Biochimie xxx (xxxx) xxx

Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Grapevine aquaporins: Diversity, cellular functions, and ecophysiological perspectives

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ARTICLE INFO

Article history:
Received 29 March 2021
Received in revised form
23 May 2021
Accepted 7 June 2021
Available online xxx

Keywords:
Vitis vinifera L.
Water channels
Gating
Stopped-flow spectroscopy
Climate changes
Viticulture

ABSTRACT

High-scored premium wines are typically produced under moderate drought stress, suggesting that the water status of grapevine is crucial for wine quality. Aquaporins greatly influence the plant water status by facilitating water diffusion across the plasma membrane in a tightly regulated manner. They adjust the hydraulic conductance of the plasma membrane rapidly and reversibly, which is essential in specific physiological events, including adaptation to soil water scarcity. The comprehension of the sophisticated plant-water relations at the molecular level are thus important to optimize agricultural practices or to assist plant breeding programs. This review explores the recent progresses in understanding the water transport in grapevine at the cellular level through aquaporins and its regulation. Important aspects, including aquaporin structure, diversity, cellular localization, transport properties, and regulation at the cellular and whole plant level are addressed. An ecophysiological perspective about the roles of grapevine aquaporins in plant response to drought stress is also provided.

Contents

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https://doi.org/10.1016/j.biochi.2021.06.004
0300-9084/

Please cite this article as: F. Sabir, O. Zarrouk, H. Noronha *et al.*, Grapevine aquaporins: Diversity, cellular functions, and ecophysiological perspectives, Biochimie, https://doi.org/10.1016/j.biochi.2021.06.004

1. Introduction

Grapevine (*Vitis vinifera* L.) is a widely cultivated plant across arid and semi-arid ecosystems. They are considered as a good woody tree/fleshy fruit plant model to study water stress. It is generally considered a 'drought avoiding' species [1]. A moderate water deficit irrigation technique is commonly practiced in vineyards to achieve high-quality wine grapes without affecting their yield [2]. Premium wine grapes are produced within very narrow climate ranges, and fluctuations in temperature or precipitation may affect the wine flavor and aroma [3]. Prolonged water scarcity and high temperature, due to ongoing global warming, affect the yield and also disturb the fragile equilibrium of water, sugar, tannin, and flavor in berries [3]. The understanding of the molecular events that govern water relations in grapevine is important under both the scientific and agricultural perspectives.

Aquaporins, belonging to the major intrinsic family (MIP), are considered the molecular entry point of water in the cells and regulate numerous physiological phenomena [4]. These water channels are highly conserved and are found in almost all living forms. They are involved in intra/intercellular water and ion homeostasis. Alteration of aquaporins expression has been shown under various stresses like drought and nutrient deficiency/toxicity, suggesting their important role in plant response to environmental constraints [5]. Depending on their sequence homology and subcellular localization, plant aquaporins are classified into five major subfamilies: plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodulin 26-like proteins (NIPs), small basic intrinsic proteins (SIPs), and uncharacterized intrinsic proteins (XIPs) [6,7]. Apart from water, these multifunctional channels are also involved in the transport of a wide range of other small solutes, including micronutrients like ammonia (NH_3), boron (B), glycerol, silicon (Si), selenium (Se) and urea, exchange of O_2 and CO_2, and mobilization of reactive oxygen species (ROS) like H_2O_2 [4,8]. They also mediate the uptake, translocation, and extrusion of toxic metalloids like arsenium (As), germanium (Ge), aluminium (Al), and antimony (Sb) [9]. Some aquaporins are specific water transporters, while others show low/null intrinsic water permeability but may facilitate the transport of other small solutes. Aquaporin transport activity can be regulated by different signals like pH, phosphorylation, cations, membrane tension, among others [5,10,11].

Although numerous reviews have summarized the role of aquaporins in grapevine cultivars and rootstocks [12,13], their exploration at the molecular level is still missing. This review addresses the diversity of grapevine aquaporins and the importance of heterologous expression systems to characterize their function. The role of grapevine aquaporins at the whole-plant level and their tissue-specific functions included in shoots, roots, leaves, and berries is likewise considered. An ecophysiological perspective on the role of specific aquaporins in plant-environment interaction is also provided. The accumulated scientific knowledge is of pivotal importance at the agronomic level in the context of the ongoing climate changes, ultimately enabling the optimization of agricultural practices.

2. Grapevine aquaporins diversity

The diversity and number of aquaporins are much higher in plants than in any other life form [4,14]. Up to 121 AQP isoforms can be found in a single plant species, with different physiological roles during biotic and abiotic stresses, whereas only 15 types of AQP isoforms have been found in mammals [14,15]. Gene duplication, polyploidy, and horizontal gene transfer (HGT) [16,17] are likely to account for the exceptional abundance of aquaporins in plants. Likewise, different types of gene duplication events, either specific (tandem, segmental and proximal) or disperse, contributing to the overall size and structure of the MIP gene family, were recently revealed in grapevine [18]. Recent genome-wide studies identified a total of 121 aquaporin isoforms in *Brassica napus* [19], 35 in *Arabidopsis* genome [7,20], while in grapevine, 33 MIP sequences were identified, four of which are recognised as incomplete [18,21].

2.1. Phylogeny and classification of grapevine aquaporins

A phylogenetic relationship between *Vitis vinifera* and *Arabidopsis thaliana* MIPs is depicted in Fig. 1, which shows that grapevine aquaporins are divided into five subfamilies of PIPs, TIPs, NIPs, SIPs, and XIPs. Additionally, sequences of hAQP1 (an orthodox water selective human aquaporin) [22] and GlpF (glycerol uptake facilitator protein of *E. coli*) [23] were included in this tree showing that water transporters PIPs and TIPs cluster with hAQP1 whereas NIPs and SIPs share high sequence similarity with GlpF (Fig. 1).

Different studies further grouped the aquaporin subfamilies in various ortholog clusters, according to their branching. In each cluster, the distinct functionality of the homologous gene and their evolution by gene duplication was elucidated [24–26]. In this

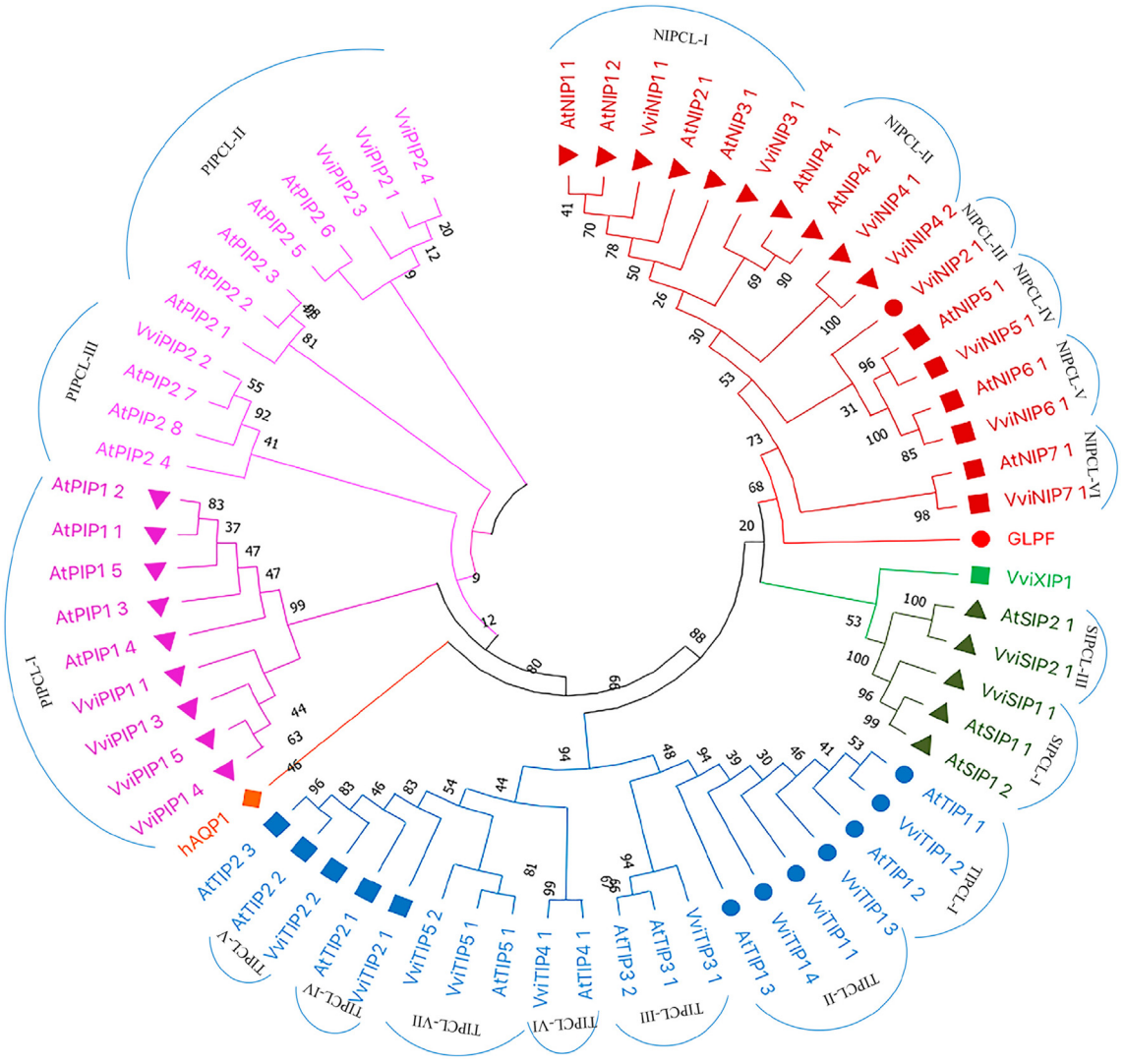

Fig. 1. Phylogenetic tree containing *Vitis vinifera* L. aquaporins and the previously identified *Arabidopsis thaliana* aquaporins. Human AQP1 (hAQP1) and *E. coli* GLPF (a glycerol facilitator protein) are also included as outgroups. The tree was generated by the Neighbor-Joining method [27] on 66 amino acid sequences. The bootstrap consensus tree inferred from 1000 replicates [28] was used to represent the evolutionary history of the taxa analysed [28]. Evolutionary analyses were conducted in MEGA X [29]. The aquaporin subfamilies are labelled with different colours: PIPs with fuchsia, TIPs with blue, NIPs with red, SIPs with green.

review, we organized the clusters of grapevine aquaporins using *Arabidopsis* subfamilies clusters (Fig. 1).

2.1.1. The VviPIP subfamily

PIPs are aquaporins with approximately 30 kDa and an isoelectric point of 9.0 due to the numerous basic amino acids in the C-terminal [19,30]. They are highly conserved and constitute the largest plant aquaporin subfamily, widespread in all plant species, including algae, ferns, gymnosperms, monocots, and dicots, indicating their early emergence during evolution [26]. In grapevine, this subfamily consists of eight members, which are split into two subgroups, *Vvi*PIP1s and *Vvi*PIP2s. *Vvi*PIP1s AQPs (PIP1; 1, PIP1; 3, PIP1; 4 and PIP1; 5) cluster together in a single branch (PIPCLI), while *Vvi*PIP2s (PIP2; 1, PIP2; 2, PIP2; 3 and PIP2; 4) cluster into two separate branches (PIPCLII and PIPCLIII) (Fig. 1), suggesting the origin of PIPs from three types of ancestor PIPs in the common ancestor of mono and dicots [25].

2.1.2. The VviTIP subfamily

TIPs are 25–28 kDa aquaporins with an acidic isoelectric point from 4 to 6.7 [19]. They are highly conserved in land plants and may have evolved from a PIP ancestor [7]. In grapevine, the ten members of TIPs are divided into five subgroups (TIP1 to TIP5), as proposed for other plant TIPs [25]. These subgroups are organized into seven clusters, in which *Vvi*TIP1s (TIP1; 1, TIP1; 2, TIP1; 3 and TIP1; 4) are grouped into two clusters (TIPCL-I and TIPCL-II) with *Arabidopsis* TIP1s (Fig. 1). On the other hand, *Vvi*TIP2s (TIP2; 1 and TIP2; 2) are distributed in two clusters (TIPCL-IV and TIPCL-V), but AtTIP2; 3, which belongs to TIPCL-IV, does not group with other members of this cluster. The remaining grapevine TIP3; 1, TIP4; 1 and TIP5s (TIP5; 1 and TIP5; 2) cluster with the corresponding *Arabidopsis* members in TIPCL-III, TIPCL-VI, and TIPCL-VII, respectively (Fig. 1). The observed internal family-specific gene duplications in each TIP subgroup were previously proposed [20,26].

2.1.3. The VviNIP subfamily

NIPs constitute a highly divergent subfamily. Soybean NIP was the first member of the plant MIPs family to be identified [31] and this subfamily is abundant in the plant kingdom, from mosses to flowering plants [9]. Although still matter of debate, it is believed that NIPs were originated by horizontal gene transfer from bacterial ancestors due to its higher phylogenetic similarity with bacterial

AqpZ than with the other plant aquaporins [16]. A total of seven NIP members were identified in grapevine, distributed into three groups NIP—I, NIP-II, and NIP-III [32]. These groups were further organized into six clusters (Fig. 1). The NIP—I group in NIPCL-I (NIP1; 1 and NIP3; 1) and NIPCL-II (NIP4; 1 and NIP4; 2), NIP-II group in NIPCL-IV (NIP5; 1), NIPCL-V (NIP6; 1), and NIPCL-VI (NIP7; 1) clusters (Fig. 1), whereas the NIP-III group, which belongs to NIPCL-III (NIP2; 1) cluster was absent in *Arabidopsis* MIP sequences as well as in other flowering plant species [19,20].

2.1.4. The VviSIP subfamily

*Vvi*SIP is the smallest subfamily found in seed plants (spermatophytes) and mosses [20] with only two members identified in grapevine [33] and classified into two subgroups: SIPCL-I (SIP1; 1) and SIPCL-III (SIP2; 1), much like *Arabidopsis* SIPs (Fig. 1). The SIPCL-II cluster is missing in both plants. SIPs are small proteins of 26 kDa like TIPs, but with a higher isoelectric point (~9.0) due to basic amino acids in C-terminal [19,24].

2.1.5. The VviXIP subfamily

Its evolutionary history suggests that the XIP subfamily has undergone a relatively recent lineage-specific expansion, generating different clades with distinct functions [34]. The transcript variants of *XIP1* gene were identified only in *Solanaceae*, likely due to alternative splicing, which resulted in slightly different proteins [35]. In *Nicotiana* species, the splice variant XIP1; 1α is one amino acid shorter than XIP1; 1β splice variant.

The XIP subfamily was initially associated with the NIP subfamily due to their sequence similarity and their overlapping substrate profile [6,36]. XIPs were found in fungi, mosses, and dicots, but interestingly, no homologs were identified in *Brassicaceae*, including *Arabidopsis thaliana* [6,36]. After their recent recognition, XIPs were phylogenetically divided into four subgroups (I-IV) [37]. In grapevine, only one homolog *Vvi*XIP1 was identified (Fig. 1) [38].

2.2. Structure and conserved sites of grapevine aquaporins

So far, the high-resolution structure of plant aquaporins by X-ray crystallography, is available only for SoPIP2; 1 [39] and AtTIP2; 1 [40]. The topology prediction of grapevine aquaporins showed the general feature of the MIP family, which includes six transmembrane spanning helices (TMH1-TMH6), connected with five intra- and extracytosolic loops (LA-LE), and N- and C-termini, facing the cytosolic side [7] (Fig. 2). The membrane aquaporin holoprotein is assembled by four monomers, each comprising an individual transmembrane pore, which are stabilized by hydrogen bonds and interaction between the loops of monomers [5]. The central pore constriction is formed by the highly conserved Asparagine-Proline-Alanine (NPA) residues present at the two hydrophobic loops, LB and LE. These loops fold back into the membrane, positioning the NPA motifs at the center of the pore of aquaporins, which appear like an hourglass [7]. The NPA motif forms the first constriction of the pore and is considered as Filter 1, allowing the movement of one single row of water molecules. Furthermore, four aromatic/arginine (ar/R) residues at the cytosolic opening of the pore form the second and narrowest constriction [8]. The ar/R residues are highly conserved among the subfamilies, defining their substrate specificity. Likewise, Froger's position residues (P1—P5) throughout the aquaporin, have been described as relevant to distinguish aquaporins from aquaglyceroporins [41], and in the interaction with other substrates and their selectivity (Fig. 2).

Unique characteristics of each subfamily of grapevine aquaporins are described in the following sections.

2.2.1. The AEF (Ala-Glu-Phe) motif

The AEFXXT motif, located in the first helix (TMH1), is highly conserved in almost all grapevine MIPs. Although the first two amino acids (AE) were found in all aquaporins, the motif was different in *Vvi*TIP2; 2 (SEFXXT) and *Vvi*TIP4; 1 (MEFXXT). The third amino acid in the motif was not conserved in NIPs and varied to either V/M/L, which are always hydrophobic, as suggested by Hove and Bhave (2011) [8]. This motif was not conserved in *Vvi*SIPs, but sequence alignment showed G/S-D-G/F-XXX-T/A residues in both SIPs of grapevine (Fig. 2). The exact function of the AEFXXT motif is still unclear, but it was suggested that it interacts between Filter 1 and Filter 2 due to their close vicinity [8].

2.2.2. Length of N-and C-terminal and loops

Grapevine MIPs showed distinct variations in their N-and C-terminal length, similar to the previously reported plant MIPs. Despite having similar sequences, PIP1s and PIP2s showed different characteristics due to their N-and C-terminal length (reviewed by Ref. [14]). *Vvi*PIP1 has a longer N-terminal with a shorter C-terminal than *Vvi*PIP2s [42] (Fig. 2). *Vvi*NIPs and *Vvi*XIPs have distinguishably longer N-and C-termini, while the shortest N- and C-terminal were observed in *Vvi*SIPs (Fig. 2). The cytosolic termini are critical for various regulatory functions and trafficking of aquaporins, which will be discussed further in the relevant sections.

Among all five loops, LE is the most conserved in grapevine MIPs. In general, a very short LD was observed in grapevine MIPs, except in *Vvi*PIPs and *Vvi*XIPs (Fig. 2). These two subfamilies showed a distinguishable long loop D, which was proposed to have an essential role in the gating of aquaporins [34,39]. Like other plant XIPs, *Vvi*XIP showed distinctly a very short LB but a very long LC, as [34,38], and contains a highly conserved LGGC motif in loop C [6]. The LC is considered to connect loop B and loop E and also to interact with monomers in the tetrameric structure, which is functionally necessary for water permeability (reviewed by Ref. [5]).

2.2.3. NPA motif

The NPA motifs, present in both loops LB and LE, are considered as the most crucial feature in all aquaporins to maintain their function, and they are highly conserved in MIPs [7]. In plants, these two motifs are highly conserved in PIPs and TIPs, while the third residue (alanine) shows a variation in NIPs, SIPs, and XIPs (reviewed by Refs. [8,14]). Similarly, these motifs are conserved in grapevine PIPs and TIPs subfamilies [42]. Among *Vvi*NIPs, the alanine in the NPA motif of loop B motif is replaced by serine (S) in *Vvi*NIP5; 1 and *Vvi*NIP7; 1 of NIP-II, whereas the other member of this subgroup, *Vvi*NIP6; 1, has conserved NPA motif (Fig. 2). The motif in loop E possesses a valine instead of alanine in all these NIP-II members [32]. On the other hand, NIP—I and NIP-II do not show any variation in these motifs. *Vvi*SIPs and *Vvi*XIP showed variation only in the first NPA motif (loop B). Alanine was replaced by threonine in *Vvi*SIP1; 1, leucine in *Vvi*SIP2; 1, and valine in *Vvi*XIP1 (Fig. 2) [33,38]. Different studies have shown the role of NPA motifs specifically in water transport, but, paradoxically, mutations in these residues could not significantly affect the transport of other substrates (reviewed by Ref. [8]). Precise spacing (108 amino acids) between these motifs was shown to be a prerequisite for silicon selectivity in NIPs [43]. More recently, the silicon transporter, *Vvi*NIP2; 1, showed exactly 108 amino acid spacing between the two motifs [44]. Besides affecting the channel activity, NPA motifs have also been shown to regulate membrane trafficking during aquaporin biogenesis in animal aquaporins [45].

2.2.4. Aromatic/arginine (ar/R) selectivity filter

The ar/R residues, in combination with the NPA motif, define the

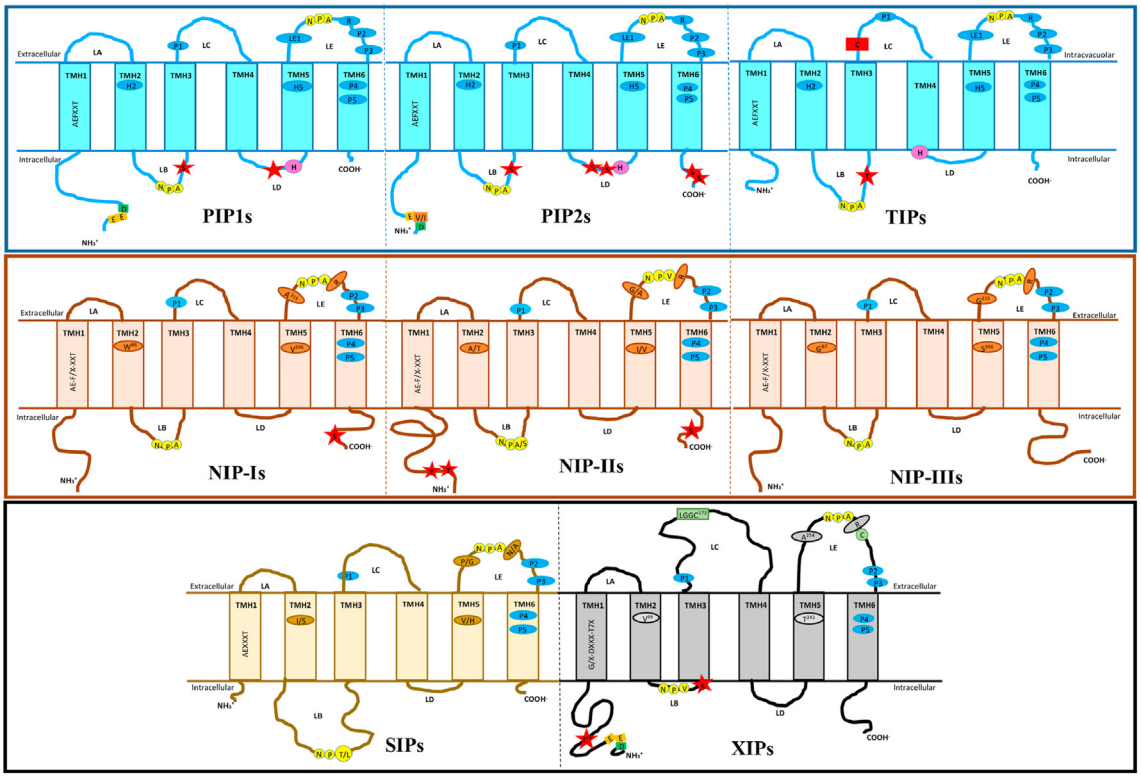

Fig. 2. Topological structure of grapevine aquaporin subfamilies based on the sequences alignment of identified sequences with the previously reported representatives of other plant species [42]. Six transmembrane domains (TMH1—TMH6), five connecting loops (LA—LE) and N- and C-termini are shown. Yellow circle: NPA motifs (Filter 1), P1—P5 blue ovals: Froger's position residues, ovals: aromatic/arginine (ar/R) residues (Filter 2), red stars: Putative phosphorylation sites, pink circles: pH regulation sites, green and yellow rectangles: Methylation and sorting signals, red rectangle: Mercury binding site in loop C of TIPs, green rectangle: conserved site in loop C of XIPs.

substrate selectivity in plant aquaporins. They determine the hydrophobicity/philicity and size of the pore and eventually decide the substrate's size and nature [32]. Contrary to NPA motifs, ar/R selectivity filters are highly variable, except in PIPs (reviewed by Refs. [8,14]). In grapevine PIPs, highly conserved hydrophilic residues FHTR were identified, which are similar to the mammalian water channel AQP1 (reviewed by Ref. [14]). On the other hand, *Vvi*TIPs showed group-specific diversity in ar/R residues, *Vvi*TIP1s have HIAV, *Vvi*TIP2s have HIGR, and *Vvi*TIP4; 1 has HVAR, which are similar to TIP groups from other plants [19], suggesting diverse substrate selectivity [46]. Interestingly, X-ray crystallography of *Arabidopsis* TIP2; 1 showed an extended selectivity filter with the fifth residue (H^{131}) located in loop C [40]. This residue is likely to participate directly in the substrate interactions in the selectivity region and is conserved in the members of the TIP2 group. In TIP1s, H^{131} is replaced by phenylalanine (F). A similar pattern of conservation of these residues was observed in grapevine TIPs (Fig. 2).

Significant variations in the ar/R region have been reported in NIPs. Based on the ar/R amino acid composition, NIPs are divided into three groups, NIP—I, NIP-II, and NIP-III, possessing distinct estimated pore sizes, which allows the selection of a broad range of substrates [47]. The amino acid residues in the ar/R region of NIP—I (W—V/I-A-R), NIP-II (T/A-A/I/V-G/A-R), and NIP-III (G-S-G-R) are group-specific, and changes in these residues significantly affect the NIPs functional efficiency in grapevine [48] and in other plants [49,50].

The ar/R residues in grapevine *Vvi*SIP1 were identified in a 3D computer simulation study using SoPIP2; 1 [39] as a template [33], showing that *Vvi*SIP1 has IVPN residues at the ar/R filter. All the analysed SIP1 proteins showed a conserved asparagine (N) at LE2 position, which is entirely different from most PIPs, TIPs, and NIPs, which contain an arginine (R). However, SIP1s from the *Brassicaceae* family have isoleucine (I) at the same position. Two conserved characteristics in the SIP1 group were identified: i) proline (P) at LE1 position, and ii) a highly conserved AFGWAYI motif in loop E [33]. On the other hand, *Vvi*SIP2 has SHGA residue at the ar/R filter, which is highly conserved in other plants also [19].

Similar to NIPs, an extensive variation of amino acids at the ar/R filter of XIPs has also been identified [36]. The ar/R filter in grapevine XIP1 is composed of VTAR. Amino acid residues in the ar/R region of some XIPs were found similar to the residues of some plant NIPs, while in other XIPs, these residues were more hydrophobic, facilitating the transport of hydrophobic and bulky substrates [36]. Additionally, a cysteine residue located after the second NPA motif (NPARC) was also identified as the signature sequence of plant XIPs [6].

3. Localization and molecular functions of grapevine aquaporins

3.1. Cellular/sub-cellular localization

As previously described, PIPs, NIPs, and XIPs are mainly localized at the plasma membrane [36,51], while TIPs and SIPs are localized at tonoplast and endoplasmic reticulum (ER), respectively. Although PIPs are generally considered as markers of the plant plasma membrane, some isoforms, especially PIP1s, are also found intracellularly [52,53]. It has been proposed that PIP2 isoforms are required for proper trafficking of PIP1s to the plasma membrane [54], but stress-induced re-localization of PIPs to intracellular vesicles was also described (reviewed by Ref. [55]).

Different mechanisms may account for the complex pattern of

aquaporin subcellular localization. In eukaryotes, the diacid motif (D/E)-X-(E/D), present in the N-terminal of the transmembrane proteins, is involved in the trafficking of proteins to the plasma membrane through the endoplasmic reticulum (ER)–Golgi secretory pathway [56]. Alignment of grapevine PIPs showed that EED residues in *Vvi*PIP1s and D-V/I-E residues in *Vvi*PIP2s are conserved, as previously reported in PIPs of maize [57] and *Arabidopsis* [58]. Mutation in these residues resulted in the retention of ZmPIP2s and AtPIP2; 1 in the ER, further suggesting the role of diacid motifs as ER export signals. However, diacid motifs are not the exclusive regulatory signal for the trafficking of aquaporins because ZmPIP1s localize at the ER despite having this motif, and ZmPIP2; 1 localizes at the plasma membrane but lacks the motif [57]. Moreover, when the N-terminal portion of the maize ZmPIP2; 5, with a functional diacid motif, was introduced into ZmPIP1; 2, the chimeric protein was not targeted to the plasma membrane [57]. Similarly, *Vvi*PIP2; 2 lacks this sorting motif (D/E)-X-(E/D) (Fig. 1), but it is targeted to the plasma membrane in heterologous expression in yeast [59]. Thus, because different proteins lacking this diacid motif are successfully trafficked to the plasma membrane, other mechanisms are likely to be involved in aquaporin sorting [51,55,60].

The transmembrane helix 3 (TMH3) has also been involved in the membrane trafficking of aquaporins. The motif ^{127}L-XXX-A^{131} present in the TMH3 of ZmPIP2s was demonstrated to regulate the trafficking of the aquaporin [61]. However, this motif is present only in *Vvi*PIP2; 1 and not in other members of grapevine PIP2s group. Interestingly, these residues are not confined to PIP2s and are also present in TIP1s and some members of NIPs of grapevine and *Arabidopsis*.

Cellular localization of aquaporins in grapevine is depicted in Fig. 3. The heterologous expression of GFP-tagged grapevine PIPs, TIPs, and NIPs showed that most of these proteins are localized in the plasma membrane of *S. cerevisiae* [32,42,44,59]. However, some fractions of the GFP-tagged proteins were retained at intracellular structures, most likely at the endoplasmic reticulum or vesicles of the secretory pathway, as previously observed (reviewed by Ref. [10]).

After heterologous expression in yeast, the grapevine SIP1-tagged with GFP was localized at endoplasmic reticulum as confirmed by Western blot analysis with specific ER antibodies [33]. In agreement, when transiently expressed in tobacco epidermal cells, *Vvi*SIP1-tagged with RFP localized at the ER.

The lysine-rich residues in the C-terminal of plants and mammalian ER aquaporins have been identified by many researchers [30,62,63]. This motif was suggested to be responsible for retrograde Golgi-to-ER transport and ER retention of these aquaporins. Grapevine SIP (*Vvi*SIP1) also shows an ER dibasic retention signal (KQKK) in its C-terminus [33].

Furthermore, transient expression of *Vvi*XIP1-RFP in *N. benthamiana* epidermal cells, co-localized with the ER marker YFP-HDEL and with the tonoplast marker YFP-ZmTIP2; 1 [38]. Contrary to *Vvi*XIP1, all the previously studied plant XIPs were localized in the plasma membrane [35,36]. Indeed, the true subcellular localization of some aquaporins remains puzzling. AtNIP1; 1 was previously localized in the plasma membrane [64,65], but a recent study revealed its unusual occurrence in ER and tonoplast, suggesting its crucial role in metalloids [As (III)] homeostasis at the sub-cellular level [66].

3.2. Membrane transport and substrate selectivity

Xenopus laevis oocytes has been the primary model to functionally characterize grapevine aquaporins [21,67,68], although some disadvantages of this model have been reported (reviewed by Ref. [7]). On the other hand, *aqy-null S. cerevisiae* strains is a powerful tool that has unveiled multifaceted roles of grapevine aquaporins (Table 1). Transport activity of aquaporins has also been studied in membrane vesicles purified from transformed yeast cells or in artificial membrane vesicles after protein purification [33,69].

3.2.1. Water conductance

Functional characterization of many grapevine aquaporins (five *Vvi*PIPs, four *Vvi*TIPs, four NIPs, one *Vvi*SIP, and one *Vvi*XIP) has been performed by stopped-flow spectroscopy [75] in yeast cells

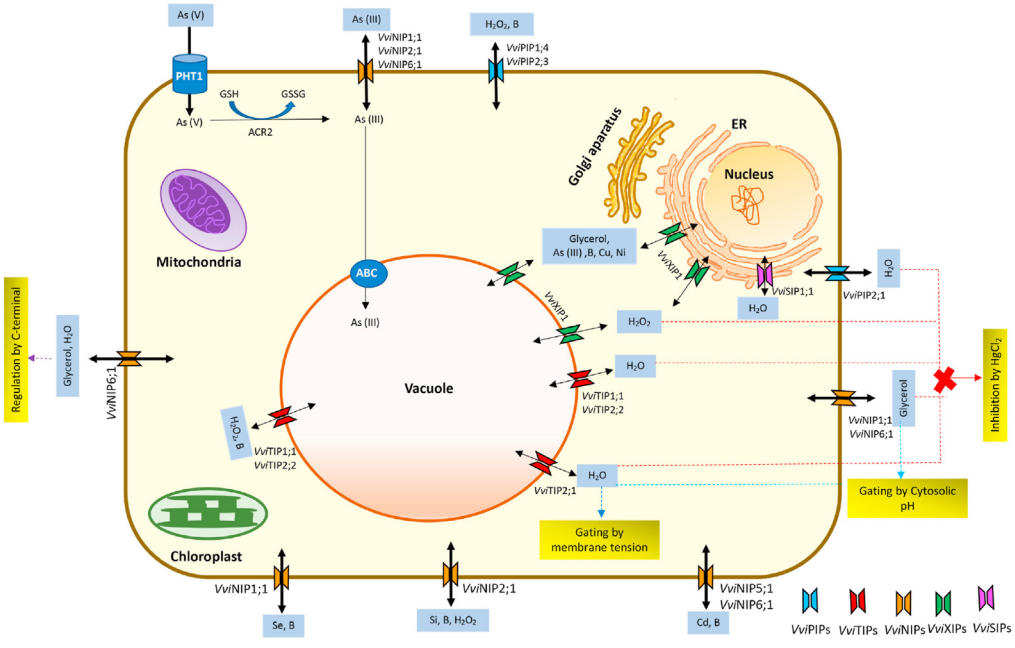

Fig. 3. Sub-cellular localization of grapevine aquaporin isoforms, substrate specificity and regulatory mechanisms. Abbreviations: PHT1-inorganic phosphate transporter, ACR2-arsenate reductase, GSH-glutathione, GSSG-glutathione disulphide and ER-Endoplasmic reticulum.

Table 1
Specific sequence features and conserved sites of grapevine aquaporins, functionally characterized in different expression systems.

Grapevine aquaporin isoforms		NCBI Accession number	Protein length (Amino acid)	Termini length (Amino acid)		Filter 1 (NPA motifs)		Filter 2 (ar/R residues)				Functional characterization		Regulation
				N-terminal	C-terminal	NPA1 (LB)	NPA2 (LE)	H2	H5	LE1	LE2	Expression system	Substrates	
PIPs	*Vvi*PIP1; 1	HQ913643	286	5	9	NPA	NPA	F^{95}	H^{225}	T^{233}	R^{239}	Yeast	ND [59]	–
	*Vvi*PIP1; 4	KJ697714	286	53	9	NPA	NPA	F^{94}	H^{223}	T^{232}	R^{238}	Yeast	H_2O_2, Boron (B) [42]	–
	*Vvi*PIP2; 1	KJ697715	284	37	17	NPA	NPA	F^{84}	H^{213}	T^{222}	R^{228}	Yeast	Water [42]	Inhibition by $HgCl_2$ [32]
	*Vvi*PIP2; 2	HQ913642	279	37	17	NPA	NPA	F^{79}	H^{208}	T^{217}	R^{223}	Yeast	ND [42]	–
	*Vvi*PIP2; 3	KJ697716	287	39	17	NPA	NPA	F^{67}	H^{196}	T^{205}	R^{211}	Yeast	H_2O_2, Boron (B) [42]	–
TIPs	*Vvi*TIP1; 1	KJ697717	251	21	12	NPA	NPA	H^{65}	I^{187}	A^{196}	V^{202}	Yeast	Water, H_2O_2, Boron (B) [42]	Inhibition by $HgCl_2$ [32]
	*Vvi*TIP2; 1	HQ913640	249	19	12	NPA	NPA	H^{63}	I^{185}	G^{194}	R^{200}	Yeast	Water [59]	Cytosolic pH-dependent gating [59] Turgor-induced membrane tension [73] Inhibition by $HgCl_2$ [59]
	*Vvi*TIP2; 2	KJ697718	250	24	13	NPA	NPA	H^{63}	I^{185}	G^{194}	R^{200}	Yeast	Water, H_2O_2, Boron (B) [42]	Inhibition by $HgCl_2$ [32]
	*Vvi*TIP4; 1	KJ697719	253	19	17	NPA	NPA	H^{60}	V^{183}	A^{192}	R^{198}	Yeast	Boron (B) [42]	–
NIPs	*Vvi*NIP1; 1	MN723560	282	48	26	NPA	NPA	W^{86}	V^{206}	A^{215}	R^{221}	Yeast	Water, Glycerol, Arsenite (As), Boron (B), Selenium (Se) [32]	Cytosolic pH-dependent gating [32] Inhibition by $HgCl_2$ [32]
	*Vvi*NIP2; 1	100265406	294	49	38	NPA	NPA	G^{87}	S^{206}	G^{215}	R^{221}	Yeast and Xenopus oocytes	Silicon (Si), Arsenite (As), Boron (B), H_2O_2 [32,44]	–
	*Vvi*NIP5; 1	MN723561	298	78	18	NPS	NPV	A^{111}	I^{230}	G^{239}	R^{245}	Yeast	Boron (B), Cadmium (Cd) [32]	–
	*Vvi*NIP6; 1	MN723562	312	84	65	NPA	NPV	T^{118}	I^{239}	A^{248}	R^{254}	Yeast	Glycerol, Arsenite (As), Boron (B), Cadmium (Cd) [32]	Cytosolic pH-dependent gating [32] Regulation by C-terminal [32] Inhibition by $HgCl_2$ [32]
SIP	*Vvi*SIP1; 1	DQ086835	238	10	7	NPT	NPA	I^{52}	V^{175}	P^{184}	N^{190}	Yeast ER vesicles, proteoliposomes and Xenopus oocytes	Water [33]	–
XIP	*Vvi*XIP1	F6I152 (XP_010647342)	320	61	26	NPV	NPA	V^{99}	T^{243}	A^{254}	R^{260}	Yeast membrane vesicles	Glycerol, H_2O_2, Arsenite (As), Boron (B), Heavy metals-Copper (Cu) and Nickle (Ni) [38]	Inhibition by $HgCl_2$ [38]

– Not determined, ND- Not detected.

(Table 1) [32,33,38,42,44,59]. Among PIPs, only *Vvi*PIP2; 1 showed. Water conductance [42,59]. The remaining members, *Vvi*PIP2; 2 and *Vvi*PIP2; 3, and *Vvi*PIP1; 1 and *Vvi*PIP1; 4, did not affect yeast water transport despite their correct localization in the plasma membrane [42,59].

Although PIPs share similar residues in the selectivity filter, PIP1s and PIP2s show distinct substrate specificity. For instance, most PIP1s showed null or very low water conductance when expressed in heterologous systems [52,70–72], even though their expression analysis suggests a crucial role in root hydraulic conductance in tobacco [74] and *Arabidopsis* [75] and in developing pea seed coat during water imbibition [76].

Results have shown that water permeability of grapevine *Vvi*-TIPs is generally higher than *Vvi*PIPs, as observed for other heterologously expressed plant TIPs and PIPs [77,78]. This high water permeability exhibited by TIPs may allow the cells to rapidly recruit the vacuolar space to maintain cellular integrity in case of osmotic fluctuations caused by rapid water exchange [77,79].

Water transport activity of grapevine NIPs (NIP1; 1, NIP2; 1, NIP5; 1, and NIP6; 1) was also measured after heterologous expression in yeast [32,44]. This MIP subfamily showed no or lower intrinsic water permeability than PIPs and TIPs. Among all NIPs, only *Vvi*NIP1; 1 showed significant water channel activity. This isoform belongs to the NIP–I group, which includes the archetype GmNod26 that has been characterized as a water channel [49]. The ar/R filter of the NIP–I group is composed of hydrophilic amino acid residues, which tend to form a narrower pore (diameter ~2.8 Å) [80]. A wider pore aperture was proposed for NIP-II and NIP III groups [49] that include *Vvi*NIP5; 1, *Vvi*NIP6; 1 (NIPII) and *Vvi*NIP2; 1 (NIP-III), but these proteins were not able to mediate water

transport when expressed in yeast [32,44]. Numerous NIP-III group members share the same ar/R filter, but their water permeability differs from each other. For instance, OsLsi1 (OsNIP2; 1) and HvNIP2; 1 were able to transport water [50,81], while the other functional Si transporters of pumpkin (CmLsi1) and grapevine *Vvi*NIP2; 1 were unable to mediate water transport despite possessing the similar NPA and Ar/R filters [82]. These NIP members contain selectivity filters with more hydrophobic residues, and their incapacity to transport water is likely due to different gating mechanisms or to their inability to organize the water molecules in the pore [80,83].

*Vvi*SIP1 showed water transport activity measured by stopped-flow using ER vesicles isolated from yeast cells overexpressing *Vvi*SIP1 and in reconstituted proteoliposomes [33]. *Vvi*SIP1 has an NPT-NPA signature, as in *Arabidopsis* AtSIP1; 1 and AtSIP1; 2, that has also been associated with water transport [84]. Nonetheless, the physiological relevance of water transport at the ER still remains to be deciphered. On the other hand, increased water permeability was not detected in membranes isolated from yeast cells overexpressing *Vvi*XIP, in agreement with previous data from *Nicotiana tabacum* [36] and in *Hevea brasiliensis* [85]. Interestingly, *Populus trichocarpa* has two XIP members, from a subfamily of six, that showed small water transport activity when expressed in *Xenopus* oocytes [86].

3.2.2. Transport of other substrates

In general, PIP isoforms have highly conserved and narrow pores quite specific for water transport, while TIPs, NIPs, and XIPs show a more diverse pore configuration (Table 1, Fig. 3). Different reports have suggested that PIPs and TIPs are orthodox water channels, while NIPs and XIPs are aquaglyceroporins permeating both water and glycerol [4,7,87]. But the specificity of other aquaporins is rather uncommon, being able to permeate H_2O_2, silicon, arsenite [4,8].

3.2.2.1. Glycerol transport. Glycerol transport activity of aquaporins expressed in yeast has been evaluated through stopped-flow spectroscopy by measuring the yeast cell volume changes triggered by glycerol osmotic gradients [32]. This approach showed that *Vvi*NIP1; 1 is an aquaglyceroporin with a higher capacity to transport glycerol and water, much like it has been observed in other members of the NIP—I group, including in the archetype GmNod26 and *Arabidopsis* NIPs [88], while *Vvi*NIP6; 1 was able to mediate the transport of glycerol but not of water as observed in Arabidopsis AtNIP6; 1 [49]. NIP6; 1 protein belongs to NIP-II cluster (Fig. 1). The enormous variation in its ar/R region and the presence of hydrophobic amino acid residues and of a wider pore (diameter ~3.4 Å) [89] may have resulted in their broad substrate specificity.

Recently, a decline in membrane glycerol permeability of grapevine NIPs was observed when the ar/R residues at H2/H5 helices positions were switched between grapevine *Vvi*NIP1; 1 and *Vvi*NIP6; 1, but the functional properties of each NIP were not significantly changed in particular in what regards water transport [48].

However, homology modelling suggested that NIP6; 1 has a much wider pore than NIP1; 1, which could accommodate more than one water molecule [48,49]. Similarly, switch the ar/R residue between the aquaglyceroporin LIMP2 and the water selective aquaporin LIMP1 did not enhance the water permeability of LIMP2 [90].

Furthermore, homology modelling analyses suggested that amino acids at the entrance of the channel, which influence its physicochemical properties, may also play relevant roles in determining NIP substrate selectivity [48].

Besides NIPs, grapevine XIP1 also showed glycerol transport when studied by stopped-flow spectroscopy in vesicles purified from yeast cells overexpressing *Vvi*XIP1 [38]. The ar/R filter of *Vvi*XIP (VTAR) is similar to the ones of *Nicotiana tabacum* and of NIP-III isoforms that have been shown to transport uncharged molecules like glycerol, urea and metalloids [36].

While in yeasts, glycerol plays an important role as osmoprotectant [91], its physiological relevance in plants is still puzzling, although other polyols, like mannitol and myo-inositol, are important compatible solutes in grapevine [92]. Glycerol may act as a carbon source in plants [93] and its exogenous application was shown to improve plant growth and root development [94]. In symbiotic nodules, the aquaporin GmNod26 is potentially involved in glycerol and NH_3 uptake during the nitrogen fixation [88].

3.2.2.2. H_2O_2 transport. Growth assays of yeasts, overexpressing different grapevine aquaporins, in the presence of toxic concentrations of H_2O_2 have shown that PIPs and TIPs are permeable to this compound, much like their counterparts in *Arabidopsis* [95,96] and maize [97].

In plant cells, H_2O_2 is a harmful molecule that induces oxidative stress but also plays important physiological roles as a signalling molecule [75]. During stress conditions, it can be compartmentalized intracellularly or exported into the apoplast [98]. It has been suggested that PIPs are involved in apoplastic exclusion of excess H_2O_2, while TIPs compartmentalize H_2O_2 into vacuoles, resulting in their further detoxification by vacuolar peroxidases [99].

Furthermore, functional studies with the ROS sensitive probe 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate acetyl ester (CM-H_2DCFDA) and with a Clark electrode, monitoring the rate of O_2 release by transformed yeast cells elicited with H_2O_2, suggested that NIP2; 1 [44] and XIP1 [38] mediate H_2O_2 uptake. Although H_2O and H_2O_2 are structurally and electrostatically similar, these aquaporins were remarkably able to distinguish both substrates because they are unable to transport water.

3.2.2.3. Boron transport. Boron is a plant micronutrient with relevant roles in preserving the cell wall integrity [100], and aquaporin-mediated uptake of boron in plants has been described under limited boron conditions [101]. PIPs of squash [102] and barley [103] root were able to mediate boron transport when heterologously expressed. Additionally, two *Arabidopsis* NIPs, AtNIP5; 1 and AtNIP6; 1, members of the NIP-II group, were also characterized as boric acid channels [101,104]. It was suggested that AtNIP5; 1 mediates uptake of boron from the soil by root cells, which is loaded into the xylem by a borate exporter BOR1, thereafter AtNIP6; 1 mediates the xylem-phloem transfer of boric acid at the nodal regions in the aerial part of the plants [104].

Growth assays have shown that strains overexpressing grapevine PIPs, TIPs, NIPs, and XIPs exhibited sensitivity when challenged with boric acid [38,42,44,48], suggesting that they mediate boric acid uptake. Among grapevine NIPs, the NIP-II member *Vvi*NIP6; 1 is likely the most prominent boron transporter, but members of other groups such as *Vvi*NIP1; 1 of the NIP—I group and *Vvi*NIP2; 1 of the NIP-III are also likely able to mediate boron uptake [32]. The significance of the ar/R residue at the H5 position has been established in the NIP-III group [50]. Recently, our study suggested that the ar/R residue at the H5 position is crucial for boron uptake by *Vvi*NIP2; 1. Thus the substitution of a serine, a small polar amino acid, by the bulkier and non-polar isoleucine (S206I) resulted in the complete loss of yeast growth sensitivity towards boron. However, the sensitivity was unaffected by the substitution at the H2 position (G87A) [32].

3.2.2.4. Arsenite transport. In higher plants, phosphate transporters (PHT1) non-specifically transport non-toxic form As (V),

which is rapidly reduced to the toxic form As (III) by arsenate reductase (ACR2), and subsequently sequestered in vacuole by ABC transporters [105]. Alternatively, As (III) can be exported to the extracellular space by NIPs and PIPs. A recent report showed that yeast strains expressing *Vvi*NIP1; 1 or *Vvi*NIP6; 1 are able to mediate both influx or efflux of As (III) [32]. The observed bidirectional flux of As (III) through grapevine NIPs was previously demonstrated in *Arabidopsis*, rice, and lotus [64,106]. Besides, *Vvi*-NIP2; 1 was also permeable to arsenite [As (III)] when expressed in oocytes [44], similarly to rice Lsi1 and barley HvNIP2; 1 [81,106,107]. Nonetheless, the main form of this compound in aerated soils, where grapevine generally grows, is arsenate [As(V)]. Thus, it appears that arsenite transport capacity seems to be an ancestral feature shared by NIP2; 1 with no, or very limited, biological significance in grapevine. Previous studies demonstrated that the selectivity of NIPs to As (III) is not strictly dependent on ar/R residue, as different groups with distinct ar/R residues showed selectivity for As (III) [50,108]. However, the mutational study of VviNIP2; 1 indicated that similar to boron, the residue at the H5 position is crucial for the transport of As (III) in grapevine [48].

3.2.2.5. Silicon transport. Supplementation of Silicon (Si) is beneficial for plants under biotic and abiotic stresses [109,110]. Studies in rice mutants allowed the identification of the first Si channel in plants, Lsi1 [111], and since then, similar proteins have been identified in different plant as NIP2 aquaporins [44]. Specific features are needed for a NIP2 being able to transport Si, including the presence of NPA motifs separated by 108 amino acids and a GSGR ar/R selectivity filter [43]. Indeed, *Vvi*NIP2; 1 is a *bona fide* silicon transporter in grapevine, highly expressed in roots, and localized at the plasma membrane. Besides Si, it also mediates the uptake and Ge (Si analog) when expressed in *Xenopus* oocytes [44]. Although grapevine is considered a non Si-accumulator, own-rooted plants accumulated >0.2% Si (DW) in leaves when irrigated with 1.5 mM Si for one month.

4. Gating and functional regulation

A high transmembrane water conductance through plant aquaporins is a prerequisite for plant growth and development and response to environmental cues. The inter/intracellular water flux is adjusted by distinct regulatory mechanisms that control the aquaporin expression, function, and gating. Aquaporins regulation at the transcriptional level is mainly associated with their differential expression, which is greatly altered by environmental stress. On the other hand, different co- and post-translational modifications such as methylation, phosphorylation, and heteromerization, regulate the aquaporin trafficking and, subsequently, their functionality [4]. A faster regulatory mechanism to control the opening and closing of the aquaporin pore is called gating and may be triggered by different mechanisms [5], including phosphorylation [39], organization in heterotetramers [112,113] membrane tension and turgor [73], and protonation [59].

A plethora of studies has been performed in grapevine, showing the differential expression of aquaporin transcripts under various stress conditions, especially under water stress [13,67,114–116]. However, only a few studies revealed the underlying molecular mechanisms for gating and functional regulation of grapevine aquaporins (Table 1, Fig. 3).

4.1. Gating by cytosolic pH

A pH-dependent gating of heterologously expressed *Vvi*TIP2; 1 showed that acidification of yeast cytosol resulted in the loss of activity [59]. The molecular basis of pH-dependent gating was explained by the presence of a His^{131} in cytosolic loop D, which is considered as a pH-sensor in PIPs [39,117]. The molecular mechanism of gating was unveiled by the pioneering work based on the atomic structure of closed and open conformations of SoPIP2; 1. Results showed that protonation of the conserved His^{193} residue changes the conformation of loop D, which occludes the pore. Both in spinach (His^{193} in SoPIP2; 1) and in *Arabidopsis* (His^{197} in AtPIP2; 1), protonation of histidine residue by cytosolic acidification resulted in reduced water flow, which is considered crucial to close the pore during anoxic stress conditions like under flooding [39,117]. Although this residue is not conserved in other aquaporin isoforms, altered membrane permeability was observed under different pH conditions. For instance, an enriched fraction of tonoplast membrane vesicles of *B. vulgaris* showed reduced permeability at acidic pH [118], whereas AtTIP5; 1 permeability was dependent on extracellular pH [119] and permeability of *Vvi*TIP1; 1 was unaltered under different pH conditions [21]. A recent report showed that water and glycerol permeabilities of grapevine NIPs are also reduced by cytosolic acidification [32]. The lack of any His residue in loop D suggests that other cytosolic acidic amino acids are involved in the intracellular pH-sensitivity of aquaporins. For instance, cytosol facing amino acids such as R^{194} and D^{195} in *Arabidopsis* [117] and Leu^{206} in *B. vulgaris* aquaporins [120] have been suggested to participate in their pH dependent gating.

4.2. Gating by membrane surface tension

Gating of grapevine *Vvi*TIP2; 1 by turgor-induced membrane tension was also reported [73]. Vacuoles play an essential role during osmotic cytosol fluctuations under drought conditions by changing their size and shape [77]. The quenching of *Vvi*TIP2; 1 permeability in an internal pressure-dependent manner [73] suggests that membrane tension prompts conformational changes of *Vvi*TIP2; 1 from an open to a closed state, inducing the closure of water channels to prevent rapid water loss [73]. Gating of water channels by osmotic or hydrostatic tension has also been reported in other plants, implying that high tension changes the aquaporin conformation from an open to close state or causes the structural collapse to close the protein channel [121,122]. Mechanosensitive gating was also observed in maize roots [123]. Similarly, molecular dynamic simulations of the crystal structure of yeast aquaporin, Aqy1, supported that surface tension and membrane curvature may mediate mechanosensitive gating [124].

4.3. Functional regulation by aquaporin terminals

Numerous studies have shown that the conserved phosphorylation sites generally at serine/threonine are located at N- and C-terminals of aquaporins [4,5,55]. Phosphorylation can directly regulate the aquaporin gating, trafficking, and their functionality. In grapevine, direct evidence of phosphorylation-mediated channel regulation is not available. However, one of our recent studies demonstrated that C-terminal extension in *Vvi*NIP6; 1 improved the water and glycerol permeability [32]. The sequence analysis showed that besides the presence of a conserved phosphorylation site of GmNod26 at Ser^{262} [125], six additional putative phosphorylation sites were also predicted in the extended C-terminal. This study suggested that additional phosphorylation sites are involved in gating and improved targeting of the channels to the membrane, as observed in C-terminal extension of *Arabidopsis* AtNIP7; 1 [88]. Sequence analysis in this review showed conserved phosphorylation sites in C-and N-termini of the respective groups of NIPs, as mentioned by Wallace et al.(2006) [88] (Fig. 2). Recently, N-terminal phosphorylation at threonine in the conserved TPG (Threonine-Proline-Glycine) repeat of *Arabidopsis* AtNIP5; 1 was shown to

regulate the polar localization of this boron channel in the plasma membrane of root cells [126]. We identified this conserved sequence in both *Vvi*NIP5; 1 and *Vvi*NIP6; 1 of grapevine in this review (Fig. 2).

Our previous study also analysed the conserved phosphorylation sites in grapevine PIPs and TIPs [42]. A serine residue in loop B, identified as phosphorylation site (Ser^{115}) in spinach SoPIP2; 1 [127], was shown to be conserved in all plant PIPs and some TIPs [128], including grapevine PIPs, TIPs [42] and *Vvi*XIP1 (Fig. 2). Additionally, serine in consensus sequences of loop D (N/SARDSHVP) and C-terminal (Lys-x-xx- Ser-x-Arg) was identified in grapevine PIPs [71,129]. Unlike PIPs, TIPs, and NIPs, regulatory mechanisms of XIPs have been scarcely explored. A recent mass spectrometry study confirmed the phosphorylation sites at five residues (four serine and one threonine) in the N-terminal of *Nb*XIP1; 1a in *Nicotiana benthamiana* [35]. However, sequence analysis showed that none of the serine residues was present at equivalent positions in grapevine *Vvi*XIP1 except Thr^{26} (Fig. 2). The unusual retention of *Vvi*XIP1 in the endoplasmic reticulum may be explained by the lack of sufficient phosphorylation sites at the N- and C-termini of this protein [38].

4.4. Inhibition by mercury chloride

Mercury ($HgCl_2$) is frequently used as blocker of aquaporin transport activity (reviewed by Ref. [7]). All grapevine aquaporins functionally characterized up to now, showed significant mercury-dependent inhibition for water, glycerol, and H_2O_2 transport [32,38,42,59]. Mercurial compounds bind to the thiol group of the cysteine residue located downstream to the first NPA motif, which blocks the pore and subsequently shuts-down the aqueous pathway [4,42]. In grapevine, this cysteine residue was conserved in loop C of TIPs, whereas in PIPs, two cysteine residues in the second and third transmembrane helices were implied for mercury binding [42,130]. Due to cell toxicity induced by mercury, researchers are in continuous urge to find out safer and specific potent aquaporin blockers (reviewed by Ref. [5]).

5. Ecophysiological perspectives: role of aquaporins in grapevine response to drought stress

To cope with water stress, grapevine developed efficient adaptation mechanisms to transfer water from roots to their growing aerial organs mediating an efficient stomatal control of transpiration [1] and an efficient xylem embolism avoidance system [67,131,132], as well as osmotic adjustment ability [133,134]. Aquaporins are deeply involved in the non-vascular transport of water through inter-and intracellular pathways [7] within the plant. They contribute to rapid and reversible regulation of cells hydraulic conductance in several organs by adjusting the membrane water permeability [67,135,136] and play an essential role in the adaptation to drought condition by maintaining water and ion homeostasis of vines.

The large heterogeneity existing across *Vitis vinifera* L. genotypes results in differences in plant drought responses, in terms of their leaf stomatal aperture (iso and anisohydric behavior), hydraulic response (at root and leaf levels), modulated in part by aquaporin activities, and resulting in different water use efficiency [1,137–140]. However, the subject is far to be simple, since several grapevine genotypes showed the ability to switch its strategy by balancing from a near-isohydric behavior when the soil water content is low, to near-anisohydric behavior when levels of soil water content increase, thanks to aquaporins activities [67]. This leads several authors to consider aquaporins as a central master in the transduction of chemical signals (namely abscisic acid ABA) into hydraulic signals [116,141].

In addition to *Vitis vinifera* L. genotypes, differences among *Vitis* spp. rootstocks in terms of aquaporins expression under drought or in term of the proportion of conductance under the control of aquaporins have also been reported [12,142,143]. This was attributed to the contrasting ability to produce ABA among rootstocks under water stress [144] and the effect of ABA on the expression and activity of aquaporins [145,146]. Considering the importance of the chemical (namely ABA) and hydraulic (as well as aquaporins) signals exchanged between the rootstock and the scion, in particular under water deficit condition [139,147], and the correlation existing between root aquaporin expression and plant hydraulic conductance and transpiration (*E*) [67], the interpretation of aquaporin expression pattern in a grafted plant is much more complex, and scion effects should be considered [143,148]. In addition, the different function and regulation of aquaporins are highly variable among the distinct isoforms and across different genotypes and organs, impairing the interpretation of the role of an individual aquaporin [67].

5.1. Physiological roles of grapevine aquaporins in the leaves

Aquaporins have been linked to grapevine stomatal regulation, which varies between varieties [147,149,150]. Through hormonal and hydraulic mechanisms, stomata tightly regulate their aperture, measured by stomatal conductance (g_s) [151–153], in response to environmental conditions, and aquaporins have a key role in this process [11,146,154]. Rapid and reversible changes in leaf hydraulic conductance K_{leaf} involving aquaporins have been first observed in *Vitis* rootstock under water stress [155]. Later studies, reported the concomitance between the decrease in K_{leaf} (*c.a* 30%) and the down-regulation of some PIPs and TIPs isoforms, in particular *VviTIP2;1*, in the anisohydric Chardonnay genotype [156].

In the near-anisohydric Touriga Nacional grapevines, an up-regulation of aquaporins isoforms *VviTIP1;1* and *VviPIP2;1* in leaves was observed concomitant with a decline in leaf water potential, suggesting the role of aquaporins in maintaining plant water status during high transpiration [116]. Conversely, a synchrony between *VviTIP2;1* down-regulation and the increase in leaf ABA and xylem sap pH was observed, without any changes in K_{leaf}, suggesting a role of hormonal signalling. Stomatal activities depend on the conjugation of metabolism of abscisic acid (ABA), hydraulic signals, and the regulation of aquaporin activity. The ABA accumulation in leaves reduces the activity and/or expression of some aquaporins in the bundle sheath cells [157], which induce a decrease in K_{leaf} and exerts a feed-forward signal to stomata to close [158,159]. The involvement of ABA in the post-transcriptional regulation of aquaporin isoform (PIP2; 1), e.g., by OST1-dependent phosphorylation, in ABA-triggered stomatal closure was previously confirmed [146]. The involvement of *VviERF055* transcription factor in aquaporin expression, concomitant with the increase of ABA was also reported in *Vitis riparia* leaves in response to dehydration [160]. Altogether, this suggests the key role of aquaporins in the transduction of chemical signals into hydraulic signals and in the differentiation between the isohydric and anisohydric strategies [141]. Discrepancies in aquaporins expressions among genotypes under water stress could be accounted to isohydric strategy being linked to an interaction between hydraulic and ABA signalling, whereas anisohydric genotypes appear not to respond to such interaction [161,162].

Aquaporin expression in the anisohydric Chardonnay petioles (*VviPIP2;1* and *VviPIP2;2*) tend to be more down-regulated than in the isohydric Grenache petioles in stressed vines [13]. They also reported a significant down-regulation of leaf *VviPIP2;1* and *VviTIP2;1* in both cultivars under water stress correlated with significant decreases in leaf water potential but not with hydraulic

conductivity measurements of Grenache, suggesting their role in regulating petiole and leaf hydraulics only in anisohydric cultivars. Additionally, a differential circadian changes in aquaporins expression between both varieties, both in well-watered and water-stressed vines was observed [13], which could account for the iso- and anisohydric behavior of the cultivars. Recently, Dayer et al. (2020) [158] showed the correlation of *VviPIP1;1*, *VviPIP2;1* and *VviTIP2;1* with K_{leaf} in the isohydric Grenache but not in the near-anisohydric Syrah, attributing the result to a stronger leaf hydraulic control in the isohydric Grenache to changes in transpiration (E) which could account to stronger regulation of aquaporins.

Latest reports by Cochetel et al. (2020) [163], investigating transcriptomic responses to water stress of four genotypes: *Vitis champinii*, *Vitis riparia*, *Vitis vinifera*, and SC2 (*Vitis vinifera* x *Vitis girdiana*), showed upregulation of *VviPIP1;3* among all studied genotypes, while the only *PIP1;2* and *TIP1;3*, were decreased by dehydration especially in *Vitis champinii* leaves. In addition, *VviXIP2;2* expression was detected mainly in *Vitis champinii* and *Vitis riparia* mostly in leaves, while it was not detected in *Vitis vinifera* and *SC2,* and its expression was only significantly repressed in *Vitis champinii* leaves by the water stress.

In grapevine, reports show the positive correlation between fine-root K_{root} and leaf area and transpiration [12]. The underlying mechanisms governing these processes are not yet elucidated, however, changes in K_{root} by aquaporins could in turn modulate K_{leaf} and hydraulic conductivity of whole plants, impacting leaf growth [164]. Aquaporins have been suggested to regulate water transport across roots to ensure the transpirational demand need by a root water transport capacity [67]. Recent RNAseq data generated from *Vitis riparia* roots and shoots indicate that the only two aquaporins expressed in roots (VIT_08s0040g018 90, VIT_14S0108g00700) were upregulated under water stress, while the 16 aquaporins expressed in shoots were down-regulated, suggesting that aquaporins contributed to water uptake in the root while contributed to limit water loss in the shoot [165].

5.2. Physiological roles of grapevine aquaporins at root level

Aquaporins play a major role in the regulation of root hydraulic conductance (Lp_r) at the cellular level. In grapevine, the examination of four different rootstocks put in evidence the importance of metabolic control in root hydraulic adjustment and root embolism recovery, being higher in the genotypes known as more resistant to water stress [142,148]. Corroborating these data, a higher expression of *VviPIP2;1* and *VviPIP2;2* and an increase of Lp_r (approximately 60% increase) in the high-vigour rootstocks comparing with the low-vigour and drought-susceptible ones under well-watered conditions was reported [12]. Also, the involvement of root aquaporins in the regulation of Lp_r as a response to changes in plant E through shoot-to-root signalling was suggested [166]. In contrast, the control of *Lp*r of grapevine rootstock was suggested to be related to the activity of aquaporins in leaves, since no relation between root aquaporins and *Lp*r was found [155]. In this topic, in own-rooted Touriga Nacional grapevines, an increase in leaf temperature (linked to decrease in E) was observed with an increase in chemical signal (e.g. root [ABA] and xylem sap' pH) in water-stressed vines, concomitant with the downregulation of *VviPIP2;1* and *VviTIP2;2* in the roots and a decrease in Lp_r [116]. Recently, the stronger adjustment of Lp_r and greater sensitivity of stomata to (ABA) xylem sap were indicated keys to the greater conservation of soil moisture and rootstock resistance to drought [158]. Thus, like in leaves, that root hydraulic conductance is also a function of ABA levels (reviewed by Ref. [167]). Meggio et al. (2014) [168] suggested that under water deficit, roots of the more anisohydric vines are more able to adjust their osmolality as well as to maintain their cell integrity and consequently more able to sustain plant E than the isohydric vines. This observation is in agreement with the findings of [166], who observed a contribution of root aquaporins in Chardonnay to sustain leaf E, but not in Grenache, under water stress conditions. Besides, a decrease in Lp_r in Grenache was observed without any change in aquaporin expressions, concluding that in isohydric genotype, Lp_r reduction is mainly resulting *via* the apoplastic pathway [158]. In contrast, an upregulation of the root *VviPIP1;1* and *VviPIP2;1* was observed with a constant Lp_r in the near-anisohydric Syrah, suggesting a contribution of aquaporins to water transport *via* the symplastic pathway in the anisohydric genotypes. Cochetel et al. (2020) [163] showed an identical pattern of decrease in the expression of *VviPIP2;4*, *VviTIP2;1* and *VviTIP1;4* after 2 weeks of water stress in the different *Vitis* species, even though the down-regulation of these aquaporins was observed earlier in *Vitis champinii*. These data indicate a different sensitivity to water stress intensity in the aquaporin response among *Vitis* species.

Considering the importance of hydraulic conductivity of roots in cell division, elongation, and differentiation in the root tip, changes impacting *Lp*r by aquaporin expression and activities will impact root growth and root ability to absorb water. Consistent with this, artificial down-regulation of aquaporins resulted in compensatory increases in root size and *L*pr [74,75,169], suggesting the existence of a feedback mechanism connecting aquaporins to *Lp*r and root growth. On the other hand, in grapevine, the contribution of *Lp*r in the meristematic and elongation zones is greater than that in the secondary growth zone of fine roots, where abrupt drop in aquaporin expression was observed [115]. In fact, several PIPs (*VviPIP1;1*, *VviPIP1;2/1;4*, *VviPIP1;3/1;5*, *VviPIP2;1*, *VviPIP2;2*, *VviPIP2;3* and *VviPIP2;4*) were more expressed in root tips than in more mature suberized zones of the roots where the radial hydraulic conductivity is lower [12,115], suggesting that root tip are more prone to take up soil water [115]. However, the greater expression of aquaporin in root tip was only observed in the drought-resistant rootstock and not in the drought-susceptible rootstock [12,115]. This suggests that drought resistance in grapevine rootstock is related to aquaporin activity to maintain *Lp*r in the root tip. Consistent with this, recent reports by Cuneo et al. (2021) [170] demonstrate the ability of drought-resistant rootstocks to rapidly re-establish growth and Lp_r near the root tip upon re-watering by limiting competing sites along with the root cylinder.

In addition, new evidence showed the post-translational regulation of aquaporins (PIP2) by phosphorylation in maize upon the arbuscular mycorrhizal symbiosis subjected to water stress conditions [171], suggesting their role in the cell hydraulic conductivity maintenance under water stress. In grapevine, arbuscular mycorrhiza-colonized roots can exhibit more efficient water uptake and allow grapevine to cope with water stress [172]. Studies have suggested that increased root hydraulic conductivity in mycorrhizal roots could be the result of increased cell-to-cell water flux *via* aquaporins. However, the mechanisms of improved water uptake are still unclear, and no arbuscular mycorrhiza-inducible aquaporin gene has been identified so far.

5.3. Physiological roles of grapevine aquaporins at berry level

Different studies reported the involvement of aquaporins in the ripening processes in grape berries [135,136]. Aquaporins were either related to the changes in xylem hydraulic resistance (R_h) along maturation stages or to the accumulation of sugars at post-veraison stages.

At veraison, a hydraulic buffering of grape berries from the parent plant occurs and is mainly associated with the changes in R_h [136,173], suggesting that variation in berry R_h could be mediated

by changes in the expression and activity of aquaporins. Authors found that a peak of expression of predominant PIP isoforms, in particular, *VviPIP1;3* and *VviPIP2;1*, occurs at veraison, concomitant with a harsh decrease in R_h. The up-regulation of these aquaporins is in alignment with data previously reported by Fouquet et al. (2008) [135], and could be related to an interaction between these aquaporins to increase membrane permeability. After veraison, R_h increases to attain a maximum level at the maturation stage, negatively correlating with the expression of predominant aquaporins, in particular, *VviPIP1;3* and *VviPIP2;1*, while other non-predominant isoforms showed high levels of expression along maturation stages. The authors [136] proposed that the influence of aquaporins on berry hydraulics (R_{berry}) will depend on aquaporin tissue localization and suggested that significant changes in aquaporin expression in underrepresented tissues could be masked by changes taking place in tissues having minimal impact on berry hydraulics in general.

On the other hand, aquaporin expression may not significantly impact R_{berry} but may contribute to the ripening process in other ways. Recent studies have demonstrated a large increase in hydrogen peroxide production postveraison [174], which could be transported by aquaporins across membranes. In addition, the ripening process in general is associated with large increases in sugar transport and accumulation, changes in cell wall metabolism, and changes in turgor, all of which are certainly impacted by aquaporins and their modulation of membrane water permeability. In this context, it was described that PIPs and TIPs are highly expressed in expanding berry cells and their expression is upregulated concomitantly with the increase of expression in sugar transporters, suggesting the existence of a link between sugar and water transports [135]. In an earlier study by Picaud et al., 2003 [175], the expression of PIP1 aquaporins increased in post-veraison berries concomitant with the accumulation of sugars in the mesocarp. More recently, Coetzee et al. [176] confirmed the tight association between sugar accumulation and *VviPIP2;1* expression in Shiraz berries, suggesting sugar loading as the main osmotic driver for the accumulation of water in the grape berry along ripening.

A close analysis of aquaporin expression was examined by Wong et al. (2018) [18] highlighted the relationships between aquaporins and functional categories involved in cell wall modification and transport, as well as with other aquaporins revealing a strong co-regulation within the family itself. Consistent with these results, the increase of berry size was associated with high levels of transcripts related to aquaporins, as well as transcripts related to cell wall modification, after ethylene application [177].

This later study suggests the involvement of aquaporins in hormonal response during grape berry ripening. In this regard, Espinoza et al. (2006) [178] showed the potential role of gibberellins in the control of PIP and TIP gene expression in berries after veraison as a way to sustain the water flow into berries during berry growth. Ziliotto (2012) [179]showed the regulation of *VviPIP1* by auxins in berries during ripening and reports by Pilati et al. (2007) [174] suggested the possible link between ABA and expression of several *VviPIP* isoforms during ripening.

On the other hand, the expression pattern of aquaporins is highly dependent on berry phenological stages. In a previous study, two isoforms assigned to subfamily PIP1 were specifically up-regulated after veraison [175]. Similarly, Choat et al. (2009) [136] demonstrated that both PIP1 and PIP2 were upregulated after veraison. Pilati et al. (2007) [174] demonstrate that several PIPs, TIPs, and NIPs were highly expressed before veraison, while other PIP2 were up-regulated during maturation. More recently, Wong et al. (2018) [18] showed evidence of the existence of a set of aquaporins more related to young green berries (e.g *VviTIP1;2*, *VviTIP2;1*, *VviPIP1;2*, *VviPIP1;3*, *VviPIP2;1*, *VviPIP2;3*), while another set was linked to maturation stages (*VviTIP1;2*, *VviTIP1;3*, *VviPIP2;3* and *VviPIP2;5*). These authors also showed the dependence of aquaporin expression on the berry tissues (exocarp or mesocarp), in consistence with previous data [180], indicating the time-lapse between the up-regulation of aquaporins in mesocarp and in the skin at veraison.

Curiously, only a few reports showed the effect of water stress in grape berry aquaporins [181]. micro-array analysis observed a decrease in PIP aquaporin abundance of berries under water stress, and Noronha et al. (2014) [33] showed a slight down-regulation for *VviSIP1* in mature berries. In addition, interesting data by Noronha et al. (2014) [33] showed the dependence of single aquaporin on the genotype. These authors showed that expression of *VviSIP1* increase from green to mature berries in Vinhão genotype, while the opposite trend of expression was observed in Aragones genotype.

In addition, some reports have speculated that aquaporins may facilitate small ion transport and/or osmoregulation [182]. However, the details of mineral transport across the plasma membrane and the tonoplast into the mesocarp cells are not yet well elucidated. It became clear that still information is needed in what concerns the expression and activity of aquaporins in the different berry tissues, namely concerning key processes that drive aquaporin modulation and link with water accumulation into berry pericarp cells.

6. Concluding remarks

Grapevine response to drought stress is a complex network of different physiological and biochemical mechanisms, but aquaporins certainly play pivotal roles in the regulation of the plant hydraulic conductance at molecular level. They have been identified and found in different plant compartments in grapevine, and are expressed in all grapevine tissues. When expressed in yeast, grapevine aquaporins showed diverse substrate selectivity, and their gating is regulated by cytosolic pH, membrane tension, among other mechanisms. Despite the role of aquaporins is relatively well-known in a broad range of living organisms, our knowledge is still limited on how they cooperate at organ and tissue specific levels and how they are regulated in grapevine response to environmental stress, particularly against drought. It is known that under water stress conditions, the differential expression pattern of aquaporins isoforms has been shown among different genotypes with different drought tolerance strategies, as well as between tissues and portions of the same tissues. This pinpoints the versatility and diversity of aquaporins within the plant and their involvement in several different physiological processes in grapevine, but the puzzle is far from being completed. Thus, the involvement of aquaporins in the regulation of root hydraulic conductance (*Lpr*) under drought remains unclear, which requires further investigation of aquaporins expression and activity during drought and recovery stage in different root regions. Furthermore, exploring the role of aquaporins in arbuscular mycorrhiza-colonized roots will provide clues about plant-microbe interaction ultimately aiming the improvement of drought stress tolerance in grapevine.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by national funds through

FCT—Fundação para a Ciência e a Tecnologia, I.P., within DL 57/2016/CP1382/CT0012 employment contract and postdoctoral grant (SFRH/BPD/89427/2012) to F.S., in the scope of the project Linking Landscape, Environment, Agriculture and Food Research Centre (Ref. UIDB/04129/2020) and the project Centre of Molecular and Environmental Biology (Ref. UIDB/04050/2020). The work was also supported by FCT, CCDR-N (Norte Portugal Regional Coordination and Development Commission) and European Funds (FEDER/POCI/COMPETE2020) through the project AgriFoodXXI (NORTE-01-0145-FEDER-000041) and the research projects BerryPlastid (PTDC/BIA-FBT/28165/2017) and MitiVineDrought (PTDC/BIAFBT/30341/2017). HN was supported by postdoctoral grant from FCT (SFRH/BPD/115518/2016). OZ is supported by employment contract from CENTRO2020 (Regional Operational Program of the Center) within "Contratação de Recursos Humanos Altamente Qualificados" (Ref. CENTRO-04-3559-FSE-000093).

References

[1] M.M. Chaves, O. Zarrouk, R. Francisco, J.M. Costa, T. Santos, A.P. Regalado, M.L. Rodrigues, C.M. Lopes, Grapevine under deficit irrigation: hints from physiological and molecular data, Ann. Bot. 105 (2010) 661–676.
[2] G. Gutiérrez-Gamboa, W. Zheng, F.M. de Toda, Current viticultural techniques to mitigate the effects of global warming on grape and wine quality: a comprehensive review, Food Res. Int. (2020) 109946.
[3] M.R. Mozell, L. Thach, The impact of climate change on the global wine industry: challenges & solutions, Wine Economics and Policy 3 (2014) 81–89.
[4] C. Maurel, L. Verdoucq, D.-T. Luu, V. Santoni, Plant aquaporins: membrane channels with multiple integrated functions, Annu. Rev. Plant Biol. 59 (2008) 595–624.
[5] R. Kapilan, M. Vaziri, J.J. Zwiazek, Regulation of aquaporins in plants under stress, Biol. Res. 51 (2018) 1–11.
[6] J.Å. Danielson, U. Johanson, Unexpected complexity of the aquaporin gene family in the moss Physcomitrella patens, BMC Plant Biol. 8 (2008) 45.
[7] C. Maurel, Y. Boursiac, D.-T. Luu, V. Santoni, Z. Shahzad, L. Verdoucq, Aquaporins in plants, Physiol. Rev. 95 (2015) 1321–1358.
[8] R.M. Hove, M. Bhave, Plant aquaporins with non-aqua functions: deciphering the signature sequences, Plant Mol. Biol. 75 (2011) 413–430.
[9] B. Pommerrenig, T.A. Diehn, G.P. Bienert, Metalloido-porins: essentiality of Nodulin 26-like intrinsic proteins in metalloid transport, Plant Sci. 238 (2015) 212–227.
[10] F. Chaumont, M. Moshelion, M.J. Daniels, Regulation of plant aquaporin activity, Biol. Cell. 97 (2005) 749–764.
[11] F. Chaumont, S.D. Tyerman, Aquaporins: highly regulated channels controlling plant water relations, Plant Physiol. 164 (2014) 1600–1618.
[12] G.A. Gambetta, C.M. Manuck, S.T. Drucker, T. Shaghasi, K. Fort, M.A. Matthews, M.A. Walker, A.J. McElrone, The relationship between root hydraulics and scion vigour across Vitis rootstocks: what role do root aquaporins play? J. Exp. Bot. 63 (2012) 6445–6455.
[13] M.C. Shelden, R. Vandeleur, B.N. Kaiser, S.D. Tyerman, 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 (2017) 1893.
[14] J.P. Bezerra-Neto, F.C. de Araújo, J.R. Ferreira-Neto, M.D. da Silva, V. Pandolfi, F.F. Aburjaile, T. Sakamoto, R.L. de Oliveira Silva, E.A. Kido, L.L. Barbosa Amorim, Plant aquaporins: diversity, evolution and biotechnological applications, Curr. Protein Pept. Sci. 20 (2019) 368–395.
[15] T. Laloux, B. Junqueira, L.C. Maistriaux, J. Ahmed, A. Jurkiewicz, F. Chaumont, Plant and mammal aquaporins: same but different, Int. J. Mol. Sci. 19 (2018) 521.
[16] R. Zardoya, X. Ding, Y. Kitagawa, M.J. Chrispeels, Origin of plant glycerol transporters by horizontal gene transfer and functional recruitment, Proc. Natl. Acad. Sci. Unit. States Am. 99 (2002) 14893–14896.
[17] K.L. Adams, J.F. Wendel, Polyploidy and genome evolution in plants, Curr. Opin. Plant Biol. 8 (2005) 135–141.
[18] D.C.J. Wong, L. Zhang, I. Merlin, S.D. Castellarin, G.A. Gambetta, Structure and transcriptional regulation of the major intrinsic protein gene family in grapevine, BMC Genom. 19 (2018) 248.
[19] H. Sonah, R.K. Deshmukh, C. Labbé, R.R. Bélanger, Analysis of aquaporins in Brassicaceae species reveals high-level of conservation and dynamic role against biotic and abiotic stress in canola, Sci. Rep. 7 (2017) 1–17.
[20] F. Abascal, I. Irisarri, R. Zardoya, Diversity and evolution of membrane intrinsic proteins, Biochim. Biophys. Acta Gen. Subj. 1840 (2014) 1468–1481.
[21] M.C. Shelden, S.M. Howitt, B.N. Kaiser, S.D. Tyerman, Identification and functional characterisation of aquaporins in the grapevine, Vitis vinifera, Funct. Plant Biol. 36 (2009) 1065–1078.
[22] K. Murata, K. Mitsuoka, T. Hirai, T. Walz, P. Agre, J.B. Heymann, A. Engel, Y. Fujiyoshi, Structural determinants of water permeation through aquaporin-1, Nature 407 (2000) 599–605.
[23] D. Fu, A. Libson, L.J. Miercke, C. Weitzman, P. Nollert, J. Krucinski, R.M. Stroud, Structure of a glycerol-conducting channel and the basis for its selectivity, Science 290 (2000) 481–486.
[24] U. Johanson, M. Karlsson, I. Johansson, S. Gustavsson, S. Sjövall, L. Fraysse, A.R. Weig, P. Kjellbom, The complete set of genes encoding major intrinsic proteins in Arabidopsis provides a framework for a new nomenclature for major intrinsic proteins in plants, Plant Physiol. 126 (2001) 1358–1369.
[25] G. Soto, K. Alleva, G. Amodeo, J. Muschietti, N.D. Ayub, New insight into the evolution of aquaporins from flowering plants and vertebrates: orthologous identification and functional transfer is possible, Gene 503 (2012) 165–176.
[26] A. Hussain, R. Tanveer, G. Mustafa, M. Farooq, I. Amin, S. Mansoor, Comparative phylogenetic analysis of aquaporins provides insight into the gene family expansion and evolution in plants and their role in drought tolerant and susceptible chickpea cultivars, Genomics 112 (2020) 263–275.
[27] N. Saitou, M. Nei, The neighbor-joining method: a new method for reconstructing phylogenetic trees, Mol. Biol. Evol. 4 (1987) 406–425.
[28] J. Felsenstein, Confidence limits on phylogenies: an approach using the bootstrap, Evolution 39 (1985) 783–791.
[29] S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura, Mega X: molecular evolutionary genetics analysis across computing platforms, Mol. Biol. Evol. 35 (2018) 1547–1549.
[30] D. Gomes, A. Agasse, P. Thiébaud, S. Delrot, H. Gerós, F. Chaumont, Aquaporins are multifunctional water and solute transporters highly divergent in living organisms, Biochim. Biophys. Acta Biomembr. 1788 (2009) 1213–1228.
[31] M.G. Fortin, N.A. Morrison, D.P.S. Verma, Nodulin-26, a peribacteroid membrane nodulin is expressed independently of the development of the peribacteroid compartment, Nucleic Acids Res. 15 (1987) 813–824.
[32] F. Sabir, S. Gomes, M.C. Loureiro-Dias, G. Soveral, C. Prista, Molecular and functional characterization of grapevine nips through heterologous expression in aqy-null Saccharomyces cerevisiae, Int. J. Mol. Sci. 21 (2020) 663.
[33] H. Noronha, A. Agasse, A.P. Martins, M.C. Berny, D. Gomes, O. Zarrouk, P. Thiebaud, S. Delrot, G. Soveral, F. Chaumont, The grape aquaporin VvSIP1 transports water across the ER membrane, J. Exp. Bot. 65 (2014) 981–993.
[34] H. Ampah-Korsah, Y. Sonntag, A. Engfors, A. Kirscht, P. Kjellbom, U. Johanson, Single amino acid substitutions in the selectivity filter render NbXIP1; 1α aquaporin water permeable, BMC Plant Biol. 17 (2017) 61.
[35] H. Ampah-Korsah, H.I. Anderberg, A. Engfors, A. Kirscht, K. Nordén, S. Kjellstrom, P. Kjellbom, U. Johanson, The aquaporin splice variant NbXIP1; 1α is permeable to boric acid and is phosphorylated in the N-terminal domain, Front. Plant Sci. 7 (2016) 862.
[36] G.P. Bienert, M.D. Bienert, T.P. Jahn, M. Boutry, F. Chaumont, Solanaceae XIPs are plasma membrane aquaporins that facilitate the transport of many uncharged substrates, Plant J. 66 (2011) 306–317.
[37] J. Venkatesh, J.-W. Yu, D. Gaston, S.W. Park, Molecular evolution and functional divergence of X-intrinsic protein genes in plants, Mol. Genet. Genom. 290 (2015) 443–460.
[38] H. Noronha, D. Araújo, C. Conde, A.P. Martins, G. Soveral, F. Chaumont, S. Delrot, H. Gerós, 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 (2016), e0160976.
[39] S. Törnroth-Horsefield, Y. Wang, K. Hedfalk, U. Johanson, M. Karlsson, E. Tajkhorshid, R. Neutze, P. Kjellbom, Structural mechanism of plant aquaporin gating, Nature 439 (2006) 688–694.
[40] A. Kirscht, S.S. Kaptan, G.P. Bienert, F. Chaumont, P. Nissen, B.L. de Groot, P. Kjellbom, P. Gourdon, U. Johanson, Crystal structure of an ammonia-permeable aquaporin, PLoS Biol. 14 (2016), e1002411.
[41] A. Froger, D. Thomas, C. Delamarche, B. Tallur, Prediction of functional residues in water channels and related proteins, Protein Sci. 7 (1998) 1458–1468.
[42] F. Sabir, M.J. Leandro, A.P. Martins, M.C. Loureiro-Dias, T.F. Moura, G. Soveral, C. Prista, 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 (2014), e102087.
[43] R.K. Deshmukh, J. Vivancos, G. Ramakrishnan, V. Guérin, G. Carpentier, H. Sonah, C. Labbé, P. Isenring, F.J. Belzile, R.R. Bélanger, A precise spacing between the NPA domains of aquaporins is essential for silicon permeability in plants, Plant J. 83 (2015) 489–500.
[44] H. Noronha, A. Silva, N. Mitani-Ueno, C. Conde, F. Sabir, C. Prista, G. Soveral, P. Isenring, J.F. Ma, R.R. Bélanger, The VvNIP2; 1 aquaporin is a grapevine silicon channel, J. Exp. Bot. 71 (21) (2020) 6789–6798.
[45] X.-G. Guan, W.-H. Su, F. Yi, D. Zhang, F. Hao, H.-G. Zhang, Y.-J. Liu, X.-C. Feng, T.-H. Ma, NPA motifs play a key role in plasma membrane targeting of aquaporin-4, IUBMB Life 62 (2010) 222–226.
[46] A.K. Azad, N. Yoshikawa, T. Ishikawa, Y. Sawa, H. Shibata, Substitution of a single amino acid residue in the aromatic/arginine selectivity filter alters the transport profiles of tonoplast aquaporin homologs, Biochim. Biophys. Acta Biomembr. 1818 (2012) 1–11.
[47] P. Rougé, A. Barre, A molecular modeling approach defines a new group of Nodulin 26-like aquaporins in plants, Biochem. Biophys. Res. Commun. 367 (2008) 60–66.
[48] F. Sabir, A. Di Pizio, M.C. Loureiro-Dias, A. Casini, G. Soveral, C. Prista, Insights into the selectivity mechanisms of grapevine NIP aquaporins, Int. J. Mol. Sci. 21 (2020) 6697.
[49] I.S. Wallace, D.M. Roberts, Distinct transport selectivity of two structural

subclasses of the nodulin-like intrinsic protein family of plant aquaglyceroporin channels, Biochemistry 44 (2005) 16826–16834.
[50] N. Mitani-Ueno, N. Yamaji, F.-J. Zhao, J.F. Ma, The aromatic/arginine selectivity filter of NIP aquaporins plays a critical role in substrate selectivity for silicon, boron, and arsenic, J. Exp. Bot. 62 (2011) 4391–4398.
[51] M.M. Wudick, D.-T. Luu, C. Maurel, A look inside: localization patterns and functions of intracellular plant aquaporins, New Phytol. 184 (2009) 289–302.
[52] F. Chaumont, F. Barrieu, R. Jung, M.J. Chrispeels, Plasma membrane intrinsic proteins from maize cluster in two sequence subgroups with differential aquaporin activity, Plant Physiol. 122 (2000) 1025–1034.
[53] N. Uehlein, B. Otto, D.T. Hanson, M. Fischer, N. McDowell, R. Kaldenhoff, Function of Nicotiana tabacum aquaporins as chloroplast gas pores challenges the concept of membrane CO2 permeability, Plant Cell 20 (2008) 648–657.
[54] E. Zelazny, J.W. Borst, M. Muylaert, H. Batoko, M.A. Hemminga, F. Chaumont, FRET imaging in living maize cells reveals that plasma membrane aquaporins interact to regulate their subcellular localization, Proc. Natl. Acad. Sci. Unit. States Am. 104 (2007) 12359–12364.
[55] A.S. Chevalier, F. Chaumont, Trafficking of plant plasma membrane aquaporins: multiple regulation levels and complex sorting signals, Plant Cell Physiol. 56 (2015) 819–829.
[56] S. Padmanabhan, M.R. Biswal, R. Manjithaya, M.K. Prakash, Exploring the context of diacidic motif DE as a signal for unconventional protein secretion in eukaryotic proteins, Wellcome Open Research 3 (2018).
[57] E. Zelazny, U. Miecielica, J.W. Borst, M.A. Hemminga, F. Chaumont, An N-terminal diacidic motif is required for the trafficking of maize aquaporins ZmPIP2; 4 and ZmPIP2; 5 to the plasma membrane, Plant J. 57 (2009) 346–355.
[58] M. Sorieul, V. Santoni, C. Maurel, D.-T. Luu, Mechanisms and effects of retention of over-expressed aquaporin AtPIP2; 1 in the endoplasmic reticulum, Traffic 12 (2011) 473–482.
[59] L. Leitão, C. Prista, T.F. Moura, M.C. Loureiro-Dias, G. Soveral, Grapevine aquaporins: gating of a tonoplast intrinsic protein (TIP2; 1) by cytosolic pH, PloS One 7 (2012), e33219.
[60] C. Hachez, A. Besserer, A.S. Chevalier, F. Chaumont, Insights into plant plasma membrane aquaporin trafficking, Trends Plant Sci. 18 (2013) 344–352.
[61] A.S. Chevalier, G.P. Bienert, F. Chaumont, A new LxxxA motif in the transmembrane Helix3 of maize aquaporins belonging to the plasma membrane intrinsic protein PIP2 group is required for their trafficking to the plasma membrane, Plant Physiol. 166 (2014) 125–138.
[62] P. Cosson, F. Letourneur, Coatomer interaction with di-lysine endoplasmic reticulum retention motifs, Science 263 (1994) 1629–1631.
[63] K. Ishibashi, Aquaporin subfamily with unusual NPA boxes, Biochim. Biophys. Acta Biomembr. 1758 (2006) 989–993.
[64] T. Kamiya, M. Tanaka, N. Mitani, J.F. Ma, M. Maeshima, T. Fujiwara, NIP1; 1, an aquaporin homolog, determines the arsenite sensitivity of Arabidopsis thaliana, J. Biol. Chem. 284 (2009) 2114–2120.
[65] R. Ji, L. Zhou, J. Liu, Y. Wang, L. Yang, Q. Zheng, C. Zhang, B. Zhang, H. Ge, Y. Yang, Calcium-dependent protein kinase CPK31 interacts with arsenic transporter AtNIP1; 1 and regulates arsenite uptake in Arabidopsis thaliana, PloS One 12 (2017), e0173681.
[66] F. Barozzi, P. Papadia, G. Stefano, L. Renna, F. Brandizzi, D. Migoni, F.P. Fanizzi, G. Piro, G.-P. Di Sansebastiano, Variation in membrane trafficking linked to SNARE AtSYP51 interaction with aquaporin NIP1; 1, Front. Plant Sci. 9 (2019) 1949.
[67] R.K. Vandeleur, G. Mayo, M.C. Shelden, M. Gilliham, B.N. Kaiser, S.D. Tyerman, 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 (2009) 445–460.
[68] I. Perrone, G. Gambino, W. Chitarra, M. Vitali, C. Pagliarani, N. Riccomagno, R. Balestrini, R. Kaldenhoff, N. Uehlein, I. Gribaudo, The grapevine root-specific aquaporin VvPIP2; 4N controls root hydraulic conductance and leaf gas exchange under well-watered conditions but not under water stress, Plant Physiol. 160 (2012) 965–977.
[69] A. Madeira, T.F. Moura, G. Soveral, Detecting aquaporin function and regulation, Frontiers in Chemistry 4 (2016) 3.
[70] A. Biela, K. Grote, B. Otto, S. Hoth, R. Hedrich, R. Kaldenhoff, The Nicotiana tabacum plasma membrane aquaporin NtAQP1 is mercury-insensitive and permeable for glycerol, Plant J. 18 (1999) 565–570.
[71] K. Fetter, V. Van Wilder, M. Moshelion, F. Chaumont, Interactions between plasma membrane aquaporins modulate their water channel activity, Plant Cell 16 (2004) 215–228.
[72] Y. Temmei, S. Uchida, D. Hoshino, N. Kanzawa, M. Kuwahara, S. Sasaki, T. Tsuchiya, Water channel activities of Mimosa pudica plasma membrane intrinsic proteins are regulated by direct interaction and phosphorylation, FEBS (Fed. Eur. Biochem. Soc.) Lett. 579 (2005) 4417–4422.
[73] L. Leitão, C. Prista, M.C. Loureiro-Dias, T.F. Moura, G. Soveral, The grapevine tonoplast aquaporin TIP2; 1 is a pressure gated water channel, Biochem. Biophys. Res. Commun. 450 (2014) 289–294.
[74] F. Siefritz, M.T. Tyree, C. Lovisolo, A. Schubert, R. Kaldenhoff, PIP1 plasma membrane aquaporins in tobacco: from cellular effects to function in plants, Plant Cell 14 (2002) 869–876.
[75] P. Martre, R. Morillon, F. Barrieu, G.B. North, P.S. Nobel, M.J. Chrispeels, Plasma membrane aquaporins play a significant role during recovery from water deficit, Plant Physiol. 130 (2002) 2101–2110.
[76] J.A. Schuurmans, J.T. van Dongen, B.P. Rutjens, A. Boonman, C.M. Pieterse, A.C. Borstlap, Members of the aquaporin family in the developing pea seed coat include representatives of the PIP, TIP, and NIP subfamilies, Plant Mol. Biol. 53 (2003) 655–667.
[77] C. Maurel, J. Reizer, J.I. Schroeder, M.J. Chrispeels, The vacuolar membrane protein gamma-TIP creates water specific channels in Xenopus oocytes, EMBO J. 12 (1993) 2241–2247.
[78] M.J. Daniels, T.E. Mirkov, M.J. Chrispeels, The plasma membrane of Arabidopsis thaliana contains a mercury-insensitive aquaporin that is a homolog of the tonoplast water channel protein TIP, Plant Physiol. 106 (1994) 1325–1333.
[79] P. Kjellbom, C. Larsson, I. Johansson, M. Karlsson, U. Johanson, Aquaporins and water homeostasis in plants, Trends Plant Sci. 4 (1999) 308–314.
[80] B. Wu, E. Beitz, Aquaporins with selectivity for unconventional permeants, Cell. Mol. Life Sci. 64 (2007) 2413–2421.
[81] M. Katsuhara, S. Sasano, T. Horie, T. Matsumoto, J. Rhee, M. Shibasaka, Functional and molecular characteristics of rice and barley NIP aquaporins transporting water, hydrogen peroxide and arsenite, Plant Biotechnol. (2014), 0421, 14.
[82] N. Mitani, N. Yamaji, Y. Ago, K. Iwasaki, J.F. Ma, Isolation and functional characterization of an influx silicon transporter in two pumpkin cultivars contrasting in silicon accumulation, Plant J. 66 (2011) 231–240.
[83] Q. Liu, H. Wang, Z. Zhang, J. Wu, Y. Feng, Z. Zhu, Divergence in function and expression of the NOD26-like intrinsic proteins in plants, BMC Genom. 10 (2009) 313.
[84] F. Ishikawa, S. Suga, T. Uemura, M.H. Sato, M. Maeshima, Novel type aquaporin SIPs are mainly localized to the ER membrane and show cell-specific expression in Arabidopsis thaliana, FEBS (Fed. Eur. Biochem. Soc.) Lett. 579 (2005) 5814–5820.
[85] D. Lopez, M.B. Amira, D. Brown, B. Muries, N. Brunel-Michac, S. Bourgerie, B. Porcheron, R. Lemoine, H. Chrestin, E. Mollison, The Hevea brasiliensis XIP aquaporin subfamily: genomic, structural and functional characterizations with relevance to intensive latex harvesting, Plant Mol. Biol. 91 (2016) 375–396.
[86] D. Lopez, G. Bronner, N. Brunel, D. Auguin, S. Bourgerie, F. Brignolas, S. Carpin, C. Tournaire-Roux, C. Maurel, B. Fumanal, Insights into Populus XIP aquaporins: evolutionary expansion, protein functionality, and environmental regulation, J. Exp. Bot. 63 (2012) 2217–2230.
[87] P. Kjellbom, C. Larsson, I. Johansson, M. Karlsson, U. Johanson, Aquaporins and water homeostasis in plants, Trends Plant Sci. 4 (1999) 308–314.
[88] I.S. Wallace, W.-G. Choi, D.M. Roberts, The structure, function and regulation of the nodulin 26-like intrinsic protein family of plant aquaglyceroporins, Biochim. Biophys. Acta Biomembr. 1758 (2006) 1165–1175.
[89] Y. Wang, K. Schulten, E. Tajkhorshid, What makes an aquaporin a glycerol channel? A comparative study of AqpZ and GlpF, Structure 13 (2005) 1107–1118.
[90] I.S. Wallace, D.M. Wills, J.F. Guenther, D.M. Roberts, Functional selectivity for glycerol of the nodulin 26 subfamily of plant membrane intrinsic proteins, FEBS (Fed. Eur. Biochem. Soc.) Lett. 523 (2002) 109–112.
[91] S. Hohmann, M. Krantz, B. Nordlander, Yeast osmoregulation, in: Methods in Enzymology, Elsevier, 2007, pp. 29–45.
[92] A. Conde, A. Regalado, D. Rodrigues, J.M. Costa, E. Blumwald, M.M. Chaves, H. Gerós, Polyols in grape berry: transport and metabolic adjustments as a physiological strategy for water-deficit stress tolerance in grapevine, J. Exp. Bot. 66 (2015) 889–906.
[93] S. Aubert, E. Gout, R. Bligny, R. Douce, Multiple effects of glycerol on plant cell metabolism. Phosphorus-31 nuclear magnetic resonance studies, J. Biol. Chem. 269 (1994) 21420–21427.
[94] J. Hu, Y. Zhang, J. Wang, Y. Zhou, Glycerol affects root development through regulation of multiple pathways in Arabidopsis, PloS One 9 (2014), e86269.
[95] G.P. Bienert, A.L. Møller, K.A. Kristiansen, A. Schulz, I.M. Møller, J.K. Schjoerring, T.P. Jahn, Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes, J. Biol. Chem. 282 (2007) 1183–1192.
[96] M. Dynowski, G. Schaaf, D. Loque, O. Moran, U. Ludewig, Plant plasma membrane water channels conduct the signalling molecule H2O2, Biochem. J. 414 (2008) 53–61.
[97] G.P. Bienert, R.B. Heinen, M.C. Berny, F. Chaumont, Maize plasma membrane aquaporin ZmPIP2; 5, but not ZmPIP1; 2, facilitates transmembrane diffusion of hydrogen peroxide, Biochim. Biophys. Acta Biomembr. 1838 (2014) 216–222.
[98] C.H. Foyer, G. Noctor, Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria, Physiol. Plantarum 119 (2003) 355–364.
[99] G.P. Bienert, J.K. Schjoerring, T.P. Jahn, Membrane transport of hydrogen peroxide, Biochim. Biophys. Acta Biomembr. 1758 (2006) 994–1003.
[100] K. Miwa, T. Fujiwara, Boron transport in plants: co-ordinated regulation of transporters, Ann. Bot. 105 (2010) 1103–1108.
[101] J. Takano, M. Wada, U. Ludewig, G. Schaaf, N. Von Wirén, T. Fujiwara, The Arabidopsis major intrinsic protein NIP5; 1 is essential for efficient boron uptake and plant development under boron limitation, Plant Cell 18 (2006) 1498–1509.
[102] C. Dordas, M.J. Chrispeels, P.H. Brown, Permeability and channel-mediated transport of boric acid across membrane vesicles isolated from squash roots, Plant Physiol. 124 (2000) 1349–1362.

[103] K.L. Fitzpatrick, R.J. Reid, The involvement of aquaglyceroporins in transport of boron in barley roots, Plant Cell Environ. 32 (2009) 1357–1365.
[104] M. Tanaka, I.S. Wallace, J. Takano, D.M. Roberts, T. Fujiwara, NIP6; 1 is a boric acid channel for preferential transport of boron to growing shoot tissues in Arabidopsis, Plant Cell 20 (2008) 2860–2875.
[105] E. Maciaszczyk-Dziubinska, D. Wawrzycka, R. Wysocki, Arsenic and antimony transporters in eukaryotes, Int. J. Mol. Sci. 13 (2012) 3527–3548.
[106] G.P. Bienert, M. Thorsen, M.D. Schüssler, H.R. Nilsson, A. Wagner, M.J. Tamás, T.P. Jahn, A subgroup of plant aquaporins facilitate the bi-directional diffusion of as (OH) 3 and Sb (OH) 3 across membranes, BMC Biol. 6 (2008) 26.
[107] J.F. Ma, N. Yamaji, N. Mitani, X.-Y. Xu, Y.-H. Su, S.P. McGrath, F.-J. Zhao, Transporters of arsenite in rice and their role in arsenic accumulation in rice grain, Proc. Natl. Acad. Sci. Unit. States Am. 105 (2008) 9931–9935.
[108] J.E. Hayes, M. Pallotta, U. Baumann, B. Berger, P. Langridge, T. Sutton, Germanium as a tool to dissect boron toxicity effects in barley and wheat, Funct. Plant Biol. 40 (2013) 618–627.
[109] J.F. Ma, N. Mitani, S. Nagao, S. Konishi, K. Tamai, T. Iwashita, M. Yano, Characterization of the silicon uptake system and molecular mapping of the silicon transporter gene in rice, Plant Physiol. 136 (2004) 3284–3289.
[110] D. Coskun, R. Deshmukh, H. Sonah, J.G. Menzies, O. Reynolds, J.F. Ma, H.J. Kronzucker, R.R. Bélanger, in: Defence of the Selective Transport and Role of Silicon in Plants, 2019.
[111] J.F. Ma, K. Tamai, N. Yamaji, N. Mitani, S. Konishi, M. Katsuhara, M. Ishiguro, Y. Murata, M. Yano, A silicon transporter in rice, Nature 440 (2006) 688–691.
[112] A. Yaneff, L. Sigaut, M. Marquez, K. Alleva, L.I. Pietrasanta, G. Amodeo, Heteromerization of PIP aquaporins affects their intrinsic permeability, Proc. Natl. Acad. Sci. Unit. States Am. 111 (2014) 231–236.
[113] C. Jozefkowicz, L. Sigaut, F. Scochera, G. Soto, N. Ayub, L.I. Pietrasanta, G. Amodeo, F.L.G. Flecha, K. Alleva, PIP water transport and its pH dependence are regulated by tetramer stoichiometry, Biophys. J. 110 (2016) 1312–1321.
[114] S. Delrot, S. Picaud, J.P. Gaudillere, Water transport and aquaporins in grapevine, in: Molecular Biology & Biotechnology of the Grapevine, Springer, 2001, pp. 241–262.
[115] G.A. Gambetta, J. Fei, T.L. Rost, T. Knipfer, M.A. Matthews, K.A. Shackel, M.A. Walker, A.J. McElrone, Water uptake along the length of grapevine fine roots: developmental anatomy, tissue-specific aquaporin expression, and pathways of water transport, Plant Physiol. 163 (2013) 1254–1265.
[116] O. Zarrouk, I. Garcia-Tejero, C. Pinto, T. Genebra, F. Sabir, C. Prista, T.S. David, M.C. Loureiro-Dias, M.M. Chave, Aquaporins isoforms in cv. Touriga Nacional grapevine under water stress and recovery—regulation of expression in leaves and roots, Agric. Water Manag. 164 (2016) 167–175.
[117] C. Tournaire-Roux, M. Sutka, H. Javot, E. Gout, P. Gerbeau, D.-T. Luu, R. Bligny, C. Maurel, Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins, Nature 425 (2003) 393–397.
[118] M. Sutka, K. Alleva, M. Parisi, G. Amodeo, Tonoplast vesicles of Beta vulgaris storage root show functional aquaporins regulated by protons, Biol. Cell. 97 (2005) 837–846.
[119] G. Soto, R. Fox, N. Ayub, K. Alleva, F. Guaimas, E.J. Erijman, A. Mazzella, G. Amodeo, J. Muschietti, TIP5; 1 is an aquaporin specifically targeted to pollen mitochondria and is probably involved in nitrogen remobilization in Arabidopsis thaliana, Plant J. 64 (2010) 1038–1047.
[120] A. Canessa Fortuna, G. Zerbetto De Palma, L. Aliperti Car, L. Armentia, V. Vitali, A. Zeida, D.A. Estrin, K. Alleva, Gating in plant plasma membrane aquaporins: the involvement of leucine in the formation of a pore constriction in the closed state, FEBS J. 286 (2019) 3473–3487.
[121] M. del Carmen Martínez-Ballesta, V. Martínez, M. Carvajal, Regulation of water channel activity in whole roots and in protoplasts from roots of melon plants grown under saline conditions, Funct. Plant Biol. 27 (2000) 685–691.
[122] Q. Ye, B. Wiera, E. Steudle, A cohesion/tension mechanism explains the gating of water channels (aquaporins) in Chara internodes by high concentration, J. Exp. Bot. 55 (2004) 449–461.
[123] X. Wan, E. Steudle, W. Hartung, Gating of water channels (aquaporins) in cortical cells of young corn roots by mechanical stimuli (pressure pulses): effects of ABA and of HgCl2, J. Exp. Bot. 55 (2004) 411–422.
[124] G. Fischer, U. Kosinska-Eriksson, C. Aponte-Santamaría, M. Palmgren, C. Geijer, K. Hedfalk, S. Hohmann, B.L. De Groot, R. Neutze, K. Lindkvist-Petersson, Crystal structure of a yeast aquaporin at 1.15 \AA reveals a novel gating mechanism, PLoS Biol. 7 (2009), e1000130.
[125] J.F. Guenther, N. Chanmanivone, M.P. Galetovic, I.S. Wallace, J.A. Cobb, D.M. Roberts, Phosphorylation of soybean nodulin 26 on serine 262 enhances water permeability and is regulated developmentally and by osmotic signals, Plant Cell 15 (2003) 981–991.
[126] S. Wang, A. Yoshinari, T. Shimada, I. Hara-Nishimura, N. Mitani-Ueno, J.F. Ma, S. Naito, J. Takano, Polar localization of the NIP5; 1 boric acid channel is maintained by endocytosis and facilitates boron transport in Arabidopsis roots, Plant Cell 29 (2017) 824–842.
[127] M. Nyblom, A. Frick, Y. Wang, M. Ekvall, K. Hallgren, K. Hedfalk, R. Neutze, E. Tajkhorshid, S. Törnroth-Horsefield, Structural and functional analysis of SoPIP2; 1 mutants adds insight into plant aquaporin gating, J. Mol. Biol. 387 (2009) 653–668.
[128] A.K. Azad, Y. Sawa, T. Ishikawa, H. Shibata, Characterization of protein phosphatase 2A acting on phosphorylated plasma membrane aquaporin of tulip petals, Biosci. Biotechnol. Biochem. 68 (2004) 1170–1174.
[129] S. Prak, S. Hem, J. Boudet, G. Viennois, N. Sommerer, M. Rossignol, C. Maurel, V. Santoni, Multiple phosphorylations in the C-terminal tail of plant plasma membrane aquaporins: role in subcellular trafficking of AtPIP2; 1 in response to salt stress, Mol. Cell. Proteomics 7 (2008) 1019–1030.
[130] S. Suga, M. Maeshima, Water channel activity of radish plasma membrane aquaporins heterologously expressed in yeast and their modification by site-directed mutagenesis, Plant Cell Physiol. 45 (2004) 823–830.
[131] C. Lovisolo, W. Hartung, A. Schubert, Whole-plant hydraulic conductance and root-to-shoot flow of abscisic acid are independently affected by water stress in grapevines, Funct. Plant Biol. 29 (2002) 1349–1356.
[132] G.A. Gambetta, J.C. Herrera, S. Dayer, Q. Feng, U. Hochberg, S.D. Castellarin, The physiology of drought stress in grapevine: towards an integrative definition of drought tolerance, J. Exp. Bot. 71 (2020) 4658–4676.
[133] M.L. Rodrigues, M.M. Chaves, R. Wendler, M.M. David, W.P. Quick, R.C. Leegood, M. Stitt, J.S. Pereira, Osmotic adjustment in water stressed grapevine leaves in relation to carbon assimilation, Funct. Plant Biol. 20 (1993) 309–321.
[134] A. Patakas, B. Nortsakis, Mechanisms involved in diurnal changes of osmotic potential in grapevines under drought conditions, J. Plant Physiol. 154 (1999) 767–774.
[135] R. Fouquet, C. Léon, N. Ollat, F. Barrieu, Identification of grapevine aquaporins and expression analysis in developing berries, Plant Cell Rep. 27 (2008) 1541–1550.
[136] B. Choat, G.A. Gambetta, K.A. Shackel, M.A. Matthews, Vascular function in grape berries across development and its relevance to apparent hydraulic isolation, Plant Physiol. 151 (2009) 1677–1687.
[137] M.M. Chaves, P.C. Harley, J.D. Tenhunen, O.L. Lange, Gas exchange studies in two Portuguese grapevine cultivars, Physiol. Plantarum 70 (1987) 639–647.
[138] B.J. Bota, J. Flexas, H. Medrano, Genetic variability of photosynthesis and water use in Balearic grapevine cultivars, Ann. Appl. Biol. 138 (2001) 353–361.
[139] C.J. Soar, J. Speirs, S.M. Maffei, A.B. Penrose, M.G. McCarthy, B.R. Loveys, Grape vine varieties Shiraz and Grenache differ in their stomatal response to VPD: apparent links with ABA physiology and gene expression in leaf tissue, Aust. J. Grape Wine Res. 12 (2006) 2–12.
[140] J. Flexas, J. Galmés, A. Gallé, J. Gulías, A. Pou, M. Ribas-Carbo, M. Tomàs, H. Medrano, Improving water use efficiency in grapevines: potential physiological targets for biotechnological improvement, Aust. J. Grape Wine Res. 16 (2010) 106–121.
[141] M. Moshelion, O. Halperin, R. Wallach, R.A.M. Oren, D.A. Way, Role of aquaporins in determining transpiration and photosynthesis in water-stressed plants: crop water-use efficiency, growth and yield, Plant Cell Environ. 38 (2015) 1785–1793.
[142] C. Lovisolo, S. Tramontini, J. Flexas, A. Schubert, Mercurial inhibition of root hydraulic conductance in Vitis spp. rootstocks under water stress, Environ. Exp. Bot. 63 (2008) 178–182.
[143] L. Rossdeutsch, Contribution du métabolisme de l'ABA et de la conductivité hydraulique à la réponse de la transpiration en situation de contrainte hydrique chez la Vigne: variabilité génétique et effets du greffage, PhD Thesis, Université de Bordeaux, 2015.
[144] L. Rossdeutsch, E. Edwards, S.J. Cookson, F. Barrieu, G.A. Gambetta, S. Delrot, N. Ollat, ABA-mediated responses to water deficit separate grapevine genotypes by their genetic background, BMC Plant Biol. 16 (2016) 1–15.
[145] R. Finkelstein, Abscisic acid synthesis and response, Arabidopsis Book 11 (2013), e0166.
[146] A. Grondin, O. Rodrigues, L. Verdoucq, S. Merlot, N. Leonhardt, C. Maurel, Aquaporins contribute to ABA-triggered stomatal closure through OST1-mediated phosphorylation, Plant Cell 27 (2015) 1945–1954.
[147] R.K. Vandeleur, G. Mayo, M.C. Shelden, M. Gilliham, B.N. Kaiser, S.D. Tyerman, 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 (2009) 445–460.
[148] S. Tramontini, M. Vitali, L. Centioni, A. Schubert, C. Lovisolo, Rootstock control of scion response to water stress in grapevine, Environ. Exp. Bot. 93 (2013) 20–26.
[149] A. Coupel-Ledru, S.D. Tyerman, D. Masclef, E. Lebon, A. Christophe, E.J. Edwards, T. Simonneau, Abscisic acid down-regulates hydraulic conductance of grapevine leaves in isohydric genotypes only, Plant Physiol. 175 (2017) 1121–1134.
[150] M. Vitali, H. Cochard, G. Gambino, A. Ponomarenko, I. Perrone, C. Lovisolo, VvPIP2; 4N aquaporin involvement in controlling leaf hydraulic capacitance and resistance in grapevine, Physiol. Plantarum 158 (2016) 284–296.
[151] A. Christmann, E.W. Weiler, E. Steudle, E. Grill, A hydraulic signal in root-to-shoot signalling of water shortage, Plant J. 52 (2007) 167–174.
[152] S.A. McAdam, F.C. Sussmilch, T.J. Brodribb, Stomatal responses to vapour pressure deficit are regulated by high speed gene expression in angiosperms, Plant Cell Environ. 39 (2016) 485–491.
[153] M.M. Chaves, J.M. Costa, O. Zarrouk, C. Pinheiro, C.M. Lopes, J.S. Pereira, Controlling stomatal aperture in semi-arid regions—the dilemma of saving water or being cool? Plant Sci. 251 (2016) 54–64.
[154] R.B. Heinen, Q. Ye, F. Chaumont, Role of aquaporins in leaf physiology, J. Exp. Bot. 60 (2009) 2971–2985.
[155] J. Galmés, A. Pou, M.M. Alsina, M. Tomas, H. Medrano, J. Flexas, Aquaporin expression in response to different water stress intensities and recovery in Richter-110 (Vitis sp.): relationship with ecophysiological status, Planta 226

(2007) 671–681.
[156] A. Pou, H. Medrano, J. Flexas, S.D. Tyerman, 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 (2013) 828–843.
[157] A. Shatil-Cohen, Z. Attia, M. Moshelion, Bundle-sheath cell regulation of xylem-mesophyll water transport via aquaporins under drought stress: a target of xylem-borne ABA? Plant J. 67 (2011) 72–80.
[158] S. Dayer, J.D. Scharwies, S.A. Ramesh, W. Sullivan, F.C. Doerflinger, V. Pagay, S.D. Tyerman, Comparing hydraulics between two grapevine cultivars reveals differences in stomatal regulation under water stress and exogenous ABA applications, Front. Plant Sci. 11 (2020) 705.
[159] C. Maurel, K. Prado, Aquaporins and leaf water relations, in: Plant Aquaporins, Springer, 2017, pp. 155–165.
[160] D.W. Hopper, R. Ghan, K.A. Schlauch, G.R. Cramer, Transcriptomic network analyses of leaf dehydration responses identify highly connected ABA and ethylene signaling hubs in three grapevine species differing in drought tolerance, BMC Plant Biol. 16 (2016) 1–20.
[161] F. Tardieu, T. Simonneau, Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours, J. Exp. Bot. (1998) 419–432.
[162] Á. Gallé, J. Csiszár, D. Benyó, G. Laskay, T. Leviczky, L. Erdei, I. Tari, Isohydric and anisohydric strategies of wheat genotypes under osmotic stress: biosynthesis and function of ABA in stress responses, J. Plant Physiol. 170 (2013) 1389–1399.
[163] N. Cochetel, R. Ghan, H.S. Toups, A. Degu, R.L. Tillett, K.A. Schlauch, G.R. Cramer, Drought tolerance of the grapevine, Vitis champinii cv. Ramsey, is associated with higher photosynthesis and greater transcriptomic responsiveness of abscisic acid biosynthesis and signaling, BMC Plant Biol. 20 (2020) 1–25.
[164] Y. Wang, Z. Zhao, F. Liu, L. Sun, F. Hao, Versatile roles of aquaporins in plant growth and development, Int. J. Mol. Sci. 21 (2020) 9485.
[165] V.S. Khadka, K. Vaughn, J. Xie, P. Swaminathan, Q. Ma, G.R. Cramer, A.Y. Fennell, Transcriptomic response is more sensitive to water deficit in shoots than roots of Vitis riparia (Michx.), BMC Plant Biol. 19 (2019) 1–20.
[166] R.K. Vandeleur, W. Sullivan, A. Athman, C. Jordans, M. Gilliham, B.N. Kaiser, S.D. Tyerman, Rapid shoot-to-root signalling regulates root hydraulic conductance via aquaporins, Plant Cell Environ. 37 (2014) 520–538.
[167] G.A. Gambetta, T. Knipfer, W. Fricke, A.J. McElrone, Aquaporins and root water uptake, in: Plant Aquaporins, Springer, 2017, pp. 133–153.
[168] F. Meggio, B. Prinsi, A.S. Negri, G. Simone Di Lorenzo, G. Lucchini, A. Pitacco, O. Failla, A. Scienza, M. Cocucci, L. Espen, Biochemical and physiological responses of two grapevine rootstock genotypes to drought and salt treatments, Aust. J. Grape Wine Res. 20 (2014) 310–323.
[169] R. Kaldenhoff, K. Grote, J.-J. Zhu, U. Zimmermann, Significance of plasmalemma aquaporins for water-transport in Arabidopsis thaliana, Plant J. 14 (1998) 121–128.
[170] I.F. Cuneo, F. Barrios-Masias, T. Knipfer, J. Uretsky, C. Reyes, P. Lenain, C.R. Brodersen, M.A. Walker, A.J. McElrone, Differences in grapevine rootstock sensitivity and recovery from drought are linked to fine root cortical lacunae and root tip function, New Phytol. 229 (2021) 272–283.
[171] G. Quiroga, G. Erice, L. Ding, F. Chaumont, R. Aroca, J.M. Ruiz-Lozano, The arbuscular mycorrhizal symbiosis regulates aquaporins activity and improves root cell water permeability in maize plants subjected to water stress, Plant Cell Environ. 42 (2019) 2274–2290.
[172] Á. Donkó, G. Zanathy, Z. Èros-Honti, S. Villangó, G.D. Bisztray, Changes of mycorrhizal colonization along moist gradient in a vineyard of Eger (Hungary), Acta Univ. Sapientiae, Agric. Environ. 6 (2014) 13–23.
[173] M. Keller, J.P. Smith, B.R. Bondada, Ripening grape berries remain hydraulically connected to the shoot, J. Exp. Bot. 57 (2006) 2577–2587.
[174] S. Pilati, M. Perazzolli, A. Malossini, A. Cestaro, L. Demattè, P. Fontana, A. Dal Ri, R. Viola, R. Velasco, C. Moser, Genome-wide transcriptional analysis of grapevine berry ripening reveals a set of genes similarly modulated during three seasons and the occurrence of an oxidative burst at veraison, BMC Genom. 8 (2007) 1–22.
[175] S. Picaud, F. Becq, F. Dédaldéchamp, A. Ageorges, S. Delrot, Cloning and expression of two plasma membrane aquaporins expressed during the ripening of grape berry, Funct. Plant Biol. 30 (2003) 621–630.
[176] Z.A. Coetzee, R.R. Walker, S. Liao, C. Barril, A.J. Deloire, S.J. Clarke, S.D. Tyerman, S.Y. Rogiers, Expression patterns of genes encoding sugar and potassium transport proteins are simultaneously upregulated or downregulated when carbon and potassium availability is modified in shiraz (Vitis vinifera L.) berries, Plant Cell Physiol. 60 (2019) 2331–2342.
[177] C. Chervin, A. Tira-umphon, N. Terrier, M. Zouine, D. Severac, J.-P. Roustan, Stimulation of the grape berry expansion by ethylene and effects on related gene transcripts, over the ripening phase, Physiol. Plantarum 134 (2008) 534–546.
[178] A. Espinoza, D. Contreras, M. Orellana, R. Perez, C. Aguirre, A. Castro, A. Riquelme, T. Fichet, M. Pinto, P. Hinrichsen, Modulation by gibberellic acid of aquaporin genes expression during berry development of grapevine (Vitis vinifera L.), in: IX International Conference on Grape Genetics and Breeding vol. 827, 2006, pp. 355–362.
[179] F. Ziliotto, M. Corso, F.M. Rizzini, A. Rasori, A. Botton, C. Bonghi, Grape berry ripening delay induced by a pre-véraison NAA treatment is paralleled by a shift in the expression pattern of auxin-and ethylene-related genes, BMC Plant Biol. 12 (2012) 1–15.
[180] J. Schlosser, N. Olsson, M. Weis, K. Reid, F. Peng, S. Lund, P. Bowen, Cellular expansion and gene expression in the developing grape (Vitis vinifera L.), Protoplasma 232 (2008) 255.
[181] J. Grimplet, L.G. Deluc, R.L. Tillett, M.D. Wheatley, K.A. Schlauch, G.R. Cramer, J.C. Cushman, Tissue-specific mRNA expression profiling in grape berry tissues, BMC Genom. 8 (2007) 1–23.
[182] S.Y. Rogiers, Z.A. Coetzee, R.R. Walker, A. Deloire, S.D. Tyerman, Potassium in the grape (Vitis vinifera L.) berry: transport and function, Front. Plant Sci. 8 (2017) 1629.