Comparing Hydraulics Between Two Grapevine Cultivars Reveals Differences in Stomatal Regulation Under Water Stress and Exogenous ABA Applications

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Hydraulics of plants that have different strategies of stomatal regulation under water stress are relatively poorly understood. We explore how root and shoot hydraulics, stomatal conductance (g_s), leaf and root aquaporin (AQP) expression, and abscisic acid (ABA) concentration in leaf xylem sap ([ABA]$_{xylemsap}$) may be coordinated under mild water stress and exogenous ABA applications in two *Vitis vinifera* L. cultivars traditionally classified as near-isohydric (Grenache) and near-anisohydric (Syrah). Under water stress, Grenache exhibited stronger adjustments of plant and root hydraulic conductances and greater stomatal sensitivity to [ABA]$_{xylemsap}$ than Syrah resulting in greater conservation of soil moisture but not necessarily more isohydric behavior. Correlations between leaf (Ψ_{leaf}) and predawn (Ψ_{PD}) water potentials between cultivars suggested a "hydrodynamic" behavior rather than a particular iso-anisohydric classification. A significant decrease of Ψ_{leaf} in well-watered ABA-fed vines supported a role of ABA in the soil-leaf hydraulic pathway to regulate g_s. Correlations between leaf and root AQPs expression levels under water deficit could explain the response of leaf (K_{leaf}) and root (Lp_r) hydraulic conductances in both cultivars. Additional studies under a wider range of soil water deficits are required to explore the possible differential regulation of g_s and plant hydraulics in different cultivars and experimental conditions.

Keywords: *Vitis vinifera*, gas exchange, aquaporin, plasma membrane intrinsic protein, tonoplast intrinsic protein, hydraulic conductivity, isohydric, isohydrodynamic

INTRODUCTION

To withstand abiotic stresses such as drought, plants have evolved complex adaptive mechanisms that are regulated dynamically. There is an interplay between stomatal regulation of transpiration (Chaves et al., 2010) and changes in the hydraulic conductivity of roots [Lp_r; (Maurel et al., 2010)], and leaves [K_{leaf}; Sack and Holbrook (2006)]. The transpiration rate of leaves, or water flux through

stomata, is regulated by stomatal conductance (g_s) and strongly influenced by vapor pressure deficit [VPD; Aphalo and Jarvis (1991); McAdam et al. (2016)] as well as changes in K_{leaf} (Pou et al., 2013), through changes of stomatal guard cell turgor. Changes in guard cell turgor involve complex and still debated mechanisms that are mediated by chemical and/or hydraulic signals (Comstock, 2002). It is well established that under soil and/or atmospheric water deficit (WD), i.e., soil drying or high VPD, respectively, abscisic acid (ABA), a plant growth regulator, is synthesized in the leaves of plants (McAdam et al., 2016), where it induces stomatal closure (Schroeder et al., 2001; Dodd, 2003, 2005). Initially it was thought that ABA acts as a long distance signal from roots to the shoot during abiotic stress. However, Christmann et al. (2007) demonstrated that signals other than ABA might be involved in this process and supporting the role of hydraulics in modulating stomatal responses to WD. Recently, a root peptide signal was discovered in Arabidopsis during soil moisture deficit, that could induce ABA synthesis in the leaves (Takahashi et al., 2018).

Even though ABA signaling is seen as the main pathway for stomatal regulation, chemical signals other than ABA have been proposed to contribute significantly (Christmann et al., 2007; Wilkinson et al., 2007) including the recently reported γ-aminobutyric acid [GABA; Mekonnen et al. (2016)]. Christmann et al. (2007) demonstrated in Arabidopsis thaliana that changes in turgor pressure of leaf mesophyll cells occurred within minutes of root-induced osmotic stress and elicited activation of ABA biosynthesis and signaling required for stomatal closure. These observations support a role of ABA in stomatal closure but call into question whether it acts as the sole primary-long distance signal of water stress.

Hydraulic mediation of stomatal closure has been observed in studies where large diurnal fluctuations of g_s and leaf water potential (Ψ_{leaf}) were observed without substantial changes in the soil water content (Salleo et al., 2000). The co-variation of g_s and Ψ_{leaf} has been interpreted as a mechanism to protect the plant from severe dehydration and consequently, xylem cavitation and loss of hydraulic conductivity (Tyree and Sperry, 1989). Other studies have suggested the presence of hydraulic signals based on positive correlations between K_{leaf} and g_s at a relatively constant Ψ_{leaf} (Nardini et al., 2001). Evidence for the involvement of a hydraulic root-to-shoot signal has been provided by experiments where wild-type tomato plants were grafted on ABA-deficient roots (Holbrook et al., 2002). Despite the inability of the mutant roots to produce increased amounts of ABA during WD, the stomata still showed the wild-type response to WD. Recent studies in grapevine suggested that g_s was regulated to a greater degree by hydraulic rather than chemical signals during the early phases of WD, while ABA seemed to have an additive effect involved in the long-term maintenance of stomatal closure under prolonged WD (Tombesi et al., 2015). According to these studies, the involvement of both hydraulic and chemical signals seems to be a more likely explanation in the regulation of g_s under WD.

Aquaporins (AQPs), cellular membrane-bound water channel proteins and members of the major intrinsic protein (MIP) family, have been shown to play a key role in the transcellular or radial flow of water in both leaves and roots (Steudle, 2000;

Chaumont and Tyerman, 2014). AQPs are known to be regulated by cytoplasmic pH, divalent cations, and phosphorylation (Tournaire-Roux et al., 2003; Tornroth-Horsefield et al., 2006; Li et al., 2015). In certain species, the transcellular path is a major contributor to K_{leaf} (Prado and Maurel, 2013). Rapid and reversible changes in K_{leaf} involving AQPs have been observed under fluctuating environmental conditions such as solar radiation (Prado et al., 2013), WD (Galmes et al., 2007), and in response to exogenous application of ABA (Shatil-Cohen et al., 2011; Pantin et al., 2013). For example, in grapevine, K_{leaf} decreased by about 30% under water stress concomitantly with a decrease of expression of some plasma membrane intrinsic protein (PIP) and tonoplast intrinsic protein (TIP) AQP isoforms (Pou et al., 2013). Furthermore, the same study found significant positive correlations between g_s, K_{leaf} and leaf AQP expression suggesting a contribution of AQPs in regulating the flow of water. In Arabidopsis, xylem-fed ABA reduced K_{leaf} by specifically decreasing the water permeability of vascular bundle sheath cells, putatively through inactivation of PIPs (Shatil-Cohen et al., 2011). In line with that study, Pantin et al. (2013) confirmed those observations and proposed a model in which ABA closes stomata via its already known chemical effect on guard cells (Tardieu and Simonneau, 1998) as well as via an indirect hydraulic action by decreasing leaf water permeability within vascular tissues (Pantin et al., 2013). According to these findings, the hydraulic signal induced by ABA may be an important component in the mechanisms used by different species to regulate g_s under WD.

In addition to g_s and K_{leaf} variations, responses to drought include changes in root hydraulic conductivity (Lp_r). In contrast to the commonly observed reduction in K_{leaf}, ABA application and WD usually have opposite effects on Lp_r: while water stress reduces Lp_r, most studies have reported increased Lp_r with ABA (Aroca et al., 2006; Thompson et al., 2007; Parent et al., 2009). The increase in Lp_r by ABA can be interpreted as a mechanism to improve the water supply to the shoot, helping to maintain the water continuum in the plant under soil or atmospheric WD (Kudoyarova et al., 2011; Pantin et al., 2013). Diurnal changes in Lp_r have been observed under well-watered (WW) conditions concomitantly with changes in shoot transpiration (Vandeleur et al., 2009). In general, these variations correlate with the transcript abundance of root AQPs suggesting that water transport across roots is regulated by AQPs to meet the transpirational demand of the shoots (Sakurai-Ishikawa et al., 2011; Laur and Hacke, 2013; Vandeleur et al., 2014). Accordingly, these studies support the hypothesis of shoot-to-root (chemical and/or hydraulic) signaling via the xylem that regulates Lp_r in response to E that is modulated by AQPs (Vandeleur et al., 2014). Positive correlations between Lp_r, g_s, and E have also been observed in grapevines exposed to exogenous ABA applications through the soil suggesting a connection between ABA-mediated root and leaf conductances (DeGaris, 2016).

The hydraulic and chemical (ABA-mediated) mechanisms described above that operate between roots and leaves to control g_s may be important in understanding the contrasting behaviors described previously in the literature, namely "isohydry" or "anisohydry" for various species and even cultivars (varieties

or sub-species) to cope with WD (Pantin et al., 2013). Near-isohydric plants have been reported to maintain Ψ_{leaf} relatively constant under declining soil moisture availability through a tight regulation of g_s (Tardieu and Simonneau, 1998). This behavior has been reported to confer an advantage of increased drought tolerance (Schultz, 2003) and is thought to be under hydraulic and chemical (ABA) control (Tardieu and Simonneau, 1998). In contrast, it has been suggested that near-anisohydric plants maintain g_s to prioritize photosynthesis, which is related to a more variable Ψ_{leaf} (Tardieu and Simonneau, 1998). This behavior has been previously reported to operate under chemical control (Tardieu et al., 1996).

The present study aimed to elucidate how the hydraulic behavior, gas exchange and aquaporin expression in two grapevine cultivars previously classified as near-isohydric (Grenache) and near-anisohydric (Syrah) (Schultz, 2003) differed in their responses to a mild WD, recovery from WD, and exogenous ABA application to roots. We hypothesized that under mild WD or exogenous ABA application, the more "isohydric" Grenache would decrease Lp_r concomitantly with g_s to maintain homeostasis of Ψ_{leaf} mediated by a down-regulation of leaf and root AQPs. In contrast, the relatively anisohydric Syrah would maintain Lp_r under mild WD through an up-regulation of root AQPs in order to maintain homeostasis of g_s, but Ψ_{leaf} is expected to decrease.

MATERIALS AND METHODS

Experimental Site and Plant Material

The experiments were carried out in 2015 and 2016 at The Plant Accelerator®, The University of Adelaide, Waite Campus located in Urrbrae (Adelaide), South Australia (34° 58′ 17″ S, 138° 38′ 23″ E). One-year-old rootlings of own rooted grapevines (*Vitis vinifera* L.) cvs. Grenache and Syrah were planted in 4.5 L pots containing a mixture of 50% vermiculite and perlite and 50% of UC soil mix (61.5 L sand, 38.5 L peat moss, 50 g calcium hydroxide, 90 g calcium carbonate) and 100 g Nitrophoska® (12:5:14, N:P:K plus trace elements; Incitec Pivot Fertilisers, Southbank, Vic., Australia) per 100 L at pH 6.8. Plants were grown for 2 months in a temperature-controlled glasshouse (day/night: approx. 25/20°C) and irrigated to field capacity every 3 days from December 21st 2015. The vines were pruned to two shoots 10 days after bud burst (January 10th, 2016) and oriented upright during their development using wooden stakes. A liquid soil fertilizer, Megamix (13:10:15 N:P:K plus trace elements; Rutec, Tamworth, NSW, Australia), was applied at a concentration of 1.6 mL L^{-1} to bring all plants to approximately equal size. The fertilizer was applied weekly for 3 weeks once the plants had developed the first adult leaves. On the 25th February 2016, all vines were moved from the greenhouse and transferred to a DroughtSpotter (Phenospex, Netherlands) automated gravimetric watering platform where individual pots were automatically weighed continuously (15 min intervals) and watered twice daily (0600 h, 1600 h) based on the weight loss by transpiration. All plants were irrigated to their field capacity mass (determined the previous days) daily until the start

of the experiment. Day (16 h) and night (8 h) temperatures in the DroughtSpotter glasshouse were kept at 25/20°C, respectively.

Treatments

Grenache and Syrah vines were used to examine the effects of WD and recovery by re-watering (REC) on g_s and root hydraulic conductivity (Lp_r; normalized to root dry weight). A set of vines were kept as control (well-watered; WW), irrigated to field capacity by weight to replace the amount of water consumed by transpiration daily. WD was imposed starting on Day 4 by reducing the amount of irrigation until a moderate WD (target $g_s \sim 50$ mmol H_2O m^{-2} s^{-1}) was reached on Day 5 (Medrano et al., 2002). After 3 days of WD at approx. the target g_s, on Day 8, the vines were rehydrated by irrigating the pots to field capacity and recovery from water stress was examined after 7 days (Day 14). An additional treatment consisting of an exogenous application of ABA was simultaneously carried out on a separate set of vines from both cultivars. In this treatment, the vines were root-fed by applying 50 µM of ABA (Valent Biosciences Corporation, Libertyville, IL, United States) daily to the root system concurrently with irrigation. This concentration of ABA was based on prior experiments, which showed that 50 µM of ABA applied to the root system of potted vines is required to have a significant effect on g_s (DeGaris, 2016). All the pots were covered with a thick layer of perlite to minimize soil water loss through evaporation.

On the night before each measurement day, due to space constraints in the DroughtSpotter glasshouse, randomly selected vines from each treatment were moved from the DroughtSpotter glasshouse to an adjacent larger glasshouse that was at the same environmental conditions for physiological measurements and tissue sampling for gene expression analysis. The three measurement days were: (i) Day 5 for the WW and WD vines, when the WD vines reached a target g_s of between 50 and 100 mmol H_2O m^{-2} s^{-1}; (ii) Day 7 for the WW, WD, and ABA-treated vines; and, (iii) Day 14 on the WW and REC vines, 7 days after rewatering commenced in the REC vines. At each time point, three to five WW vines were used as controls to compare against the specific treatment(s).

Physiological Measurements

Leaf Gas Exchange

In order to track g_s, daily measurements of g_s were performed in all vines on the DroughtSpotter platform on fully expanded leaves (estimated minimum leaf age: Leaf Plastochron Index, LPI > 10) in the basal section of the shoots. These daily measurements were performed mid-morning (1030–1130 h) daily on two fully expanded and mature leaves per vine with a porometer (SC-1, Decagon Devices, Pullman, WA, United States). In an earlier characterization of the performance of the porometer, we observed a strong correlation of g_s measured by the porometer and g_s measured using an open system infrared gas analyzer (LI-COR, Model 6400XT).

On the specific sampling dates, g_s and E were measured concomitantly with other physiological measurements between 1000 h and 1100 h. Measurements were performed on two fully expanded, healthy leaves using an open system infrared gas

analyzer (IRGA; LI-6400XT, LI-COR Biosciences Inc., Lincoln, NE, United States) with a 6 cm^2 cuvette. An external LED light source (LI-6400-02B) attached to the cuvette was used at a fixed PAR value of 1500 μmol m^{-2} s^{-1} due to the non-saturating light levels in the glasshouse for photosynthesis (approx. 200 μmol m^{-2} s^{-1}). After gas exchange measurements were performed, the same leaf was excised to determine Ψ_{leaf}.

Leaf Water Potential and Sap Collection for ABA Analysis

Predawn, leaf and stem water potentials were measured on mature, primary leaves of vines on the specific sampling dates. Predawn leaf water potential (Ψ_{PD}) was measured before sunrise (0400–0500 h), and leaf (Ψ_{leaf}) and stem (Ψ_{stem}) water potentials around midday (1100–1200 h). Ψ_{stem} was measured after leaves equilibrated in aluminum foil and plastic bags for 2 h. One leaf per vine was measured from three to five vines per group using a Scholander-type pressure chamber (PMS Instruments Co., Albany, OR, United States).

After recording leaf water potential values, an overpressure of ∼0.5 MPa was applied to the encapsulated leaf for xylem sap collection (approx. 35 μL). The sap was collected from the cut surface of the protruding petiole using a micropipette and transferred to a pre-weighed and labeled micro tube before snap freezing in liquid nitrogen. Samples were stored at −80°C until subsequent analysis of ABA.

ABA Analysis of Xylem Sap

Abscisic acid concentration in xylem sap samples ($[ABA]_{xylemsap}$) was analyzed as described in Speirs et al. (2013). Briefly, the volume of each sample was measured using a pipette for normalization. Each sample was mixed with 30 μL of deuterated standard (Plant Biotechnology Institute, Saskatoon, SK, Canada) containing deuterium-labeled analogs of ABA, phaseic acid (PA), dihydrophaseic acid (DPA) and the glucose ester of ABA (ABA-GE) at a concentration of 100 ng mL^{-1} each. Solids were precipitated in a centrifuge at 12,470 × g for 5 min. From each sample, 20 μL supernatant was transferred to a LC/MS tube and analyzed by liquid chromatography/mass spectrometry (Agilent 6410 Triplequadropole LC-MS/MS with Agilent 1200 series HPLC, Agilent Technologies Inc., Santa Clara, CA, United States). A Phenomenex C18(2) column (75 mm × 4.5 mm × 5 μm; Phenomenex, Torrance, CA, United States) was used at 40°C and samples were eluted with a 15 min linear gradient of 10–90% acetonitrile. Nanopure water and acetonitrile were both mixed with 0.05% acetic acid. Compounds were identified by retention times and mass/charge ratio.

Hydraulic Conductance of Leaves, Plant and Roots

Leaf and whole plant hydraulic conductances (K_{leaf} and K_{plant}) were determined using the evaporative flux method (Sack et al., 2002; Flexas et al., 2013). This measurement is based on the relationship between the leaf transpiration rate (E) and the water potential gradient ($\Delta\Psi$) when leaf water potential (Ψ_{leaf}) reaches a steady state. In this case hydraulic conductance is calculated as follows: $K_{leaf} = E/(\Psi_{stem} - \Psi_{leaf})$ and $K_{plant} = E/(\Psi_{PD} - \Psi_{leaf})$. Our approach of estimating hydraulic conductances using measurements of E with an IRGA (leaf enclosed in a cuvette) rather than with a flowmeter or gravimetrically (on an unenclosed leaf) relies on the assumption that the leaf boundary layer resistance is negligible, which is addressed by adequate mixing of the air inside the cuvette.

The hydraulic conductance of the entire root system (Lp_r) was measured for the same plants using a High Pressure Flow Meter (HPFM; Dynamax, Houston, TX, United States) as previously described in Vandeleur et al. (2009). This is a destructive technique whereby the stem of the vine is cut above the soil surface, covered with filtered deionized water and the stump connected to the HPFM with a water-tight seal as quickly as possible, typically within 1 min. A transient ramp in pressure (up to 0.5 MPa at a rate of 7 kPa s^{-1}) with simultaneous recording of flow rate was used to calculate hydraulic conductance, which was normalized by dividing by total root dry weight to obtain Lp_r. Since the hydraulic conductance is inversely proportional to the viscosity of water, the measured conductance was temperature-corrected internally within the HPFM. All measurements were conducted within 5 min of shoot excision. The soil was washed from the roots before drying at 60°C for more than 48 h prior to weighing.

AQPs Expression in Roots and Leaves

Samples of leaves and roots were collected from vines immediately after physiological measurements for subsequent analysis of AQP transcript abundance by quantitative reverse transcription PCR (RT-qPCR). Leaves were immediately immersed in liquid nitrogen and stored at −80°C until analysis. Roots were carefully selected from the bottom and upper parts of the pot in order to get the thinner, white and more functional roots. The root samples were quickly washed to remove soil particles and dried with tissue paper before being submerged in liquid nitrogen.

Leaf and root material was ground to a fine powder in liquid nitrogen using a mortar and pestle. For leaves, total RNA was extracted from 100 mg of fine frozen powder using the Spectrum Plant Total RNA extraction Kit (Sigma-Aldrich, St. Louis, MO, United States). DNA contamination was avoided by digestion with the On-Column DNase I Digestion Set (Sigma-Aldrich, St. Louis, MO, United States) during RNA extraction according to manufacturer recommendations. For roots, RNA extractions were performed as described by Vandeleur et al. (2014). RNA was extracted from 200 mg of fine frozen powder with a 20 mL sodium perchlorate extraction buffer (5 M sodium perchlorate, 0.2 M Tris pH 8.3, 8.5% (w/v) polyvinylpolypyrrolidone, 2% PEG 6000, 1% (w/v) SDS, 1% (v/v) β-mercapto-ethanol) for 30 min at room temperature. The lysate was filtered through a glass wool filter and mixed with 30 mL of cold absolute ethanol before precipitation at −20°C overnight. After centrifugation at 3500 rpm for 20 min at 4°C, the pellets were washed with cold ethanol and purified using the Spectrum Plant

Total RNA Extraction Kit with on-column DNase digestion as described for leaves. Concentration and purity of total RNA were determined on a NanoDrop^{TM} 1000 Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, United States). Agarose gel electrophoresis (1.2% agarose) was done to visualize the integrity of RNA.

For cDNA synthesis, 1 μg of total RNA was reverse transcribed using iScript^{TM} cDNA Synthesis Kit for RT-qPCR (Bio-Rad, Hercules, CA, United States) as per the manufacturer's instructions.

Gene expression analysis was carried out by quantitative real time PCR (Bio-Rad iCycler iQ system; Bio-Rad) in a 20 μL mixture containing 1 μL of 1:10 diluted cDNA, 10 μL iQ SYBR Green Reaction-Mix (Bio-Rad), 0.6 μL of each primer, and 2.7 μL of DEPC water. Three biological and technical replicates were run for each sample. Thermal cycling conditions were as follows: one cycle of 30 s at 95°C followed by 40 cycles of 20 s at 95°C, 20 s at 59°C, and 20 s at 72°C. A previous standard quantification curve with five serial dilutions of cDNA was constructed for each gene to calculate amplification efficiency. The fluorescence threshold value (Ct) was calculated using the iCycle iQ system software (Bio-Rad). Overall, a mean Ct value was calculated from three independent biological replicates, each with three PCR replicates. Elongation factor (*ELF*), *GAPDH*, actin and ubiquitin were examined as possible reference genes across the treatments using the software tool NormFinder (Molecular Diagnostic Laboratory, Department of Molecular Medicine, Aarhus University Hospital, Skejby, Denmark; Andersen et al., 2004; Selim et al., 2012). Of these, ELF was the most stable and hence used for normalization. Gene expression was relative to mean of the control calculated as (E_{target}) DCT$_{target}$ (control–sample)/ (E_{ref}) DCT$_{ref}$ (control–sample) (Pfaffl, 2001), where E_{target} and E_{ref} are the efficiencies of the target gene and reference gene (ELF), respectively, determined from a dilution series. The absence of non-specific products was confirmed by both the analysis of the melt curves and by electrophoresis in 2% (w/v) agarose gel of the PCR product. The primer sequences used for the amplifications were designed by Vandeleur et al. (2009) based on published sequences of AQPs found in grapevine (**Supplementary Table S1**).

Statistical Analyses

All statistical analyses and preparation of figures were performed in the statistical language R (R Core Team). To compare differences between treatments (WW, WD, ABA, REC) and cultivars (Grenache, Syrah), linear models of the form $Y \sim Treatment \times Cultivar$ were fitted to the data and analysis of variance (ANOVA) was performed to test for general differences among groups. For pairwise comparisons, estimated marginal means were obtained using the R package "emmeans" (Lenth, 2019) and *p*-values were adjusted based on the multivariate *t* distribution (adjust = "mvt"). Significant differences ($P \leq 0.05$) are indicated by different letters in the figures and described in the text. Both linear and non-linear models were fitted to the data for correlations between two continuous variables. Comparisons of two

linear regression models were performed by analysis of covariance (ANCOVA).

RESULTS

Water Relations of a Mild Water Deficit and ABA Watered Vines

A soil moisture deficit imposed on the two cultivars in this study, Syrah and Grenache, resulted in similar values of Ψ_{PD} on Day 5 for the WD vines (**Figure 1D**) and a range of Ψ_{PD} values from −0.2 to −0.5 MPa were measured in WD vines for Days 5 and 7 (**Figures 1D,E**). However, by Day 7, 3 days after the onset of stress, a significantly lower Ψ_{PD} was measured for WD vines of Syrah compared to Grenache (**Figure 1E**). This deficit resulted in the WD vines of Grenache showing a reduction in g_s on Day 5 while Syrah did not show this trend (**Figure 1A**). On Day 7, WD vines from both cultivars showed a similar reduction of g_s relative to WW plants after the deficit was sustained for 3 days (**Figure 1B**), however, this difference was only significant for Grenache. WD vines of both Grenache and Syrah showed a similar reduction in leaf water potential (Ψ_{leaf}) on Days 5 and 7 (**Figures 1G,H**). In comparing the two cultivars, the WD vines of Syrah had, on average, a lower Ψ_{leaf} compared to Grenache (**Figure 1H**). Syrah showed a trend toward higher vine water stress based on its lower Ψ_{leaf}.
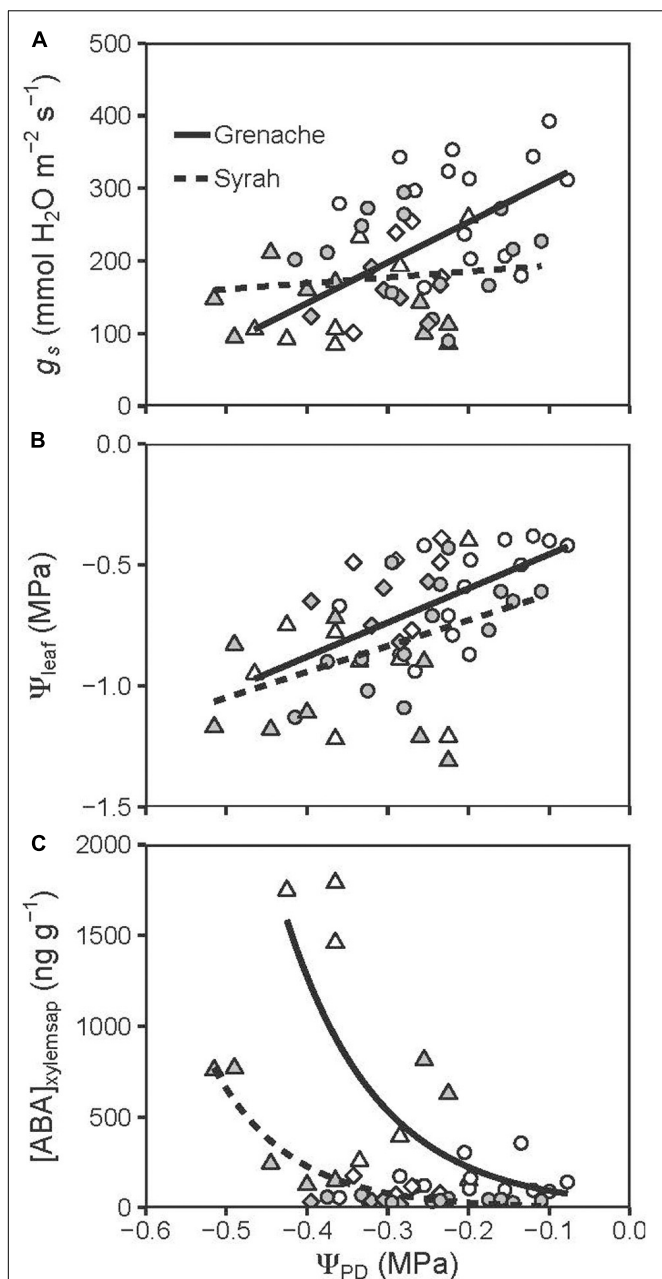
The ABA concentrations in the xylem sap were significantly higher in the WD vines from both cultivars relative to the controls, but Grenache presented a higher concentration than Syrah on Day 5 (**Figure 1J**). On Day 7, the average ABA concentration in the xylem sap was higher in WD and ABA treated vines compared to WW vines, but only ABA treated Grenache had a significantly higher [ABA]$_{xylemsap}$ concentration (**Figure 1K**). ABA-treated vines showed a significant reduction of g_s as compared to the controls as well as the WD vines (**Figure 1B**) despite adequate soil moisture and similar values of Ψ_{PD} than WW vines (**Figure 1E**). For either cultivar, ABA-treated vines had a similar Ψ_{leaf} to that of WD vines (**Figure 1H**). When WD vines were recovered (REC) for 7 days, they showed no significant differences in vine water status or [ABA]$_{xylemsap}$ compared to the WW vines (**Figures 1C,F,I,L**).
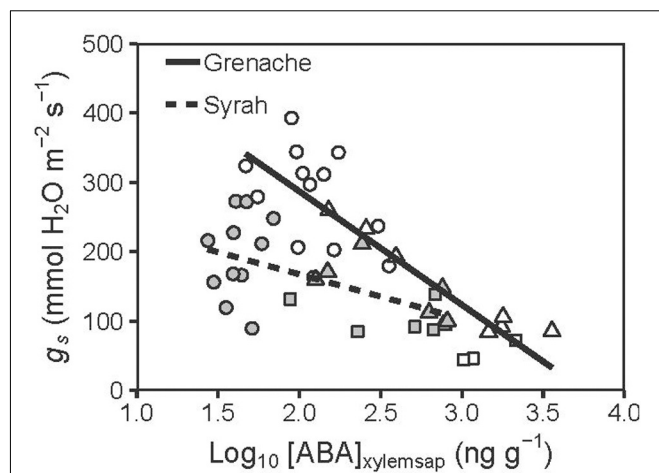
Correlations Between Physiological Variables

A closer examination between some physiological variables showed a significantly ($P = 0.017$) stronger response of g_s to changes in Ψ_{PD} in vines of Grenache compared to vines of Syrah, when Ψ_{PD} dropped from approx. −0.1 MPa to −0.5 MPa (**Figure 2A**). In contrast, Ψ_{leaf} declined similarly in response to decreasing Ψ_{PD} in both cultivars (**Figure 2B**). Different responses of stomata between cultivars were in line with differences observed for the relationship between [ABA]$_{xylemsap}$ and Ψ_{PD} (**Figure 2C**). Grenache had a steeper response to a decrease in the Ψ_{PD} than Syrah with a significant increase in
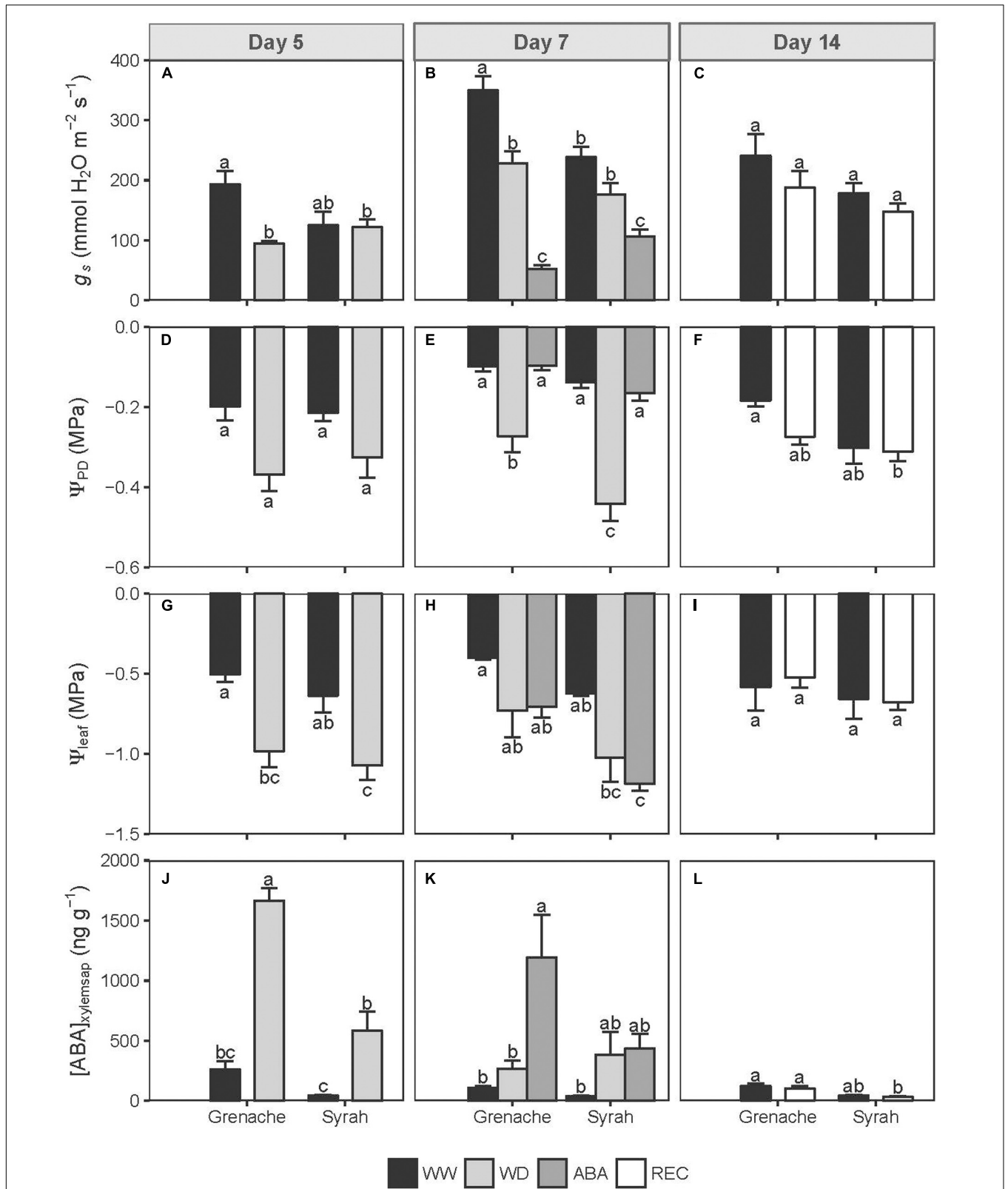
FIGURE 1 | Stomatal conductance (g_s; **A–C**), pre-dawn (Ψ_{PD}; **D–F**) and leaf (Ψ_{leaf}; **G–I**) water potentials and ABA concentration in the xylem sap ([ABA]$_{xylemsap}$; **J–L**) in Grenache and Syrah grapevines under mild water deficit (WD), exogenous application of abscisic acid (ABA) and recovery from water stress (REC) at different sampling days along the experiment. Values are means ± SE (n = 5). Different letters indicate statistically significant differences ($P \leq 0.05$) across all treatments and cultivars within the day.
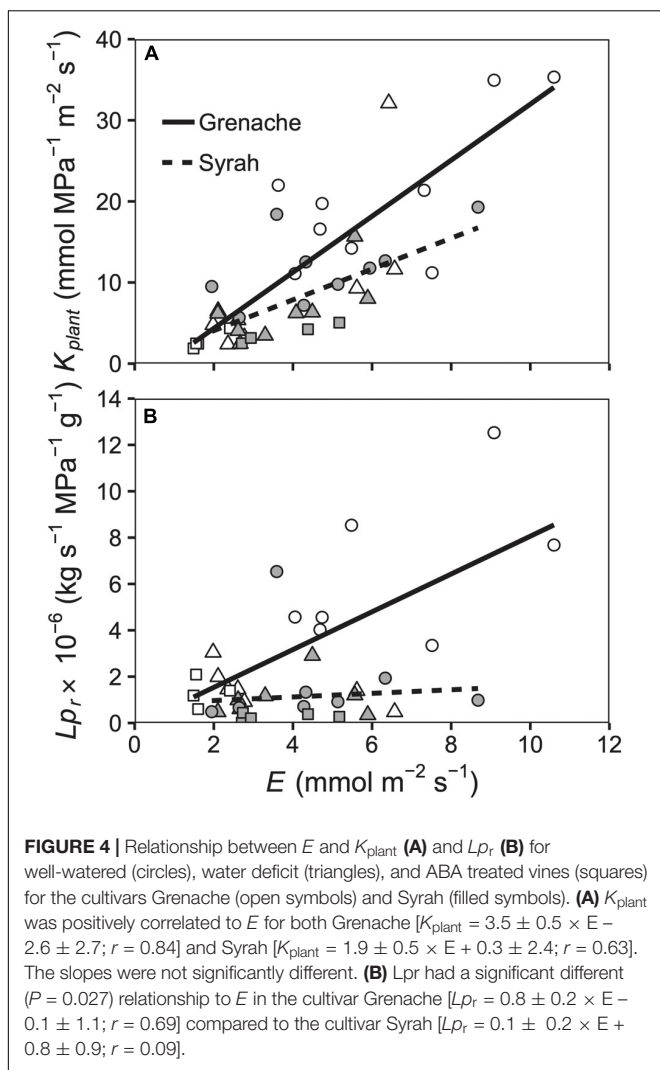
FIGURE 3 | Relationship between $[ABA]_{xylemsap}$ (Log_{10} transformed) and g_s measured for well-watered (circles), water deficit (triangles), and ABA treated vines (squares) from the cultivars Grenache (open symbols) and Syrah (filled symbols). Significant negative regressions were predicted [Grenache: $g_s = -163.4 \pm 20.9 \times \log_{10}([ABA]_{xylem}) + 613.6 \pm 53.1$; $r = -0.86$] and Syrah [$g_s = -63.9 \pm 19.6 \times \log_{10}([ABA]_{xylem}) + 295.2 \pm 42.7$; $r = -0.58$]. Both linear regressions had significantly different slopes ($P = 0.001$) and significantly different intercepts ($P < 0.001$).

FIGURE 2 | Correlation between Ψ_{PD} and gs **(A)**, Ψ_{leaf} **(B)**, and $[ABA]_{xylemsap}$ **(C)** for well-watered (circles), water deficit (triangles), and recovery from water stress vines (diamonds) from the cultivars Grenache (open symbols) and Syrah (filled symbols). **(A)** Grenache and Syrah show a significantly different ($P = 0.017$) relationship between Ψ_{PD} and g_s: in Grenache g_s is significantly positively correlated to Ψ_{PD} ($r = 0.58$, $P = 0.002$) while no correlation was found for Syrah ($r = 0.14$, $P = 0.487$). **(B)** For both cultivars Ψ_{leaf} is significantly positively correlated to Ψ_{PD} [Grenache: $r = 0.54$, $P = 0.004$; Syrah: $r = 0.44$, $P = 0.021$]. No significant differences were found for the slope ($P = 0.580$) and intercept ($P = 0.091$) of the linear regression lines [Grenache: Ψ_{leaf} (MPa) = $1.4 \pm 0.4 \times \Psi_{PD}$ (MPa) -0.3 ± 0.1; Syrah: Ψ_{leaf} (MPa) = $1.1 \pm 0.4 \times \Psi_{PD}$ (MPa) -0.5 ± 0.1]. **(C)** Non-linear regressions between Ψ_{PD} and $[ABA]_{xylemsap}$ for Grenache ($[ABA]_{xylemsap} = 39.1 \pm 60.4$ ng g^{-1} $\times$ exp[$-8.7 \pm \pm 4.3$ (1/MPa) $\times \Psi_{PD}$ (MPa)]) and Syrah ($[ABA]_{xylemsap} = 3.3 \pm 5.7$ ng g^{-1} $\times$ exp[$-10.5 \times \pm 3.5$ (1/MPa) $\times \Psi_{PD}$ (MPa)]).

$[ABA]_{xylem}$ values below Ψ_{PD} of -0.3 MPa. In contrast, Syrah had a lower and later increment of $[ABA]_{xylemsap}$ showing values lower than 1000 ng g^{-1} over the range of Ψ_{PD} values measured in this study. The ABA catabolites, PA and DPA, were found to increase in the xylem sap of ABA-treated and WD vines compared to WW vines, which was apparent as early as Day 5 (**Supplementary Figure S3**). These catabolites decreased in concentration upon recovery from water stress on Day 14 to levels below those of WW vines.

The two cultivars were compared for the relationship between g_s and $[ABA]_{xylemsap}$ in **Figure 3**. The decline in g_s with increasing $[ABA]_{xylemsap}$ for Syrah (slope: -63.9 ± 19.6 mmol H$_2$O m^{-2} s^{-1} ng^{-1} g) was significantly ($P = 0.001$) lower than that for Grenache (slope: -163.4 ± 20.9 mmol H$_2$O m^{-2} s^{-1} ng^{-1} g) indicating a higher stomatal sensitivity of Grenache to ABA compared to Syrah. Interestingly, vines that were treated with exogenous ABA (instead of WD) showed the same relationship between $[ABA]_{xylemsap}$ of leaves and g_s for both cultivars; these data are included as square symbols in **Figure 3**.

In comparing leaf and root hydraulics, both K_{plant} and Lp_r decreased in parallel with decreased transpiration (E), but there were significant differences between Grenache and Syrah when considering Lp_r only (**Figure 4**). As compared to whole plant hydraulic conductance (K_{plant}), Lp_r of Grenache changed significantly in response to different levels of E compared to vines from the cultivar Syrah (**Figure 4B**). It should be noted that K_{plant} was determined using values of E obtained from an IRGA and therefore expected to show a strong relationship with E as observed in **Figure 4A**.

FIGURE 4 | Relationship between E and K_{plant} **(A)** and Lp_r **(B)** for well-watered (circles), water deficit (triangles), and ABA treated vines (squares) for the cultivars Grenache (open symbols) and Syrah (filled symbols). **(A)** K_{plant} was positively correlated to E for both Grenache [$K_{plant} = 3.5 \pm 0.5 \times E - 2.6 \pm 2.7$; $r = 0.84$] and Syrah [$K_{plant} = 1.9 \pm 0.5 \times E + 0.3 \pm 2.4$; $r = 0.63$]. The slopes were not significantly different. **(B)** Lpr had a significant different ($P = 0.027$) relationship to E in the cultivar Grenache [$Lp_r = 0.8 \pm 0.2 \times E - 0.1 \pm 1.1$; $r = 0.69$] compared to the cultivar Syrah [$Lp_r = 0.1 \pm 0.2 \times E + 0.8 \pm 0.9$; $r = 0.09$].

Leaf hydraulic conductance (K_{leaf}) was observed to decrease significantly in Grenache, but not in Syrah, in response to exogenous ABA application through the soil water (**Supplementary Figure S4**). Withholding water from the soil did not significantly lower K_{leaf} in either cultivar.

On Day 5, leaf hydraulic conductance (K_{leaf}) was observed to decrease in WD Grenache vines, but not in Syrah, however, these differences were not statistically significant, likely due to the small sample number and scatter in the data (**Figure 5A** and **Supplementary Figure S4**). Similarly, root hydraulic conductance (Lp_r) decreased significantly in the WD vines compared to the control vines in Grenache, but not in Syrah (**Figure 5B**).

Do Expression Levels of Certain AQPs Correlate With Changes in Hydraulic Conductance?

Several AQPs in the leaves and roots of both cultivars were analyzed by qPCR. These included: *PIP1;1*, *PIP2;1*, *PIP2;2*, *PIP2;3*, *TIP1;1*, and *TIP2;1*. Of these AQPs, only *PIP1;1* (**Figures 5C,D**),

PIP2;1 (**Figures 5E,F**), and *TIP2;1* (**Figures 5G,H**) showed differences between the treatments so are discussed in this paper. The decrease in K_{leaf} observed on Day 5 in Grenache compared to Syrah was associated with some of the AQPs expressed in the leaves. For instance, the expression of *PIP1;1* in leaves was down-regulated under WD conditions in Grenache, but not in Syrah (**Figure 5C**). In the roots, the decrease in Lp_r observed in Grenache under WD was not related to changes in AQPs levels. In contrast, *PIP2;1* was up-regulated in Syrah without any changes in Lp_r (**Figure 5F**). AQPs were also analyzed in ABA-treated grapevines, however, no differences were found between WW and ABA vines in any of the AQPs evaluated.

DISCUSSION

The traditionally so-called isohydric behavior of plants has been characterized by a limited decline in Ψ_{leaf} by the closure of stomata under WD, while anisohydric behavior has been characterized by maintenance of high g_s and consequently a greater reduction in Ψ_{leaf}. There is much interest in these different behaviors since agriculturally important plants with different degrees of stomatal regulation can have very different water demands under certain environmental conditions and therefore require different irrigation management strategies. However, the hydraulic and gas exchange properties of plants that confer either near-isohydry or anisohydry are still relatively poorly understood and controversies found in recent studies have argued that stomatal regulation is a response to the plant-environment interaction rather than a genetically determined trait (Hochberg et al., 2017; Charrier et al., 2018).

In this study, we investigated specific mechanisms that may explain the differences in iso/anisohydry, in particular, root hydraulics, leaf gas exchange, leaf and root AQP expression, and xylem ABA. We used two *V. vinifera* cultivars, Grenache and Syrah, which have previously been shown to exemplify the differences in isohydry (Schultz, 2003). Our choice of cultivars, Grenache and Syrah, have been used as models for near-isohydric and anisohydric classification, respectively, in several studies (Schultz, 2003; Soar et al., 2006; Prieto et al., 2010; Coupel-Ledru et al., 2014, 2017; Gerzon et al., 2015; Scharwies and Tyerman, 2016; Shelden et al., 2017).

To impose water stress on the vines, our experiment aimed for a target g_s of 50 mmol H_2O m^{-2} s^{-1} as this represents a moderately severe degree of plant water stress based on downregulation of net photosynthesis and electron transport rate as reported in Medrano et al. (2002). We acknowledge that this approach has limitations including the possibility of different perceptions of stress (at this g_s level) by different genotypes. There are similar drawbacks using soil moisture thresholds for drought stress experiments as different genotypes would likely have different plant responses, e.g., hydraulic and chemical signaling, to any level of soil moisture.
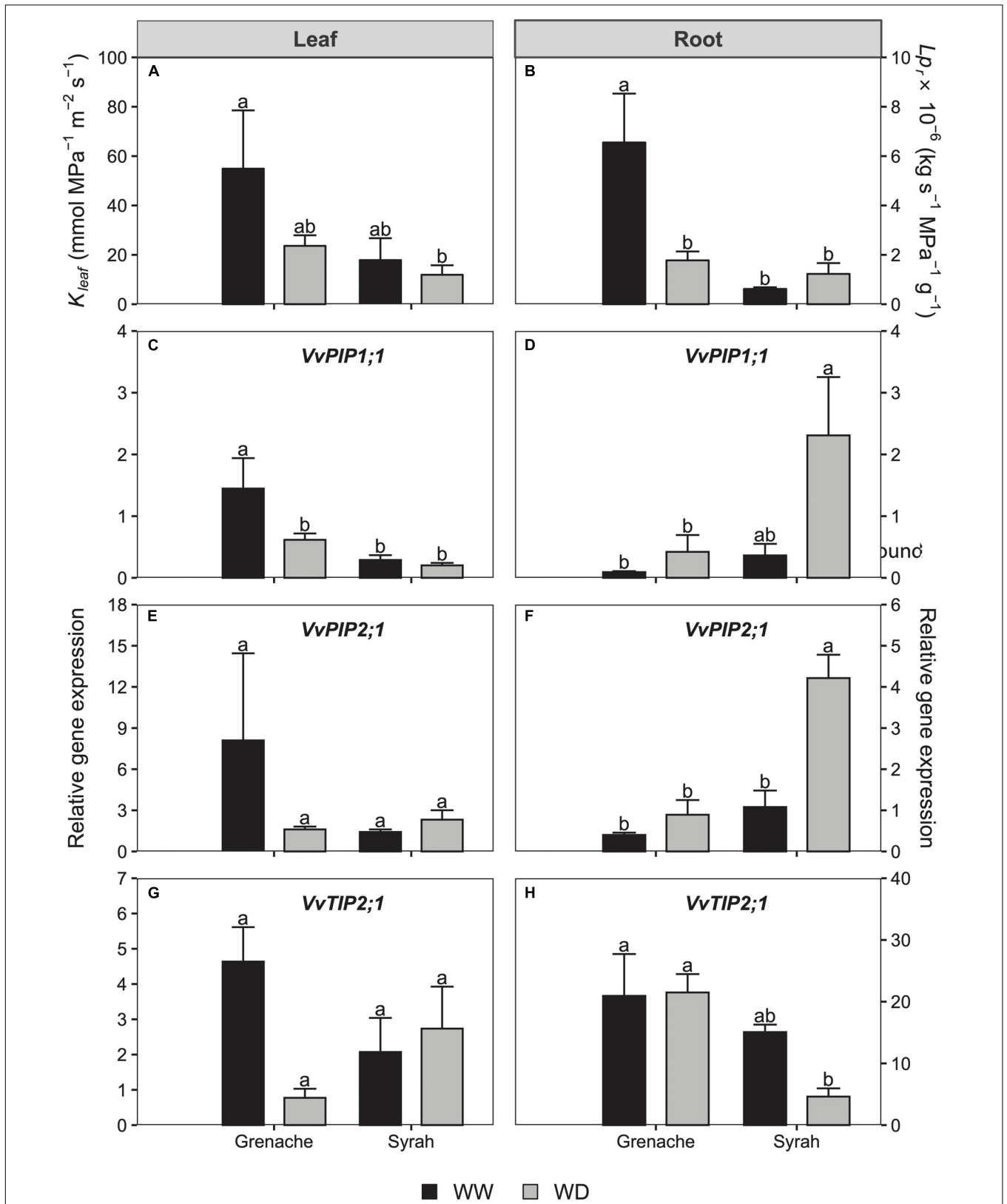
FIGURE 5 | Leaf (K_{leaf}; **A**) and root (Lp_r; **B**) hydraulic conductances and gene relative expression of *PIP1;1*, *PIP2;1,* and *TIP2;1* in the leaf **(C,E,G)** and roots **(D,F,H)** in Grenache and Syrah grapevines under well-watered (WW) and mild water deficit (WD) at Day 5 of the experiment. Values are means ± SE (*n* = 3). Different letters indicate statistically significant differences (*P* ≤ 0.05) across both treatments and cultivars.

Stomatal Sensitivity to ABA

In the present study, water-stressed Syrah vines presented a significantly lower Ψ_{PD} and Ψ_{leaf} on Day 7 of the experiment compared to Grenache that would indicate a more anisohydric behavior. However, the response of g_s to WD was only different between cultivars on Day 5 when Grenache showed an earlier reduction of g_s to WD than Syrah, indicating a more isohydric behavior, as expected (**Figure 2A**). However, this level of stomatal closure in Grenache was insufficient to maintain a homeostasis of Ψ_{leaf} as might be expected with isohydric behavior. It is important to note that only a mild WD was imposed in this experiment with Ψ_{PD} values not lower than -0.6 MPa and that measurements were performed on different days and thus different environmental conditions (e.g., VPD; **Supplementary Figure S1**). In this regard, recent studies have suggested that the iso/anisohydric classification is a continuum behavior rather than a genetic trait possessed by a cultivar and that it depends on the level of drought imposed and prevailing VPD conditions (Charrier et al., 2018). In that study, the authors observed in four different cultivars a threshold of Ψ_{PD} (~ -0.7 MPa) at which the stomata become more sensitive to VPD, and this response was similar for all cultivars. In our study, we did not achieve that Ψ_{PD} threshold so it is possible that the g_s response to Ψ_{PD} observed here might not account for small differences in VPD between different days (**Supplementary Figure S1**).

Despite this, the mild WD imposed here reveals differences in some physiological variables among cultivars, but not necessarily a more isohydric behavior. For instance, a higher sensitivity of g_s to changes in Ψ_{PD} and ABA was observed in Grenache compared to Syrah (**Figures 2A, 3**, respectively). In addition, higher $[ABA]_{xylemsap}$ was produced by Grenache leaves at decreasing water potentials than Syrah indicating a higher sensitivity of this cultivar to changes in soil water potential as well as a greater reliance on chemical signaling from roots to shoots (**Figure 2C**). These results are in agreement with other studies confirming a higher sensitivity of K_{leaf} to ABA fed via the petiole in Grenache compared to Syrah (Coupel-Ledru et al., 2017). However, Tardieu and Simonneau (1998) suggested that an isohydric behavior could be related to stomatal sensitivity to ABA being modulated by Ψ_{leaf} or E, i.e., an indirect response of g_s to ABA (e.g., via leaf AQPs), while anisohydric behavior could be due to a direct response of g_s to ABA.

Our observations of the ABA response on g_s was as expected: g_s decreased markedly on Day 7 in both cultivars in ABA-fed vines compared to WW vines (**Figure 1B**). Interestingly, we also measured a significant decrease of Ψ_{leaf} in Syrah, but not in Grenache, in response to ABA feeding (**Figure 1H**), which suggests a hydraulic involvement, i.e., water potential and/or hydraulic conductance, in Syrah. This potential role of ABA in stomatal regulation via hydraulics rather than directly as a chemical signal has been previously mentioned in Arabidopsis (Pantin et al., 2013). The down-regulation of several AQPs in response to ABA and the decrease of hydraulic conductance in

the roots, as shown in **Figure 4B** (square symbols), could be associated with this response.

Based on the assumption that ABA acted directly or indirectly on g_s, differences in the response of g_s to WD observed in the two cultivars may be due to Grenache producing more ABA or Syrah having a higher rate of ABA catabolism. The maximum ABA concentration in the xylem sap of leaves measured during the experiment in all treatments was higher in Grenache compared to Syrah (**Figure 1**). Rossdeutsch et al. (2016) found that the average ABA concentration in the xylem sap of Grenache shoots during drought stress was similar to Syrah shoots, however, the same authors measured significantly higher concentrations of DPA, a degradation product of ABA, in Syrah compared to Grenache. This may indicate that Syrah has a higher catabolism of ABA, which may lower its hypersensitivity to WD relative to Grenache, and results of the present study support this hypothesis. We observed that, on Day 7, water-stressed Syrah vines had nearly three-fold higher DPA concentration in the xylem sap of leaves compared to WW vines, similar to the difference of DPA between WW and WD Grenache vines on Day 5 (**Supplementary Figure S3**). The higher DPA levels suggest greater cumulative water stress in Syrah compared to Grenache as ABA catabolism results in accumulation of DPA and PA over time (Cutler and Krochko, 1999). ABA could also act indirectly on Ψ_{leaf} and decrease its value by decreasing K_{leaf}, which has been proposed by Shatil-Cohen et al. (2011). This putative reduction in K_{leaf} occurs as water flow through the symplastic (cell-to-cell) pathway via leaf bundle sheath cells decreases. Future research could aim to test the stomatal sensitivity to ABA in Grenache and Syrah at different and lower Ψ_{leaf} values to verify the hypothesis by Tardieu and Simonneau (1998).

Hydraulic Response to Mild WD: Any Divergent Strategy?

Regulation of hydraulic conductivities in roots, stems and leaves has been used previously to determine the iso-anisohydric cultivar behavior. Schultz (2003) suggested differences in K_{leaf} between Grenache and Syrah could be the origin of their isohydric and anisohydric behavior, respectively. In the present study, K_{leaf} was higher in Grenache which could be explained by its larger xylem vessels (Gerzon et al., 2015; Shelden et al., 2017) as compared to Syrah, however, xylem vessel sizes were not measured in this study. Different correlations between gas exchange parameters and hydraulic conductances between cultivars suggest that the hydraulic pathway in some plants could be more adaptive to changes in E, which is an important consideration since physiological adaptations of the hydraulic pathway are expected to occur over longer time scales than the evolution of water stress in the present study. For instance, significant differences in the response of Lp_r to changes in E separated the two cultivars (**Figure 4B**). This is an important new finding since little is known about root hydraulics in characterizing cultivars with expected contrasting behaviors (Lovisolo et al., 2010). Distinct differences in Lp_r have previously been linked to different expression patterns of AQPs hypothesized to result from a xylem-mediated hydraulic

signal, possibly from shoots to roots (Vandeleur et al., 2014). The linear regressions between E and Lp_r calculated in this study for Grenache were similar to the linear regressions shown by Vandeleur et al. (2009). Interestingly, Coupel-Ledru et al. (2017) found that K_{leaf} of detached leaves fed with solutions of different ABA concentrations decreased with increasing ABA concentration in Grenache, but no change in K_{leaf} was observed in Syrah. This response is similar to what we observed in Grenache leaves (**Supplementary Figure S4**), as well as roots which showed a stronger response than Syrah to changes in E (**Figure 4B**). Evidence of decreased K_{leaf} in response to ABA in Grenache in the present trial supports the finding that Ψ_{leaf} was similar between the WD vines of the two cultivars (**Figure 1H**) despite significantly different Ψ_{PD} (**Figure 1D**). This suggests that ABA had the effect of increasing the leaf hydraulic resistance or decreasing K_{leaf}. This decrease in K_{leaf} is not unexpected as it was determined using an IRGA via measurements of E. Future experiments could confirm this finding using independent measures of K_{leaf} for example with

the evaporative flux (gravimetric or flow-based) or rehydration kinetics methods (Flexas et al., 2013).

Simonin et al. (2015) suggested that a positive correlation between E and K_{leaf} could help to stabilize the gradient between Ψ_{stem} and Ψ_{leaf} for hydraulic transport to the leaf. This would cause less variation in Ψ_{leaf}, which is a feature of isohydric behavior to maximize g_s and, therefore, CO_2 uptake for photosynthesis. At the whole plant level, our results showed no significant changes in the gradient between Ψ_{PD} and Ψ_{leaf} (i.e., $\Delta\Psi_{plant}$) in relation to changes in E for both cultivars despite differences in hydraulic regulation (**Supplementary Figure S2**), a behavior termed "isohydrodynamic" by Franks et al. (2007). Furthermore, considering the correlation between Ψ_{PD} and Ψ_{leaf} found in our study, both cultivars should be classified as relatively anisohydric as the slope of the relationship between Ψ_{PD} and E was not different (**Figure 2B**). However, g_s was differentially regulated, as shown by the association between g_s and Ψ_{PD}, where Grenache had a steeper slope than Syrah (**Figure 2A**). Given that the root and plant
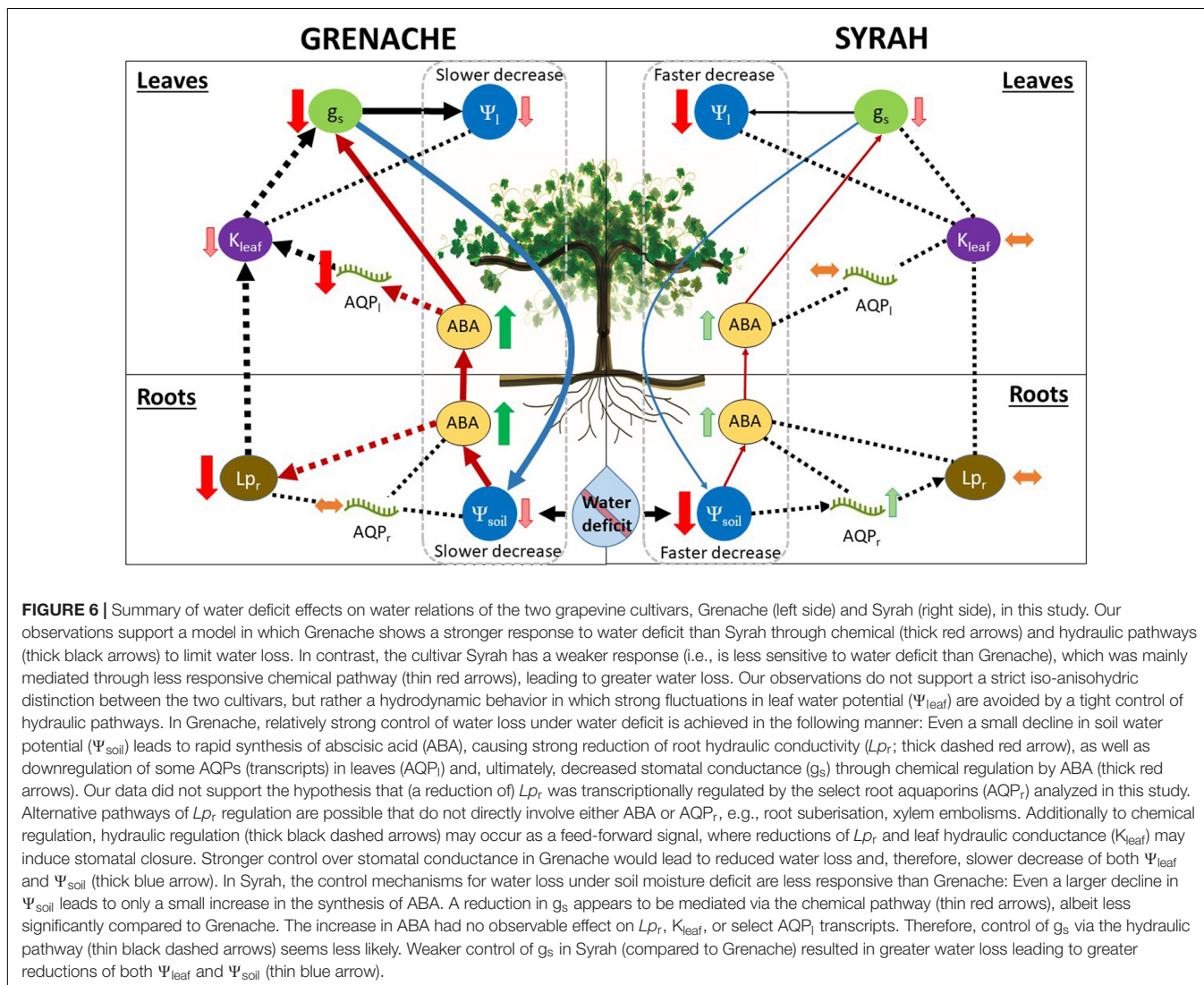
FIGURE 6 | Summary of water deficit effects on water relations of the two grapevine cultivars, Grenache (left side) and Syrah (right side), in this study. Our observations support a model in which Grenache shows a stronger response to water deficit than Syrah through chemical (thick red arrows) and hydraulic pathways (thick black arrows) to limit water loss. In contrast, the cultivar Syrah has a weaker response (i.e., is less sensitive to water deficit than Grenache), which was mainly mediated through less responsive chemical pathway (thin red arrows), leading to greater water loss. Our observations do not support a strict iso-anisohydric distinction between the two cultivars, but rather a hydrodynamic behavior in which strong fluctuations in leaf water potential (Ψ_{leaf}) are avoided by a tight control of hydraulic pathways. In Grenache, relatively strong control of water loss under water deficit is achieved in the following manner: Even a small decline in soil water potential (Ψ_{soil}) leads to rapid synthesis of abscisic acid (ABA), causing strong reduction of root hydraulic conductivity (Lp_r; thick dashed red arrow), as well as downregulation of some AQPs (transcripts) in leaves (AQP$_l$) and, ultimately, decreased stomatal conductance (g_s) through chemical regulation by ABA (thick red arrows). Our data did not support the hypothesis that (a reduction of) Lp_r was transcriptionally regulated by the select root aquaporins (AQP$_r$) analyzed in this study. Alternative pathways of Lp_r regulation are possible that do not directly involve either ABA or AQP$_r$, e.g., root suberisation, xylem embolisms. Additionally to chemical regulation, hydraulic regulation (thick black dashed arrows) may occur as a feed-forward signal, where reductions of Lp_r and leaf hydraulic conductance (K_{leaf}) may induce stomatal closure. Stronger control over stomatal conductance in Grenache would lead to reduced water loss and, therefore, slower decrease of both Ψ_{leaf} and Ψ_{soil} (thick blue arrow). In Syrah, the control mechanisms for water loss under soil moisture deficit are less responsive than Grenache: Even a larger decline in Ψ_{soil} leads to only a small increase in the synthesis of ABA. A reduction in g_s appears to be mediated via the chemical pathway (thin red arrows), albeit less significantly compared to Grenache. The increase in ABA had no observable effect on Lp_r, K_{leaf}, or select AQP$_l$ transcripts. Therefore, control of g_s via the hydraulic pathway (thin black dashed arrows) seems less likely. Weaker control of g_s in Syrah (compared to Grenache) resulted in greater water loss leading to greater reductions of both Ψ_{leaf} and Ψ_{soil} (thin blue arrow).

hydraulic conductances in Grenache were closely correlated to g_s, it is possible to consider an isohydrodynamic behavior in Grenache in which a strong stomatal control maintains relatively constant internal water potential gradients but, at the same time allows Ψ_{leaf} to fluctuate in synchrony with Ψ_{PD}. This new perspective of water transport regulation in plants was examined in a study where a theoretical framework based on the relationship between midday and predawn leaf water potentials was used to characterize plant responses to drought (Martínez-Vilalta et al., 2014). The continuum between isohydry and anisohydry requires further exploration, and their functional significance and mechanism remains under debate.

Regulation of Hydraulic Conductance by AQPs Under Mild WD

Aquaporins play a major role in the regulation of hydraulic conductance at the cellular level. As suggested by Vandeleur et al. (2014), AQPs are likely involved in the regulation of Lp_r in response to changes in E, which was explained by shoot-to-root signaling. Pou et al. (2013) found in grapevine that the expression of $TIP2;1$ in the leaf was well-correlated to changes in g_s during drought and rehydration. In Touriga Nacional grapevines, Zarrouk et al. (2015) found that specific AQPs were downregulated in the roots of water stressed vines concurrent with decreases in Lp_r, whereas the opposite trend was observed in the leaves: an upregulation of specific AQPs without changes in K_{leaf}. In the present study, three of the AQPs examined ($PIP1;1$, $PIP2;1$, $TIP2;1$) were correlated with K_{leaf} in both cultivars: they were down-regulated under WD conditions in Grenache and no changes were observed for Syrah following a similar response of K_{leaf} in each cultivar (**Figures 5C,E,G**). This difference between Grenache and Syrah could explain different responses in hydraulic conductance to changes in E. Therefore, stronger leaf hydraulic control in Grenache could be explained by stronger regulation of AQP transcripts. Previous studies have observed an overexpression of $SlTIP2;2$ in tomato that resulted in higher E in the transgenic plants (Sade et al., 2009). According to the research of Shatil-Cohen et al. (2011) and Pantin et al. (2013), AQPs modulate K_{leaf}, e.g., via the bundle sheath-mesophyll-continuum, which would send a feed-forward signal to stomata. Hence, two different hypotheses exist for the relationship between E and hydraulic conductance. Either (i) changes in E affect hydraulic conductance through the function of AQPs, or, (ii) a hydraulic feed-forward signal mediated by AQPs affects g_s. In the first case, a differential stomatal response could require alternative hydraulic regulation via AQPs, which would be the case in Grenache in the present study. In the second case, alternative hydraulic regulation by AQPs, potentially via ABA, could affect the differential stomatal behavior through feed-forward signaling to stomata. At the root level, a different situation was observed for both cultivars as the decrease in Lp_r under WD for Grenache was not followed by changes in root AQP expression levels indicating that such Lp_r reduction was mainly produced via the apoplastic pathway. In contrast, in Syrah, an up-regulation of

the root AQPs $PIP1;1$ and $PIP2;1$ was observed despite constant Lp_r suggesting a contribution of AQPs to water transport via the symplastic pathway under WD conditions. This observation is in agreement with the findings of Vandeleur et al. (2014) who observed a contribution of AQPs at the root level in Chardonnay, but not in Grenache, after a WD was imposed. A summary of our findings is provided in the schematic below (**Figure 6**). As the expression of AQPs has been shown to be highly variable depending on environmental and experimental conditions, the involvement of these water channel proteins in the regulation of stomata under drought remains unclear requiring further investigation.

CONCLUSION

A mild WD ($\Psi_{PD} \sim -0.6$ MPa) imposed on two grapevine cultivars showed a stronger reduction of g_s in Grenache as compared to Syrah. This response was supported by the higher sensitivity of Grenache to ABA concentration in the xylem sap. Despite this, these observations do not necessarily indicate a more isohydric behavior in Grenache than in Syrah since (i) the correlation between Ψ_{PD} and Ψ_{leaf} was similar for both cultivars; and, (ii) the responses observed under the range of Ψ_{PD} examined here might not apply under a more severe WD. In Grenache, the plant (K_{plant}) and particularly the root (Lp_r) hydraulic conductances showed a stronger response to the transpiration rate (E) than Syrah indicating potentially higher leaf-to-root communication in this cultivar.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

SD, VP, and ST conceived the experiment. SD, JS, VP, ST, SR, WS, and FD conducted the experiments. SD and JS wrote the manuscript. SD, JS, VP, and ST edited the manuscript.

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SUPPLEMENTARY MATERIAL

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