont, F., Tardieu, F., 2014. Circadian rhythms of hydraulic conductance and growth are enhanced by drought and improve plant performance. Nat. Commun. 5, 5365. https://doi.org/10.1038/ncomms6365.

Campos, P.R., Silva, J.F.C., Vásquez, H.M., Vittori, A., Silva, M.D., 2010. Fractions of carbohydrates and of nitrogenous compounds of tropical grasses at different cutting ages. Rev. Bras. De. Zootec. 39, 1538–1547. https://doi.org/10.1590/S1516-35982010000700021.

Carbonneau, A., 1998. Aspects qualitatifs. In: Tiercelin, J.R. (Ed.), Traité d'irrigation. Proceedings from 17th World Congress of Vine and Wine, Bratislava. Lavoisier Tec et Doc, Paris, p. 1011.

Carvalho, L.C., Coito, J.L., Colaço, S., Sangiogo, M., Amâncio, S., 2014. Heat stress in grapevine: the pros and cons of acclimation. Plant Cell Environ. 38, 777–789. https://doi.org/10.1111/pce.12445.

Carvalho, L.C., Coito, J.L., Gonçalves, E.F., Chaves, M.M., Amâncio, S., 2016. Differential physiological response of the grapevine varieties Touriga Nacional and Trincadeira to combined heat, drought and light stresses. Plant Biol. 18, 101–111. https://doi.org/10.1111/plb.12410.

Charrier, G., Delzon, S., Domec, J.C., Zhang, L., Delmas, C.E.L., Merlin, I., Corso, D., Barrios-Masias, F.H., Ojeda, H., Ollat, N., Gambetta, G.A., 2018. Drought will not leave your glass empty: low risk of hydraulic failure revealed by long-term drought observations in world's top wine regions. Sci. Adv. 4, eaao6969. https://doi.org/10.1126/sciadv.aao6969.

Chaumont, F., Tyerman, S.D., 2014. Aquaporins: highly regulated channels controlling plant water relations. Plant Physiol. 164, 1600–1618. https://doi.org/10.1104/pp.113.233791.

Chaves, M.M., Harley, P.C., Tenhunen, J.D., Lange, O.L., 1987. Gas exchange studies in two Portuguese grapevine cultivars. Physiol. Plant. 70, 639–647. https://doi.org/10.1111/j.1399-3054.1987.tb04318.x.

Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P.P., Osório, M.L., Carvalho, I., Faria, T., Pinheiro, C., 2002. How plants cope with water stress in the field: photosynthesis and growth. Ann. Bot. 89 (Spec), 907–916. https://doi.org/10.1093/aob/mcf105.

Chaves, M.M., Zarrouk, O., Francisco, R., Costa, J.M., Santos, T., Regalado, A.P., Rodrigues, M.L., Lopes, C.M., 2010. Grapevine under deficit irrigation: hints from physiological and molecular data. Ann. Bot. 105, 661–676. https://doi.org/10.1093/aob/mcq030.

Coito, J.L., Rocheta, M., Carvalho, L., Amâncio, S., 2012. Microarray-based uncovering reference genes for quantitative real time PCR in grapevine under abiotic stress. BMC Res. Notes 5, 220. https://doi.org/10.1186/1756-0500-5-220.

Conde, C., Silva, P., Fontes, N., Dias, A.C.P., Tavares, R.M., Sousa, M.J., Agasse, A., Delrot, S., Gerós, H., 2007. Biochemical changes throughout grape berry development and fruit and wine quality. Food 1, 1–22.

Coskun, D., Deshmukh, R., Sonah, H., Menzies, J.G., Reynolds, O., Ma, J.F., Kronzucker, H.J., Bélanger, R.R., 2019. In defence of the selective transport and role of silicon in plants. N. Phytol. 223, 514–516. https://doi.org/10.1111/nph.15764.

Costa, J.M., Ortuño, M.F., Lopes, C.M., Chaves, M.M., 2012. Grapevine varieties exhibiting differences in stomatal response to water deficit. Funct. Plant Biol. 39, 179–189. https://doi.org/10.1071/FP11156.

Coupel-Ledru, A., Tyerman, S.D., Masclef, D., Lebon, E., Christophe, A., Edwards, E.J., Simonneau, T., 2017. Abscisic acid down-regulates hydraulic conductance of grapevine leaves in isohydric genotypes only. Plant Physiol. 175, 1121–1134. https://doi.org/10.1104/pp.17.00698.

Damásio, M., Barbosa, M., Deus, J., Fernandes, E., Leitão, A., Albino, L., Fonseca, F., Silvestre, J., 2023. Can grapevine leaf water potential be modelled from physiological and meteorological variables? A machine learning approach. Plants 12, 4142. https://doi.org/10.3390/plants12244142.

Dayer, S., Peña, J.P., Gindro, K., Torregrosa, L., Voinesco, F., Martínez, L., Prieto, J.A., Zufferey, V., 2017. Changes in leaf stomatal conductance, petiole hydraulics and vessel morphology in grapevine (Vitis vinifera cv. Chasselas) under different light and irrigation regimes. Funct. Plant Biol. 44, 679. https://doi.org/10.1071/FP16041.

Dayer, S., Reingwirtz, I., McElrone, A.J., Gambetta, G.A., 2019. Response and recovery of grapevine to water deficit: From genes to physiology. In: Cantu, D., Walker, M.A. (Eds.), The Grape Genome, Compendium of Plant Genomes. Springer International Publishing, Cham, pp. 223–245. https://doi.org/10.1007/978-3-030-18601-2_11.

Dayer, S., Scharwies, J.D., Ramesh, S.A., Sullivan, W., Doerflinger, F.C., Pagay, V., Tyerman, S.D., 2020. Comparing hydraulics between two grapevine cultivars reveals differences in stomatal regulation under water stress and exogenous ABA applications. Front. Plant Sci. 11, 705. https://doi.org/10.3389/fpls.2020.00705.

De Deus, J., Damasio, M., Rodrigues, A., Silvestre, J., Zarrouk, O., Carvalho, L., 2023. The effect of drought memory on grapevine physiological responses to water deficit and recovery. BIO Web Conf. 68, 01014. https://doi.org/10.1051/bioconf/20236801014.

Deloire, A., Carbonneau, A., Wang, Z., Ojeda, H., 2004. Vine and water: a short review. OENO One 38 (1). https://doi.org/10.20870/oeno-one.2004.38.1.932.

Fick, S.E., Hijmans, R.J., 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. Int. J. Climatol. 37, 4302–4315. https://doi.org/10.1002/joc.5086.

Flexas, J., Bota, J., Escalona, J., Sampol, B., Medrano, H., 2002. Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. Funct. Plant Biol. 29, 461–471. https://doi.org/10.1071/PP01119.

Flexas, J., Galmés, J., Gallé, A., Gulías, J., Pou, A., Ribas-Carbo, M., Tomàs, M., Medrano, H., 2010. Improving water use efficiency in grapevines: potential physiological targets for biotechnological improvement. Aust. J. Grape Wine Res. 16, 106–121. https://doi.org/10.1111/j.1755-0238.2009.00057.x.

Gambetta, G.A., Herrera, J.C., Dayer, S., Feng, Q., Hochberg, U., Castellarin, S.D., 2020. The physiology of drought stress in grapevine: towards an integrative definition of drought tolerance. J. Exp. Bot. 71, 4658–4676. https://doi.org/10.1093/jxb/eraa245.

Greenspan, M.D., Schultz, H.R., Matthews, M.A., 1996. Field evaluation of water transport in grape berries during water deficits. Physiol. Plant. 97, 55–62. https://doi.org/10.1111/j.1399-3054.1996.tb00478.x.

Greenspan, M.D., Shackel, K.A., Matthews, M.A., 1994. Developmental changes in the diurnal water budget of the grape berry exposed to water deficits. Plant Cell Environ. 17, 811–820. https://doi.org/10.1111/j.1365-3040.1994.tb00175.x.

Grunwald, Y., Gosa, S.C., Torne-Srivastava, T., Moran, N., Moshelion, M., 2022. Out of the blue: phototropins of the leaf vascular bundle sheath mediate the regulation of leaf hydraulic conductance by blue light. Plant Cell 34, 2328–2342. https://doi.org/10.1093/plcell/koac089.

Herrera, J.C., Calderan, A., Gambetta, G.A., Peterlunger, E., Forneck, A., Sivilotti, P., Cochard, H., Hochberg, U., 2022. Stomatal responses in grapevine become increasingly more tolerant to low water potentials throughout the growing season. Plant J. 109, 804–815. https://doi.org/10.1111/tpj.15591.

Herrera, J.C., Savoi, S., Dostal, J., Elezovic, K., Chatzisavva, M., Forneck, A., Savi, T., 2024. The legacy of past droughts induces water-sparingly behaviour in Grüner Veltliner grapevines. Plant Biol. https://doi.org/10.1111/plb.13620.

Hochberg, U., Bonel, A.G., David-Schwartz, R., Degu, A., Fait, A., Cochard, H., Peterlunger, E., Herrera, J.C., 2017a. Grapevine acclimation to water deficit: the adjustment of stomatal and hydraulic conductance differs from petiole embolism vulnerability. Planta 245, 1091–1104. https://doi.org/10.1007/s00425-017-2662-3.

Hochberg, U., Degu, A., Fait, A., Rachmilevitch, S., 2013. Near isohydric grapevine cultivar displays higher photosynthetic efficiency and photorespiration rates under drought stress as compared with near anisohydric grapevine cultivar. Physiol. Plant. 147, 443–452. https://doi.org/10.1111/j.1399-3054.2012.01671.x.

Hochberg, U., Windt, C.W., Ponomarenko, A., Zhang, Y.J., Gersony, J., Rockwell, F.E., Holbrook, N.M., 2017b. Stomatal closure, basal leaf embolism, and shedding protect the hydraulic integrity of grape stems. Plant Physiol. 174, 764–775. https://doi.org/10.1104/pp.16.01816.

Holzapfel, B.P., Smith, J.P., Mandel, R.M., Keller, M., 2006. Manipulating the postharvest period and its impact on vine productivity of Semillon grapevines. Am. J. Enol. Vitic. 57, 148–157. https://doi.org/10.5344/ajev.2006.57.2.148.

Huang, Y., Li, C.Y., Biddle, K.D., Gibson, S.I., 2008. Identification, cloning and characterization of sis7 and sis10 sugar-insensitive mutants of Arabidopsis. BMC Plant Biol. 8, 104. https://doi.org/10.1186/1471-2229-8-104.

IPMA, 2021. Clima normais. 〈https://www.ipma.pt/pt/oclima/normais.clima/〉 (accessed 20 February 2025).

IPMA, 2025. Ondas de calor. 〈https://www.ipma.pt/pt/oclima/ondascalor/〉 (accessed 23 February 2025).

Jacinto, J., Jesus, J.G., Damásio, M., Silvestre, J., Máguas, C., Antunes, C., 2023. Phloem carbon isotopic signature as a valuable tool to assess physiological adjustments among European grapevine varieties under a Mediterranean climate. Agric. Water Manag. 286, 108396. https://doi.org/10.1016/j.agwat.2023.108396.

Kaldenhoff, R., Fischer, M., 2006. Functional aquaporin diversity in plants. Biochim. Et. Biophys. Acta Biomembr. 1758, 1134–1141. https://doi.org/10.1016/j.bbamem.2006.03.012.

Knipfer, T., Fei, J., Gambetta, G.A., McElrone, A.J., Shackel, K.A., Matthews, M.A., 2015. Water transport properties of the grape pedicel during fruit development: insights into xylem anatomy and function using microtomography. Plant Physiol. 168, 1590–1602. https://doi.org/10.1104/pp.15.00031.

Korver, R.A., Koevoets, I.T., Testerink, C., 2018. Out of shape during stress: a key role for auxin. Trends Plant Sci. 23, 783–793. https://doi.org/10.1016/j.tplants.2018.05.011.

Labarga, D., Mairata, A., Puelles, M., Martín, I., Albacete, A., García-Escudero, E., Pou, A., 2023. The rootstock genotypes determine drought tolerance by regulating aquaporin expression at the transcript level and phytohormone balance. Plants 12, 718. https://doi.org/10.3390/plants12040718.

Lavoie-Lamoureux, A., Sacco, D., Risse, P., Lovisolo, C., 2017. Factors influencing stomatal conductance in response to water availability in grapevine: a meta-analysis. Physiol. Plant. 159, 468–482. https://doi.org/10.1111/ppl.12530.

Levin, A.D., Williams, L.E., Matthews, M.A., 2020. A continuum of stomatal responses to water deficits among 17 wine grape cultivars (Vitis vinifera). Funct. Plant Biol. 47, 11. https://doi.org/10.1071/FP19073.

Liang, Y., Yang, X., Wang, C., Wang, Y., 2024. miRNAs: primary modulators of plant drought tolerance. J. Plant Physiol. 301, 154313. https://doi.org/10.1016/j.jplph.2024.154313.

Liu, H.Y., Sun, W.N., Su, W.A., Tang, Z.C., 2006. Co-regulation of water channels and potassium channels in rice. Physiol. Plant. 128, 58–69. https://doi.org/10.1111/j.1399-3054.2006.00709.x.

Lovisolo, C., Hartung, W., Schubert, A., 2002. Whole-plant hydraulic conductance and root-to-shoot flow of abscisic acid are independently affected by water stress in grapevines. Funct. Plant Biol. 29, 1349. https://doi.org/10.1071/FP02079.

Lovisolo, C., Perrone, I., Carra, A., Ferrandino, A., Flexas, J., Medrano, H., Schubert, A., 2010. Drought-induced changes in development and function of grapevine (Vitis spp.) organs and in their hydraulic and non-hydraulic interactions at the whole-plant level: a physiological and molecular update. Funct. Plant Biol. 37, 98. https://doi.org/10.1071/FP09191.

Lovisolo, C., Schubert, A., 1998. Effects of water stress on vessel size and xylem hydraulic conductivity in Vitis vinifera L. J. Exp. Bot. 49, 693–700. https://doi.org/10.1093/jxb/49.321.693.

Lovisolo, C., Schubert, A., 2006. Mercury hinders recovery of shoot hydraulic conductivity during grapevine rehydration: evidence from a whole-plant approach. N. Phytol. 172 (3), 469–478. https://doi.org/10.1111/j.1469-8137.2006.01852.x.

Ma, J.F., Mitani, N., Nagao, S., Konishi, S., Tamai, K., Iwashita, T., Yano, M., 2004. Characterization of the silicon uptake system and molecular mapping of the silicon transporter gene in rice. Plant Physiol. 136, 3284–3289. https://doi.org/10.1104/pp.104.047365.

Marusig, D., Petruzzellis, F., Tomasella, M., Napolitano, R., Altobelli, A., Nardini, A., 2020. Correlation of field-measured and remotely sensed plant water status as a tool to monitor the risk of drought-induced forest decline. Forests 11, 77. https://doi.org/10.3390/f11010077.

Marusig, D., Tombesi, S., 2020. Abscisic acid mediates drought and salt stress responses in Vitis vinifera—a review. Int. J. Mol. Sci. 21, 8648. https://doi.org/10.3390/ijms21228648.

Maurel, C., Boursiac, Y., Luu, D.T., Santoni, V., Shahzad, Z., Verdoucq, L., 2015. Aquaporins in plants. Physiol. Rev. 95, 1321–1358. https://doi.org/10.1152/physrev.00008.2015.

McElrone, A.J., Manuck, C.M., Brodersen, C.R., Patakas, A., Pearsall, K.R., Williams, L.E., 2021. Functional hydraulic sectoring in grapevines as evidenced by sap flow, dye infusion, leaf removal and micro-computed tomography. AoB Plants 13, plab003. https://doi.org/10.1093/aobpla/plab003.

Moshelion, M., Halperin, O., Wallach, R., Oren, R., Way, D.A., 2015. Role of aquaporins in determining transpiration and photosynthesis in water-stressed plants: crop water-use efficiency, growth and yield. Plant Cell Environ. 38, 1785–1793. https://doi.org/10.1111/pce.12410.

Munitz, S., Netzer, Y., Shtein, I., Schwartz, A., 2018. Water availability dynamics have long-term effects on mature stem structure in Vitis vinifera. Am. J. Bot. 105, 1443–1452. https://doi.org/10.1002/ajb2.1148.

Nagata, M., Yamashita, I., 1992. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. J. Food Sci. Technol. 39, 925–928. https://doi.org/10.3136/nskkk1962.39.925.

Nemhauser, J.L., Hong, F., Chory, J., 2006. Different plant hormones regulate similar processes through largely non overlapping transcriptional responses. Cell 126, 467–475. https://doi.org/10.1016/j.cell.2006.05.050.

Noronha, H., Araújo, D., Conde, C., Martins, A.P., Soveral, G., Chaumont, F., Delrot, S., Gerós, H., 2016. The grapevine uncharacterized intrinsic protein 1 (VvXIP1) is regulated by drought stress and transports glycerol, hydrogen peroxide, heavy metals but not water. PLoS One 11, e0160976. https://doi.org/10.1371/journal.pone.0160976.

Noronha, H., Silva, A., Mitani-Ueno, N., Conde, C., Sabir, F., Prista, C., Soveral, G., Isenring, P., Ma, J.F., Bélanger, R.R., Gerós, H., 2020. The grapevine NIP2;1 aquaporin is a silicon channel. J. Exp. Bot. 71, 6789–6798. https://doi.org/10.1093/jxb/eraa294.

Ohtsuka, A., Sack, L., Taneda, H., 2018. Bundle sheath lignification mediates the linkage of leaf hydraulics and venation. Plant Cell Environ. 41, 342–353. https://doi.org/10.1111/pce.13087.

Ollat, N., Gaudillère, J., 2000. Carbon balance in developing grapevine berries. Acta Hortic. (526), 345–350. https://doi.org/10.17660/ActaHortic.2000.526.37.

Opazo, I., Toro, G., Salvatierra, A., Pastenes, C., Pimentel, P., 2020. Rootstocks modulate the physiology and growth responses to water deficit and long-term recovery in grafted stone fruit trees. Agric. Water Manag. 228, 105897. https://doi.org/10.1016/j.agwat.2019.105897.

Pagay, V., Zufferey, V., Lakso, A.N., 2016. The influence of water stress on grapevine (Vitis vinifera L.) shoots in a cool, humid climate: growth, gas exchange and hydraulics. Funct. Plant Biol. 43, 827. https://doi.org/10.1071/FP16017.

Passioura, J.B., 2006. Viewpoint: the perils of pot experiments. Funct. Plant Biol. 33, 1075. https://doi.org/10.1071/FP06223.

Pou, A., Medrano, H., Flexas, J., Tyerman, S.D., 2013. A putative role for TIP and PIP aquaporins in dynamics of leaf hydraulic and stomatal conductances in grapevine under water stress and re-watering. Plant Cell Environ. 36, 828–843. https://doi.org/10.1111/pce.12019.

Ramos, T., Gonçalves, M., Martins, J., Pereira, L., 2016. Características de retenção de água no solo para utilização na rega das culturas. In: Funções de Pedotransferência Para Solos, First ed., 1. INIAV, Oeiras, Portugal.

Richardson, A.D., Duigan, S.P., Berlyn, G.P., 2002. An evaluation of noninvasive methods to estimate foliar chlorophyll content. N. Phytol. 153, 185–194. https://doi.org/10.1046/j.0028-646X.2001.00289.x.

Rienth, M., Vigneron, N., Darriet, P., Sweetman, C., Burbidge, C., Bonghi, C., Walker, R. P., Famiani, F., Castellarin, S.D., 2021. Grape berry secondary metabolites and their modulation by abiotic factors in a climate change scenario—a review. Front. Plant Sci. 12, 643258. https://doi.org/10.3389/fpls.2021.643258.

Rogiers, S.Y., Greer, D.H., Hatfield, J.M., Hutton, R.J., Clarke, S.J., Hutchinson, P.A., Somers, A., 2012. Stomatal response of an anisohydric grapevine cultivar to evaporative demand, available soil moisture and abscisic acid. Tree Physiol. 32, 249–261. https://doi.org/10.1093/treephys/tpr131.

Romero, P., Dodd, I.C., Martinez-Cutillas, A., 2012. Contrasting physiological effects of partial root zone drying in field-grown grapevine (Vitis vinifera L. cv. Monastrell) according to total soil water availability. J. Exp. Bot. 63, 4071–4083. https://doi.org/10.1093/jxb/ers088.

Rowe, J.H., Topping, J.F., Liu, J., Lindsey, K., 2016. Abscisic acid regulates root growth under osmotic stress conditions via an interacting hormonal network with cytokinin, ethylene and auxin. N. Phytol. 211, 225–239. https://doi.org/10.1111/nph.13882.

Sabir, F., Gomes, S., Loureiro-Dias, M.C., Soveral, G., Prista, C., 2020. Molecular and functional characterization of grapevine NIPs through heterologous expression in aqy-null Saccharomyces cerevisiae. Int. J. Mol. Sci. 21, 663. https://doi.org/10.3390/ijms21020663.

Sabir, F., Leandro, M.J., Martins, A.P., Loureiro-Dias, M.C., Moura, T.F., Soveral, G., Prista, C., 2014. Exploring three PIPs and three TIPs of grapevine for transport of water and atypical substrates through heterologous expression in aqy-null yeast. PLoS One 9, e102087. https://doi.org/10.1371/journal.pone.0102087.

Sabir, F., Zarrouk, O., Noronha, H., Loureiro-Dias, M.C., Soveral, G., Gerós, H., Prista, C., 2021. Grapevine aquaporins: diversity, cellular functions, and ecophysiological perspectives. Biochimie 188, 61–76. https://doi.org/10.1016/j.biochi.2021.06.004.

Sade, N., Vinocur, B.J., Diber, A., Shatil, A., Ronen, G., Nissan, H., Wallach, R., Karchi, H., Moshelion, M., 2008. Improving plant stress tolerance and yield production: is the tonoplast aquaporin SlTIP2;2 a key to isohydric to anisohydric conversion? N. Phytol. 181, 651–661. https://doi.org/10.1111/j.1469-8137.2008.02689.x.

Schultz, H.R., 2003. Differences in hydraulic architecture account for near-isohydric and anisohydric behaviour of two field-grown Vitis vinifera L. cultivars during drought. Plant Cell Environ. 26, 1393–1405. https://doi.org/10.1046/j.1365-3040.2003.01064.x.

Scoffoni, C., Albuquerque, C., Brodersen, C.R., Townes, S.V., John, G.P., Bartlett, M.K., Buckley, T.N., McElrone, A.J., Sack, L., 2017. Outside-xylem vulnerability, not xylem embolism, controls leaf hydraulic decline during dehydration. Plant Physiol. 173, 1197–1210. https://doi.org/10.1104/pp.16.01643.

Scoffoni, C., Albuquerque, C., Buckley, T.N., Sack, L., 2023. The dynamic multi-functionality of leaf water transport outside the xylem. N. Phytol. 239, 2099–2107. https://doi.org/10.1111/nph.19069.

Scoffoni, C., Albuquerque, C., Cochard, H., Buckley, T.N., Fletcher, L.R., Caringella, M.A., Bartlett, M.K., Brodersen, C., Jansen, S., McElrone, A.J., Sack, L., 2018. The causes of leaf hydraulic vulnerability and its influence on gas exchange in Arabidopsis thaliana. Plant Physiol. 178, 1584–1601. https://doi.org/10.1104/pp.18.00743.

Shatil-Cohen, A., Attia, Z., Moshelion, M., 2011. Bundle-sheath cell regulation of xylem-mesophyll water transport via aquaporins under drought stress: a target of xylem-borne ABA? Plant J. 67, 72–80. https://doi.org/10.1111/j.1365-313X.2011.04576.x.

Shelden, M.C., Vandeleur, R., Kaiser, B.N., Tyerman, S.D., 2017. A comparison of petiole hydraulics and aquaporin expression in an anisohydric and isohydric cultivar of grapevine in response to water-stress induced cavitation. Front. Plant Sci. 8, 1893. https://doi.org/10.3389/fpls.2017.01893.

Shellie, K., Kovaleski, A.P., Londo, J.P., 2018. Water deficit severity during berry development alters timing of dormancy transitions in wine grape cultivar Malbec. Sci. Hortic. 232, 226–230. https://doi.org/10.1016/j.scienta.2018.01.014.

Sims, D.A., Gamon, J.A., 2002. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. Remote Sens. Environ. 81, 337–354. https://doi.org/10.1016/S0034-4257(02)00010-X.

Skelton, R.P., Brodribb, T.J., Choat, B., 2017. Casting light on xylem vulnerability in an herbaceous species reveals a lack of segmentation. N. Phytol. 214, 561–569. https://doi.org/10.1111/nph.14450.

Sorek, Y., Greenstein, S., Netzer, Y., Shtein, I., Jansen, S., Hochberg, U., 2021. An increase in xylem embolism resistance of grapevine leaves during the growing season is coordinated with stomatal regulation, turgor loss point and intervessel pit membranes. N. Phytol. 229, 1955–1969. https://doi.org/10.1111/nph.17025.

Sperry, J.S., Donnelly, J.R., Tyree, M.T., 1988. A method for measuring hydraulic conductivity and embolism in xylem. Plant Cell Environ. 11, 35–40. https://doi.org/10.1111/j.1365-3040.1988.tb01774.x.

Stoll, M., Loveys, B., Dry, P., 2000. Hormonal changes induced by partial rootzone drying of irrigated grapevine. J. Exp. Bot. 51, 1627–1634. https://doi.org/10.1093/jexbot/51.350.1627.

Takase, T., Ishikawa, H., Murakami, H., Kikuchi, J., Sato-Nara, K., Suzuki, H., 2010. The circadian clock modulates water dynamics and aquaporin expression in Arabidopsis roots. Plant Cell Physiol. 52, 373–383. https://doi.org/10.1093/pcp/pcq198.

Tamayo, M., Sepúlveda, L., Guequen, E.P., Saavedra, P., Pedreschi, R., Cáceres-Mella, A., Alvaro, J.E., Cuneo, I.F., 2023. Hydric behavior: insights into primary metabolites in leaves and roots of Cabernet Sauvignon and Grenache grapevine varieties under drought stress. Horticulturae 9, 566. https://doi.org/10.3390/horticulturae9050566.

Taskos, D., Zioziou, E., Nikolaou, N., Doupis, G., Koundouras, S., 2020. Carbon isotope natural abundance (δ^{13}C) in grapevine organs is modulated by both water and nitrogen supply. OENO One 54 (4), 837–850. https://doi.org/10.20870/oeno-one.2020.54.4.3600.

Tombesi, S., Farinelli, D., Palliotti, A., Poni, S., Dejong, T., 2018. Xylem manipulation techniques affecting tree vigour in peach and olive trees. Acta Hortic. (1228), 91–96. https://doi.org/10.17660/ActaHortic.2018.1228.12.

Tombesi, S., Nardini, A., Farinelli, D., Palliotti, A., 2014. Relationships between stomatal behavior, xylem vulnerability to cavitation and leaf water relations in two cultivars of Vitis vinifera. Physiol. Plant. 152, 453–464. https://doi.org/10.1111/ppl.12180.

Tombesi, S., Nardini, A., Frioni, T., Soccolini, M., Zadra, C., Farinelli, D., Poni, S., Palliotti, A., 2015. Stomatal closure is induced by hydraulic signals and maintained by ABA in drought-stressed grapevine. Sci. Rep. 5, 12449. https://doi.org/10.1038/srep12449.

Tramontini, S., Döring, J., Vitali, M., Ferrandino, A., Stoll, M., Lovisolo, C., 2014. Soil water-holding capacity mediates hydraulic and hormonal signals of near-isohydric and near-anisohydric Vitis cultivars in potted grapevines. Funct. Plant Biol. 41, 1119. https://doi.org/10.1071/FP13263.

Vandeleur, R.K., Mayo, G., Shelden, M.C., Gilliham, M., Kaiser, B.N., Tyerman, S.D., 2009. The role of plasma membrane intrinsic protein aquaporins in water transport through roots: diurnal and drought stress responses reveal different strategies between isohydric and anisohydric cultivars of grapevine. Plant Physiol. 149, 445–460. https://doi.org/10.1104/pp.108.128645.

Verma, S., Negi, N.P., Pareek, S., Mudgal, G., Kumar, D., 2022. Auxin response factors in plant adaptation to drought and salinity stress. Physiol. Plant. 174, e13714. https://doi.org/10.1111/ppl.13714.

Vignesh, M.R., Palanisamy, S., 2021. Aquaporin and its effect on foliar uptake to overcome drought stress in plants. Plant Physiol. Rep. 26, 193–199. https://doi.org/10.1007/s40502-021-00567-3.

Villalobos-González, L., Muñoz-Araya, M., Franck, N., Pastenes, C., 2019. Controversies in midday water potential regulation and stomatal behavior might result from the environment, genotype, and/or rootstock: evidence from Carménère and Syrah grapevine varieties. Front. Plant Sci. 10, 1522. https://doi.org/10.3389/fpls.2019.01522.

Vitali, M., Cochard, H., Gambino, G., Ponomarenko, A., Perrone, I., Lovisolo, C., 2016. VvPIP2;4N aquaporin involvement in controlling leaf hydraulic capacitance and resistance in grapevine. Physiol. Plant. 158, 284–296. https://doi.org/10.1111/ppl.12463.

Wang, Y.T., Chen, Z.Y., Jiang, Y., Duan, B.B., Xi, Z.M., 2019. Involvement of ABA and antioxidant system in brassinosteroid-induced water stress tolerance of grapevine (Vitis vinifera L.). Sci. Hortic. 256, 108596. https://doi.org/10.1016/j.scienta.2019.108596.

Wang, M., Ding, L., Gao, L., Li, Y., Shen, Q., Guo, S., 2016. The interactions of aquaporins and mineral nutrients in higher plants. Int. J. Mol. Sci. 17, 1229. https://doi.org/10.3390/ijms17081229.

Wang, Y., Zhao, Z., Liu, F., Sun, L., Hao, F., 2020. Versatile roles of aquaporins in plant growth and development. Int. J. Mol. Sci. 21, 9485. https://doi.org/10.3390/ijms21249485.

Winkel, T., Rambal, S., 1993. Influence of water stress on grapevines growing in the field: from leaf to whole-plant response. Funct. Plant Biol. 20, 143. https://doi.org/10.1071/PP9930143.

Wong, D.C.J., Zhang, L., Merlin, I., Castellarin, S.D., Gambetta, G.A., 2018. Structure and transcriptional regulation of the major intrinsic protein gene family in grapevine. BMC Genom. 19, 248. https://doi.org/10.1186/s12864-018-4638-5.

Xiong, D., Nadal, M., 2020. Linking water relations and hydraulics with photosynthesis. Plant J. 101, 800–815. https://doi.org/10.1111/tpj.14595.

Yan, X., Zhou, M., Dong, X., Zou, S., Xiao, H., Ma, X.F., 2015. Molecular mechanisms of foliar water uptake in a desert tree. plv129 AoB Plants 7. https://doi.org/10.1093/aobpla/plv129.

Zamorano, D., Franck, N., Pastenes, C., Wallberg, B., Garrido, M., Silva, H., 2021. Improved physiological performance in grapevine (Vitis vinifera L.) cv. Cabernet Sauvignon facing recurrent drought stress. Aust. J. Grape Wine Res. 27, 258–268. https://doi.org/10.1111/ajgw.12482.

Zarrouk, O., Costa, J.M., Francisco, R., Lopes, C., Chaves, M., 2016a. Drought and water management in Mediterranean vineyards. In: Chaves, M.M., Flexas, J. (Eds.), Grapevine in a Changing Environment: A Molecular and Ecophysiological Perspective. Wiley-Blackwell, 400. https://doi.org/10.1002/9781118735985.ch3.

Zarrouk, O., Garcia-Tejero, I., Pinto, C., Genebra, T., Sabir, F., Prista, C., Soares David, T., Loureiro-Dias, M.C., Chaves, M.M., 2016b. Aquaporins isoforms in cv. Touriga Nacional grapevine under water stress and recovery—regulation of expression in leaves and roots. Agric. Water Manag. 158, 94–104. https://doi.org/10.1016/j.agwat.2015.08.013.

Zhang, Y., Hochberg, U., Rockwell, F.E., Ponomarenko, A., Chen, Y., Manandhar, A., Graham, A.C., Holbrook, N.M., 2022. Xylem conduit deformation across vascular plants: an evolutionary spandrel or protective valve? N. Phytol. 237, 1242–1255. https://doi.org/10.1111/nph.18584.

Zhang, Y., Rockwell, F.E., Graham, A.C., Alexander, T., Holbrook, N.M., 2016. Reversible leaf xylem collapse: a potential “circuit breaker” against cavitation. Plant Physiol. 172, 2261–2274. https://doi.org/10.1104/pp.16.01191.