

MANAGING PRECISION IRRIGATION IN VINEYARDS: HYDRAULIC AND MOLECULAR SIGNALING IN EIGHT GRAPEVINE VARIETIES

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Abstract:

Context and purpose of the study

Understanding the physiological and molecular bases of grapevine responses to mild to moderate water deficits is fundamental to optimize vineyard irrigation management and identify the most suitable varieties. In Mediterranean regions, the higher frequency of heat waves and droughts highlights the importance of precision irrigation to meet vine water demands and demonstrates the necessity for a deeper understanding of the different physiological responses among varieties under water stress. In this context, previous reports show an interplay between stomatal regulation of transpiration and changes in leaf hydraulic conductivity, also with the involvement of aquaporins (AQPs), particularly under water stress. However, how those signaling mechanisms are regulated in different grapevine varieties along phenological phases is unclear. We aimed to assess the impact of vine water status and phenology on stomatal and hydraulic adjustments, along with aquaporins expression, of leaves of 8 grapevine varieties (Petit Verdot (PV), Alicante Bouschet (AB), Syrah (S), Vinhão (V), Touriga Nacional (TN), Castelão (Cs), Trincadeira (TR) and Tinta Caiada (TC)) selected according to their contrasting carbon isotopic signatures.

Material and methods

Ten-year-old vines grafted on 1103P, grown in the Alentejo region (Portugal) as part of the ampelographic collection of Esporão commercial vineyard, were subjected to three different irrigation treatments since 2018: full irrigation (FI); deficit irrigation (DI, 50% of FI); no-irrigation (NI). Plant monitoring was done during 2021 at four phenological stages: pea-size (PS), veraison (VER), full-maturation (FM) and post-harvest (Post-H). Measurements included stomatal conductance (g_s), predawn leaf water potential (ψ_{pd}), hydraulic conductivity (K_{leaf}) and the expression of 10 genes coding AQPs, previously reported to transport water *in vitro*.

Results

Results show that K_{leaf} is a function of grapevine phenology and variety. In all varieties and treatments, K_{leaf} peaked at veraison and declined thereafter (circa 80%). Water stress either increased or decreased K_{leaf} , likely depending on the anisohydric (e.g. TN) or isohydric (e.g. V, S) behavior. Interestingly, post-harvest irrigation increased K_{leaf} , surpassing values observed at FM, except in TN, suggesting that varieties benefit differently from late season irrigation. Stomatal conductance was highest at VER, in all irrigation treatments, except for TN, which peaked at PS. PV maintained constant g_s throughout the season in all treatments. Non-irrigated AB, S, V, and TR maintained low and constant g_s along the season. A significant correlation between g_s and ψ_{pd} was observed, except in Cs and PV, indicating that in those varieties additional factors contribute to control stomatal aperture. Studied AQPs were all expressed at PS and VER in all varieties and water treatments, except in TC, TR and AB where some *PIPs* and *NIPs* were not expressed under water stress. *VviPIP1;2* appears to be constitutive since it is expressed at all phenological stages, irrigation treatments and varieties. Overall, DI and NI induced an up-regulation of AQP expression in anisohydric varieties at PS and VER. In contrast, AQPs were down-regulated by stress at PS in the isohydric varieties. These results indicate that AQPs expression trend is variety and phenology dependent.

Keywords: Aquaporins, Hydraulic conductivity, Stomatal conductance, Water deficit.

1. Introduction

Grapevine (*Vitis vinifera* L.) is generally considered a 'drought avoiding' species and is well adapted to semi arid lands with Mediterranean type climates, characterized by warm and dry summers. To cope with water stress, grapevine developed efficient adaptation mechanisms to transfer water from roots to growing shoots, involving an effective stomatal control of transpiration (Chaves et al., 2010) and xylem embolism avoidance system (Lovisolo et al., 2002; Vandeleur et al., 2009), as well as osmotic adjustment ability (Chaves et al., 2010). During these processes, aquaporins are deeply involved in the non-vascular transport of water, through inter- and intracellular pathways (Maurel et al., 2015) within the plant. 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. The increasing frequency of heat waves and droughts in Mediterranean regions induced a paradoxical increase in irrigation demand in vineyards. Consequently, improving the efficiency of water use in Mediterranean vineyards became imperative. Deficit irrigation emerged as a potential strategy to allow crops to withstand mild water stress (Zarrouk et al., 2016). However, this varies with the used genotype. To optimize the management of deficit irrigation, and identify the most suitable varieties, it is crucial to understand the physiological and molecular bases governing water relations and movement at the cellular/tissue level and in whole plants within grapevine genotypes. In this context, this work aimed at elucidating the impact of vine water status and phenology on stomatal and hydraulic adjustments, along with aquaporins expression, in leaves of 8 grapevine varieties selected according to their iso-or anisohydric behavior.

2. Material and methods

Plant Material and Experimental Site

The study was conducted during the growing season of 2021 at a private ampelographic collection from Esporão commercial vineyard located in the Alentejo region, Portugal (38.380098, -7.560724). Ten-year-old grapevine plants, grafted on 1103 Paulsen with 1.5 m x 3.0 m (N/S oriented) spacing and trained on a vertical shoot positioned system, were used. Eight varieties were selected and grouped according to their behavior: anisohydric, Petit Verdot (PV), Alicante Bouschet (AB), Touriga Nacional (TN) and Castelão (Cs), and isohydric, Syrah (S), Vinhão (V), Trincadeira (TR) and Tinta Caiada (TC). The region is characterized by hot and dry summers, being classified as 'Csa' (Köppen-Geiger Climate Classification (IPMA, 2021)). The soil is an Eutric Cambisol with a ApBw1Bw2C profile, derived from granite with 75-80% of sand. All varieties had been subjected to three different irrigation treatments since 2018: full irrigation (FI, 100% ETC); deficit irrigation (DI, 50% of FI); no-irrigation (NI, rain-fed). Plant monitoring was done at four phenological stages: pea-size (PS), veraison (VER), full-maturation (FM) and post-harvest (Post-H).

Grapevine Water Status

Grapevine water status was monitored along the experiment through the measurement of predawn leaf water potential (Ψ_{pd}), using a Scholander pressure chamber (Manofrígido, S.A., Lisboa, Portugal). In each sampling date, measurements were done in five plants per variety and treatment, using at least 2 leaves per vine.

Stomatal Conductance and Leaf Transpiration

Stomatal conductance (g_s) and leaf transpiration (E) measurements were performed with a Steady State Porometer (LI-1600, LI-COR Inc, Lincoln, NE, USA). Measurements were performed between 11:00 am and 12:00 pm in 2 leaves per vine, from 5 plants per variety and treatment.

Hydraulic conductance

Two leaves per vine, from five plants per variety and treatment, were submerged in water and excised, and immediately transported under water to the laboratory. Hydraulic conductance (K_h , $\text{kg s}^{-1} \text{MPa}^{-1}$) was measured in petiole segments (Sperry et al., 1988), with a high precision flow meter, XYL'EM (Embolism Meter, Bronkhorst, Montigny-lès-Cormeilles, France). Hydraulic conductance was normalized to the length of each segment (hydraulic conductivity, $\text{kg s}^{-1} \text{MPa}^{-1} \text{m}^{-1}$), and converted to leaf specific hydraulic conductivity (K_{leaf} , $\text{kg s}^{-1} \text{MPa}^{-1} \text{m}^{-1}$) by dividing the hydraulic conductivity by petiole sectional area (m^2) and by leaf area (m^2). Leaf images were acquired using a 64 Megapixel digital camera (Samsung S20, Samsung, Suwon, South Korea) and

used for the determination of leaf area (m^2) with image analysis software ImageJ (University of Wisconsin, USA).

RNA Extraction and RT-qPCR

Grapevine leaves were carefully harvested at 11 am and immediately frozen in liquid nitrogen, and transported to the lab. Total RNA was extracted using the Spectrum™ Plant Total RNA kit (Sigma-Aldrich, St. Louis, MO, USA). RNA concentration and integrity were evaluated, and cDNA was synthesized using 700 ng of total RNA with RevertAid Reverse Transcriptase (Fermentas Life Science, Helsingborg, Sweden) according to the manufacturer's protocol. Quantitative real-time PCR was performed in CFX Maestro (Bio-Rad, Hercules, CA, USA), using SsoFast EVA Green Master Mix (Bio-Rad). Three genes were used as reference, *Actin 2* (ACT), *Vitis vinifera translation initiation factor 3 subunit G* (TIF), and *Vitis vinifera translation initiation factor eIF-2B subunit alpha* (TIF-GTP). The expression of 10 aquaporins, previously reported to transport water *in vitro* (Sabir et al., 2021), was assessed: *VviPIP1;1*, *VviPIP1;2*, *VviPIP2;2*, *VviPIP2;2*, *VviTIP1;1*, *VviTIP2;1*, *VviTIP2;2*, *VviNIP2;1*, *VviNIP6;1* and *VviXIP1*. Quantification of the relative gene expression was done with the $\Delta\Delta C_q$ method, and expressed as mean and standard error of three biological replicates and 2 technical replicates.

Statistical analysis

The experimental design was a completely randomized design. An exploratory and descriptive analysis was made of all physiological and molecular measurements, followed by a one-way variance analysis (ANOVA; SPSS 15.0 statistical package; SPSS, Chicago, IL, USA) with Duncan test for mean separation ($p < 0.05$). Data are presented as averages of the four varieties of each group \pm SD (anishydric (PV, AB, TN, CS) and isohydric (S, V, TR, TC)).

3. Results and discussion

3.1. Grapevine water status and Stomatal conductance

Predawn leaf water potential (ψ_{pd}) was significantly different among irrigation treatments (Figure 1). No significant differences between FI and DI treatments were observed except at FM for isohydric varieties. Independently of the irrigation treatment, stomatal conductance (g_s) was highest at VER (Figure 2), in all varieties, except for TN, which peaked at PS. This behavior was previously reported (Costa et al., 2012) and attributed to the characteristics of the summer (July) in northern hemisphere, namely, higher irradiance, temperature and evapotranspiration (Flexas et al., 2002). As water deficit increased along the season, differences in stomatal closure among cultivars became apparent. However, no differences were observed between FI and DI. Interestingly, non-irrigated grapevines maintained low and constant g_s since PS. In this regard, cumulative long-term water stress affects the stomatal behavior of the different genotypes. This is evident at PS that showed lower g_s in NI than in FI and DI although ψ_{pd} values were above the mild stress threshold. This could indicate a drought memory effect in the drought-response phenotype in grapevine, enabling non-irrigated plants to preserve water more efficiently than irrigated ones (Tombesi et al., 2018). A significant correlation between g_s and ψ_{pd} was also observed, except for Cs and PV (data not shown), indicating that in some varieties additional factors contribute to control stomatal aperture (Medrano et al., 2002; Flexas et al., 2007).

3.2. Hydraulic conductance

Results show that K_{leaf} is a function of grapevine phenology and variety. As previously reported (Flexas et al., 2002; Lovisolo et al., 2010), K_{leaf} peaked at veraison and declined thereafter (circa 80%) in all varieties and all water treatments (Figure 3). A significant positive correlation was found between K_{leaf} and g_s in all varieties except for Cs and V (data not shown), indicating that additional signals other than hydraulic are involved in seasonal g_s dynamic. Water stress either increased or decreased K_{leaf} , likely depending on the ani- (e.g. TN) or isohydric (e.g. V, S) behavior of the genotypes. This is consistent with previous studies showing that anishydric genotypes have higher ability to maintain high hydraulic conductance under water stress, compared to isohydric genotypes (Hochberg et al., 2017). However, some caution should be taken since no significant differences among treatments were observed in the present study. Interestingly, post-harvest irrigation increased K_{leaf} , surpassing values observed at FM, except in TN. These results suggest that varieties benefit differently from late season irrigation, and indicate that hydraulic traits are more seasonally dynamic than previously thought in grapevine (Sorek et al., 2020).

3.3. Aquaporins gene expression

All the AQPs monitored were expressed in PS and VER in all varieties and water treatments, except in TC, TR and AB, where some *PIPs* and *NIPs* were not expressed under water stress. *VviPIP1;2* appears to be a constitutive AQP since it is expressed at all phenological stages, irrigation treatments and varieties. Overall, DI and NI induced an up-regulation of expressed AQPs in the anisohydric varieties at PS and VER. In contrast, AQPs were down-regulated by stress at PS in the isohydric varieties (Figure 4). These results indicate that the modulation of AQP expression is variety and phenology dependent. Aquaporins have a key role in the regulation of the opening and closure of stomata by enabling plants to rapidly and reversibly modify water permeability (Chaumont and Tyerman, 2014) which could explain the higher g_s values in anisohydric varieties at PS and VER than in isohydric ones in water stressed vines. In isohydric leaves, AQPs could be inhibited by hormonal signaling (Shatil-Cohen et al., 2011), which induce a decrease in K_{leaf} , exerting a feed-forward signal for stomata to close (Dayer et al. 2020). This also could explain the drop of g_s and K_{leaf} at the end of the season since several AQPs were down-regulated or not expressed.

4. Conclusions

The data gathered herein can contribute for the selection of well adapted varieties for specific sites and conditions. We show that hydraulic traits are seasonally dynamic and are responsive to the impacts of cumulative long-term water stress. Results indicate also, that the modulation of AQP expression is variety and phenology dependent, being up-regulated before veraison only in the anisohydric varieties.

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6. Literature cited

- Chaumont F., Tyerman S., 2014. Aquaporins: Highly regulated channels controlling plant water relations. *Plant Physiol.* 164, 1600–1618.
- Chaves M., Zarrouk O., Francisco R., Costa J., Santos T., Regalado A., Rodrigues M., Lopes C., 2010. Grapevine under deficit irrigation: hints from physiological and molecular data. *Annals of Applied Biology*, 157(2), 281-295.
- Costa J.M., Ortuño M.F., Lopes C.M., Chaves M.M., 2012. Grapevine varieties exhibiting differences in stomatal response to water deficit. *Functional Plant Biology* 39 (3), 179-189.
- Dayer S., Scharwies J., Ramesh S., Sullivan W., Doerflinger F., Pagay V., Tyerman S., 2020. Comparing Hydraulics Between Two Grapevine Cultivars Reveals Differences in Stomatal Regulation Under Water Stress and Exogenous ABA Applications. *Front Plant Sci.* 11, 705.
- Flexas J., Medrano H., 2002. Drought-inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitations revisited. *Annals of Botany.* 83, 183–189.
- Flexas J., Diaz-Espejo A., Galmés J., Kaldenhoff R., Medrano H., Ribas-Carbo M., 2007. Rapid variations of mesophyll conductance in response to changes in CO2 concentration around leaves. *Plant, Cell & Environment*, 33(7), 1268-1284.
- Hochberg U., Windt C., Ponomarenko A., Zhang Y., Gersony J., Rockwell F., Holbrook N., 2017. Stomatal closure, basal leaf embolism, and shedding protect the hydraulic integrity of grape stems. *Plant Physiology* 174, 764–775.
- 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. *Functional Plant Biology* 29 (11), 1349-1356.
- 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. *Functional Plant Biology* 37, 98–116.
- Maurel C., Boursiac Y., Luu D-T., Santoni V., Shahzad Z., Verdoucq L., 2015. Aquaporins in Plants. *Physiol Rev* 95, 1321–1358.

- Medrano H., Escalona J., Bota J., Gulías J., Flexas J.,** 2002. Regulation of Photosynthesis of C3 Plants in Response to Progressive Drought: Stomatal Conductance as a Reference Parameter. *Annals of Botany* 89 (7), 895–905.
- Pou A., Medrano H., Flexas J., Tyerman S.,** 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 & Environment* 36, 828–843.
- Sabir F., Zarrouk O., Noronha H., Loureiro-Dias M., Soveral G., Geró, H., Prista C.,** 2021. Grapevine aquaporins: Diversity, cellular functions, and ecophysiological perspectives. *Biochimie* 188, 61–76.
- 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?. *The Plant Journal* 67, 72–80.
- Sperry J., Donnelly J., Tyree M.,** 1988. A method for measuring hydraulic conductivity and embolism in xylem. *Plant Cell Environ* 11, 35–40.
- Sorek Y, Greenstein S, Netzer Y, Shtein I, Jansen S, Hochberg U.,** 2020. 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. *New Phytologist* 229, 1955–1969.
- Tombesi S., Frioni T., Poni S., Palliotti A.,** 2018. Effect of water stress “memory” on plant behavior during subsequent drought stress. *Environmental and Experimental Botany* 150, 106-114.
- Vandeleur R., Mayo G., Shelden M., Gilliam M., Kaiser B, Tyerman S.,** 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 Physiology*, 149(1), 445-460.
- Zarrouk O., Costa M., Francisco R., Lopes C., Chaves M.,** 2016. Drought and water management in Mediterranean vineyards. In *Grapevine in a Changing Environment: A Molecular and Ecophysiological Perspective*, 38-67.

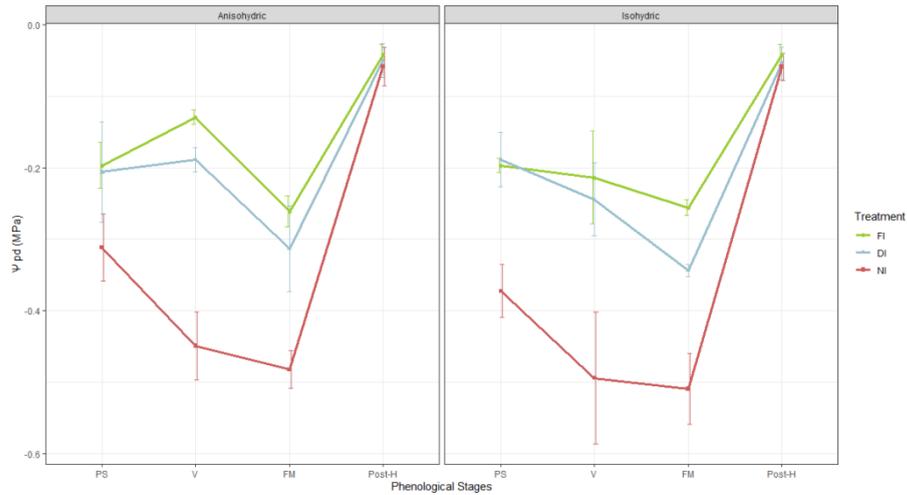


Figure 1: Predawn Leaf Water Potential (Ψ_{pd}) in Full irrigated (FI), Deficit irrigated (DI) and Non-irrigated (NI) treatments along the grapevine phenology in the anisohydric varieties (PV, AB, TN and CS) and isohydric varieties (S, V, TR and TC). Data represents averages of the 4 varieties of each group \pm SD.

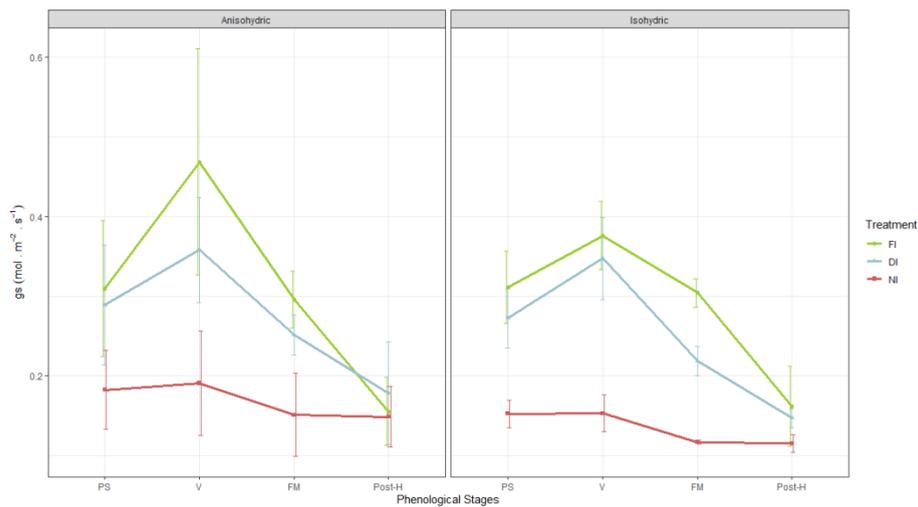


Figure 2: Leaf stomatal conductance (g_s) in Full irrigated (FI), Deficit irrigated (DI) and Non-irrigated (NI) treatments along the grapevine phenology for the anisohydric varieties (PV, AB, TN and CS) and isohydric varieties (S, V, TR and TC). Data represents averages of the 4 varieties of each group \pm SD.

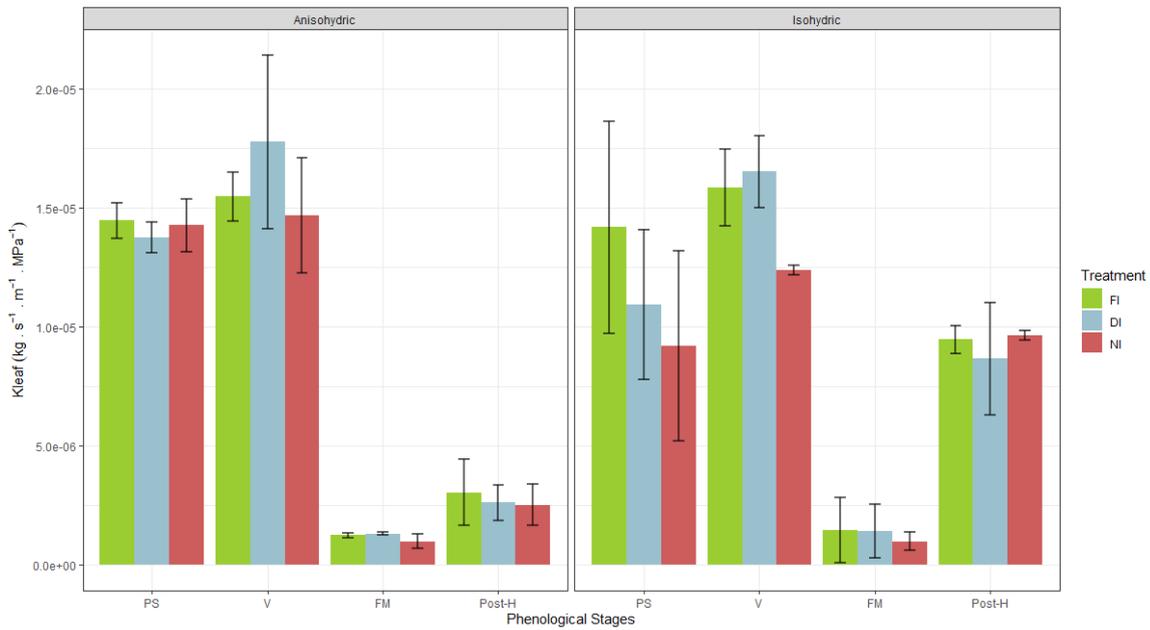


Figure 3: Leaf hydraulic conductivity (K_{leaf}) in Full irrigated (FI), Deficit irrigated (DI) and Non-irrigated (NI) treatments along the grapevine phenology for the anisohydric varieties (PV, AB, TN and CS) and isohydric varieties (S, V, TR and TC). Data represents averages of the 4 varieties of each group \pm SD.

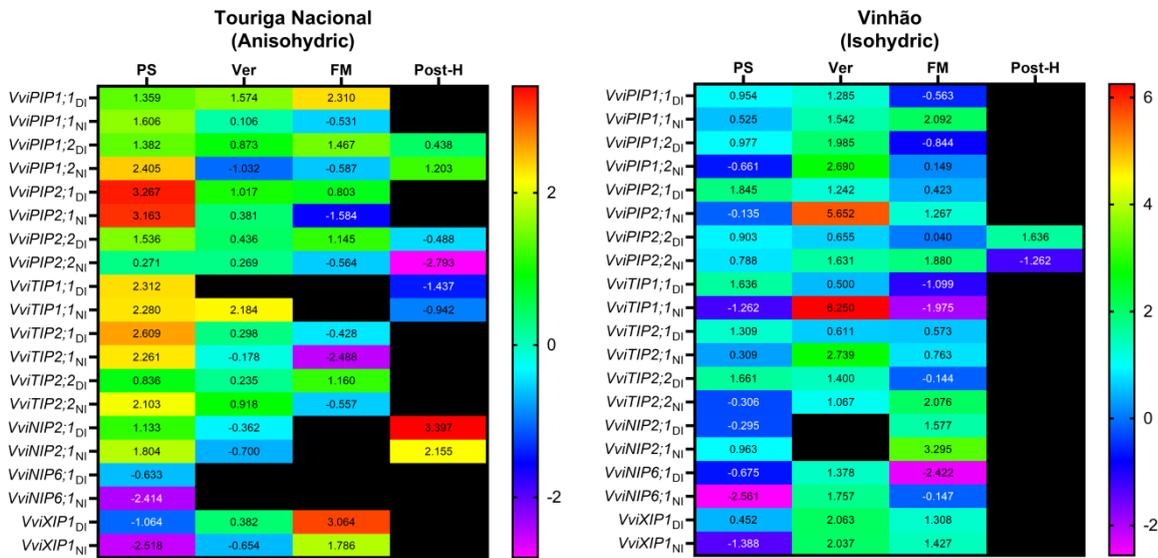


Figure 4: Aquaporin gene expression ($\log_2(\text{fold change})$) of *VviPIP1;1*, *VviPIP1;2*, *VviPIP2;2*, *VviPIP2;2*, *VviTIP1;1*, *VviTIP2;1*, *VviTIP2;2*, *VviNIP2;1*, *VviNIP6;1* and *VviXIP1* along the grapevine phenology for the anisohydric variety 'Touriga Nacional' and the isohydric variety 'Vinhão'. Relative values for the treatments DI and NI are expressed in comparison to FI.