

RESEARCH PAPER

The relationship between root hydraulics and scion vigour across *Vitis* rootstocks: what role do root aquaporins play?

G. A. Gambetta², C. M. Manuck¹, S. T. Drucker², T. Shaghasi², K. Fort², M. A. Matthews², M. A. Walker² and A. J. McElrone^{1,2,*}

¹ USDA-ARS, Crops Pathology and Genetics Research Unit, Davis CA 95616, USA

² Department of Viticulture and Enology, University of California, Davis CA 95616, USA

* To whom correspondence should be addressed. E-mail: ajmcelrone@ucdavis.edu

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Abstract

Vitis vinifera scions are commonly grafted onto rootstocks of other grape species to influence scion vigour and provide resistance to soil-borne pests and abiotic stress; however, the mechanisms by which rootstocks affect scion physiology remain unknown. This study characterized the hydraulic physiology of *Vitis* rootstocks that vary in vigour classification by investigating aquaporin (*VvPIP*) gene expression, fine-root hydraulic conductivity (Lp_r), % aquaporin contribution to Lp_r , scion transpiration, and the size of root systems. Expression of several *VvPIP* genes was consistently greater in higher-vigour rootstocks under favourable growing conditions in a variety of media and in root tips compared to mature fine roots. Similar to *VvPIP* expression patterns, fine-root Lp_r and % aquaporin contribution to Lp_r determined under both osmotic (Lp_r^{Osm}) and hydrostatic (Lp_r^{Hyd}) pressure gradients were consistently greater in high-vigour rootstocks. Interestingly, the % aquaporin contribution was nearly identical for Lp_r^{Osm} and Lp_r^{Hyd} even though a hydrostatic gradient would induce a predominant flow across the apoplastic pathway. In common scion greenhouse experiments, leaf area-specific transpiration (E) and total leaf area increased with rootstock vigour and were positively correlated with fine-root Lp_r . These results suggest that increased canopy water demands for scion grafted onto high-vigour rootstocks are matched by adjustments in root-system hydraulic conductivity through the combination of fine-root Lp_r and increased root surface area.

Key words: fine-root hydraulics, grapevines, PIPs, plasma-membrane intrinsic proteins, root aquaporins, rootstocks.

Introduction

Perennial fruit crop scions are often grafted onto rootstocks for a variety of reasons, which include providing resistance to soil-borne pests, conferring resistance to water deficit and extreme soil types, and controlling growth of the scion (e.g. Pongrácz, 1983). Grafting affords some grower control over important agronomic traits and provides flexibility in growing a particular scion across diverse soil and environmental conditions. Grapevine rootstocks are commonly characterized according to vigour characteristics conferred to the scion (i.e. biomass accumulation and yield), which influence winegrape quality (Cortell *et al.*, 2005, 2007, 2008) and are known to alter scion gas exchange and water use

efficiency (Candolfi-Vasconcelos *et al.*, 1994). Despite their common usage in agriculture, the mechanisms through which rootstocks affect scion vigour and resistance to abiotic stress are not fully understood or ambiguous across crop systems.

For some perennial crop species, altered scion vigour has been linked to differences in hydraulic parameters of the root system. Greater whole-root-system hydraulic conductance has been documented in vigour-inducing rootstocks of apple, peach, olive, and kiwi (Atkinson *et al.*, 2003; Clearwater *et al.*, 2004; Nardini *et al.*, 2006; Solari *et al.*, 2006). Most of these studies measured whole root systems using a high-pressure-flow meter

and/or the evaporative flux method, and related results to the size of the root system (i.e., biomass or surface area). Other studies have compared the vascular anatomy between low- and high-vigour rootstocks of cherry and peach trees: several of these studies demonstrated positive correlations between calculated hydraulic conductance (based on xylem vessel diameters) and vigour (Olmstead *et al.*, 2006a, b; Goncalves *et al.*, 2007), while another found a negative correlation using a ratio of the phloem and xylem areas (Iwanami *et al.*, 2009).

Despite these recent efforts to assess the role of root-system hydraulics, the contribution of fine-root hydraulic properties to known differences among rootstocks has not been deeply studied. This is surprising since radial water absorption across fine roots constitutes a large proportion of the total resistance and is known to be limiting to water uptake in the root system (Frensch and Steudle, 1989; Steudle, 2001; Steudle and Meshcheryakov, 1996). Radial water uptake across fine roots occurs via the apoplastic (flow within the cell walls) and the cell-to-cell (C–C) pathways (Steudle, 2001). Because cell membranes must be crossed in the C–C pathway, transport efficiency of this pathway is thought to be affected by the activity, density, and location of aquaporins (i.e. water-specific protein channels embedded in cell membranes) (e.g. Knipfer and Fricke, 2010). Much work over the last decade and a half has demonstrated the role of aquaporins in affecting root hydraulic properties (Javot and Maurel, 2002), yet little work has addressed whether inherent differences in aquaporin activity contribute to differences in stress resistance and vigour potential among rootstocks for perennial crop species.

Recent studies utilizing molecular tools have demonstrated the importance of aquaporins to plant vigour and water relations of herbaceous species. For transgenic tobacco growing under favourable conditions, constitutive overexpression of *Arabidopsis AtPIP1b* increased transpiration rates and plant vigour (Aharon *et al.*, 2003), while antisense suppression of tobacco *NtAQPI* resulted in decreased root hydraulic conductance but had negligible effects on transpiration (Siefritz *et al.*, 2002). More recently Sade *et al.* (2010) demonstrated that constitutively overexpressing *NtAQPI* in *Arabidopsis* and tomato plants can enhance transpiration, photosynthesis, and shoot growth rates under favourable growing conditions. Lovisolo *et al.* (2007) conducted one of the only studies to assess links between aquaporin gene expression and rootstock effects in perennial woody plants and found a positive correlation between aquaporin expression and root-specific hydraulic conductance (i.e. per gram of root dryweight), which was actually higher in olive dwarfing rootstocks. However, whole-root-system hydraulic conductance was greater in high-vigour plants due to greater root biomass (Lovisolo *et al.*, 2007). This same research group found differential aquaporin activity, measured by mercurial inhibition, under drought conditions for grapevine rootstocks derived from varied *Vitis* species parentage (Lovisolo *et al.*, 2008).

Given the link between aquaporins and root hydraulic conductance, and that aquaporins respond to many of the factors used to differentiate grapevine rootstocks (i.e. water deficit, salt, anoxia), this study investigated their role in establishing differences among commercially available *Vitis* rootstocks under

favourable (i.e. non-stressed) growing conditions. The inherent differences in aquaporin gene expression (i.e. plasma-membrane intrinsic proteins, PIPs), fine-root hydraulic conductivity (L_p), and % aquaporin contribution to L_p , between these rootstocks were characterized and the data with differences in root and shoot biomass and scion transpiration were paired.

Materials and methods

Plant material and growth conditions

Bare-root, bench-grafted grapevines were obtained from commercial nurseries in California and used in all greenhouse experiments. A common *Vitis vinifera* cv. Cabernet Sauvignon scion was grafted onto the following rootstocks: 110R, 140R, 1103P, 5BB, SO4, 101-14Mgt, and 420A. These rootstocks were chosen based on their common usage in commercial vineyards, their varying species parentage, and their differential vigour and abiotic stress tolerance. Rootstocks 110R, 140R and 1103P (all with *Vitis berlandieri* × *Vitis rupestris* parentage) are commonly characterized as high vigour and drought resistant, rootstocks 5BB and SO4 (both with *V. berlandieri* × *Vitis riparia* parentage) are characterized as moderate vigour and drought intolerant, and rootstocks 101-14Mgt (*V. riparia* × *V. rupestris* parentage) and 420A (*V. berlandieri* × *V. riparia* parentage) are characterized as low-to-moderate vigour and drought intolerant (Pongrácz, 1983). The vigour classification put forth by Pongrácz (1983) has been validated recently by a multiyear and multisite rootstock trial in California (see data summarized briefly in the Results section below), and serves as the basis for the classifications in the figures and throughout the manuscript.

Vines were acquired as dormant material and stored on wood chips at approximately 7 °C until use in the experiments. Prior to planting, all plants were rinsed with tap water, washed in a 10% sodium hypochlorite solution, and re-rinsed in a succession of water baths. All buds were removed from the stems. Stems were then dipped into a low-temperature vat of melted wax for approximately 2 seconds and cooled in an ice bath to set the wax. Care was taken to prevent wax from getting onto the roots. Vines were potted into either soil or a hydroponics system (see below) within 24 h of washing/waxing and were grown in a greenhouse.

Grapevines were grown either hydroponically in soil, fritted clay, or a continuous re-circulating drip system (RDS) modelled after Wheatley *et al.* (2009). For hydroponics, ten RDS systems were used, each system consisting of eight pots (2.83 l each). Waxed vines were planted in treepots (TPOT1, Stuewe and Sons, Tangent, OR, USA), and the pots were filled with lightweight expanded clay aggregate pebbles (General Hydroponics, Sebastopol, CA, USA). Holes in the bottoms of the treepots allowed adequate drainage of hydroponic solution. The treepots were distributed evenly in a basin covered by a square piece (0.34 m²) of polyisocyanurate insulation (R-Matte Plus 3- thickness 1.9 cm, Rmax, Dallas, TX, USA) perforated with eight square holes to hold the treepots. A drainage hole at the bottom of each basin was fitted with a barbed tub outlet fitting (National Garden Wholesale, Vancouver, WA, USA) to which a flexible black tubing drain line was attached. The tubing drained into a 113-l plastic reservoir (Newell Rubbermaid, Sandy Springs, GA, USA) filled with modified Hoagland's solution. A 1.32 kl h⁻¹ Supreme Mag Drive utility pump (Danner Manufacturing, Central Islip, NY, USA) pumped the solution up from each reservoir into two multi-outlet Maverick Head drip manifolds (DIG Irrigation, Vista, CA, USA) per basin that distributed the Hoagland's solution between eight 7.6 l h⁻¹ drip emitters (DIG Irrigation). Two drip emitters were placed in each pot 5 cm below the pebble surface and continually supplied the hydroponic solution to the grapevines through the drip emitters. As the re-circulating solution drained from the basins, it dripped into the reservoir facilitating adequate aeration of the solution. All RDS systems were set up in a greenhouse with set temperatures between 20–28 °C. The modified Hoagland's solution consisted of 2 mM Ca(NO₃)₂, 3 mM KNO₃, 1.25 mM KH₂PO₄, 1.5 mM MgSO₄, 100 μM Na₂SiO₃, 40 μM H₃BO₃, 9 μM MnSO₄, 4 μM ZnSO₄, 4 μM CuSO₄, 0.10 μM H₂MoO₄, and 100 μM NaFeDTPA. pH was adjusted to approximately 5.8 using

H₃PO₄ and KOH. Solution in each reservoir was changed in all RDS systems as needed based upon total volume losses due to transpiration and evaporation. To minimize evaporative losses from each RDS and reduce algae growth, heavy-duty aluminum foil was used to cover the reservoirs and all treepots.

For soil and fritted clay, four of the rootstocks described above (420A, 101-14Mgt, 1103P, and 110R) were grafted with a common scion (described above) and grown in either a modified UC soil mix (peat/sand/redwood compost 1:1:1, with 2.44 kg m⁻² dolomite lime) or fritted clay in 4.3 l pots under similar greenhouse conditions. These vines were irrigated regularly and fertilized weekly. Vines from this experimental setup were also used to evaluate rootstock vegetative growth in terms of leaf area and root biomass and were compared to results from recent field rootstock trials conducted across several years on multiple sites across grape growing regions of California.

Root sampling

Root sampling occurred between 10:00 and 10:30 a.m., and samples were returned to the laboratory within 20 min of harvesting. Vines were carefully removed from pots, growing media was carefully washed from the roots, and pruning razor blades were used to cut healthy fine roots from the root mass under water. All portions of the root system sampled for L_{pr} included an intact root tip ensuring a measurement of the radial contribution to L_{pr} . Roots were transferred to the lab in nutrient solution and experiments were carried out immediately. A sub-sample of fine roots for gene expression analyses were placed into 5.0 ml cryogenic vials and frozen immediately using liquid N₂. Frozen samples were taken to the lab in a transport dewar and stored in a -80 °C freezer until analysed. The remaining vine and root system was dried for a minimum of 48 h at 90 °C for biomass measurements.

For root tip and mature root analyses roots were first dissected and then immediately frozen using liquid N₂ as described above. The root tip section consisted of the first 2 cm of the root tip while mature root portions were comprised of root sections 10–20 cm proximal to the tip from which all lateral roots were removed.

Aquaporin gene expression

Gene expression analyses were carried out according to Choat *et al.* (2009). In short, total RNA was extracted, treated with DNase, and reverse transcribed following the methods described by Castellari *et al.* (2007). Quantitative real-time PCR was carried out in an ABI PRISM 7700 sequence detector (Applied Biosystems). Each reaction (20 µl) contained 1 mM of each primer, 5 µl of 1:400 or 1:4,000 diluted cDNA, and 10 µl of Power SYBR Green Master Mix (Applied Biosystems). Thermal cycling conditions were 95 °C for 10 min followed by 40 cycles of 95 °C for 30 s, 56 °C for 30 s, and 60 °C for 60 s. Both cDNA dilutions were run in duplicate. For rootstock studies gene transcripts were normalized to VvUbiquitin1 (TC32075, Institute for Genomic Research database) by comparing the cycle threshold (C_T) of the target gene with that of VvUbiquitin1 (Bogs *et al.*, 2005) via the comparative C_T method. Gene expression was expressed as mean and SE calculated over all biological replicates. For the determination of VvUbiquitin1 variation expression was quantified absolutely using genomic DNA standards (Yun *et al.*, 2006; Gambetta *et al.*, 2010). Primer pair sequences for the VvPIP isogenes and VvUbiquitin1 can be found in Supplementary Table S1 (available at JXB online) and all primer pairs were validated by isolating and sequencing their PCR products to confirm identity. Aquaporin gene expression was quantified in fine roots across rootstocks relative to VvUbiquitin1, which provided a stable reference and when expression was absolutely quantified its CV was 3.6%.

Across studies involving *Vitis*, there is confusion when integrating genomic and cDNA for a number of VvPIP isogenes making it difficult to resolve if multiple extremely closely related cDNAs represent allelic variants, true isogenes, or possibly the same gene (in the case of partial cDNAs). This is due to the high level of conservation among PIPs at the DNA level (Shelden *et al.*, 2009) and the high level of heterozygosity

present among *V. vinifera* cultivars (Myles *et al.*, 2011). To account for these issues, all available VvPIP gene sequences (Shelden *et al.*, 2009) were clustered (unpublished data) and primer pairs were designed to amplify all related gene sequences within a given cluster of extremely closely related isogene/allelic variants by designing primer sequences across regions of perfect homology. This is especially important regarding PIP1-2 and PIP1-4 which are 98% identical at the DNA level, and PIP1-3 and PIP1-5 which are 96% identical at the DNA level (Shelden *et al.*, 2009). Therefore, this study has reported expression levels as PIP1-2:1-4 and VvPIP1-3:1-5 for these putative isogenes/allelic variants.

Fine-root L_{pr}

Hydraulic conductivity (L_{pr}) was measured in fine roots using two different methods depending on the driving force used. For experiments using a hydrostatic pressure gradient, a meniscus tracking method similar to that described by Choat *et al.* (2009) was used with modifications to the apparatus based upon feasibility differences in applying pressure to fine roots versus berries (Supplementary Fig. S1). Healthy, unbranched fine roots (including tip) were excised under water (using a fresh razor blade). Fine roots were fed through the cylinder of a hard, plastic luer fitting (polypropylene 3.2 mm 200 series, Value Plastics, Fort Collins, CO, USA) such that the upper ~3 mm of the segment (i.e. the downstream end of the segment) was left exposed above the fitting. The root was sealed into the luer fitting using non-toxic, dental impression polymer (Penron Clinical Technologies, LLC, Wallingford, CT, USA) to prevent compression of the tissue (similar to details described in McElrone *et al.*, 2007). Seals were tested for each sample prior to taking measurements. Samples were then connected to additional luer connectors attached to plastic tubing fed through the lid of a pressure chamber (Soil Moisture Equipment Corp, Santa Barbara, CA, USA) and submersed in diH₂O inside the pressure chamber. The tube protruding from the lid was connected to a Swagelok reducing union that also held a microcapillary (i.d. = 20 µm) that was used to measure outflow from the sample by tracking the movement of a meniscus at the air–water interface. The first hydrostatic experiments used a single pressure of 0.2 MPa to determine fine-root L_{pr} , which was confirmed to be in the linear range (see corresponding results in Fig. 5). A follow-up experiment utilized two of the rootstocks with consistently divergent vigour ratings and VvPIP gene expression (420A and 110R) to determine pressure–flow relationships in both hydrostatic and osmotic gradients, and to confirm accuracy of the single pressure measurements in the first experiment. For the hydrostatic measurements, a range of pressures was used (0.1–0.3 MPa, in a minimum of four 0.03–0.05 MPa pressure steps; see Fig. 5). Baseline L_{pr} values were first obtained with the root submersed in diH₂O, and then measurements were repeated with 0.6 mM H₂O₂ for aquaporin chemical inhibition. Hydrogen peroxide based solutions have been used effectively as inhibitors of aquaporin activity, while providing lower toxicity than mercuric chloride (HgCl₂) (Henzler *et al.*, 2004; Ye and Steudle, 2006; McElrone *et al.*, 2007). The distance travelled by the meniscus was recorded every 60 s to calculate a volumetric flow rate. L_{pr} (m s⁻¹ MPa⁻¹) was calculated using the following equation: $L_{pr} = (Q_v/P) \times (1/A)$, where Q_v is the volumetric flow rate (m³ s⁻¹), P (MPa) is the pressure applied to the root, and A (m²) is the surface area of a cylinder calculated from fine-root segment length and radius (North and Nobel, 1991). Upon completion of L_{pr} measurements, roots were scanned using an Epson 1640 scanner (Seiko Epson Corporation, Nagano, Japan) and then analysed using WinRHIZO version 2003a (Régent Instruments, Quebec, Canada) to obtain data regarding root length, surface area, and diameter. The WinRHIZO program and this scanning method have been determined to be an accurate and effective method of obtaining root data (Himmelbauer *et al.*, 2004). Root biomass was tightly correlated with root surface area regardless of rootstock ($r^2 = 0.78$, $P < 0.001$). Root surface area was determined from root biomass according to this relationship.

For experiments using an osmotic pressure gradient, healthy unbranched fine roots (including tip) were excised under water (using a fresh razor blade) and glued into a 500-µm diameter glass capillary. The capillary and root were fed into a custom-made chamber

(Supplementary Fig. S1) in which the solution could be changed and flow through the root was quantified via the movement of the meniscus in the capillary. Roots were equilibrated for at least 1 h in diH₂O followed by measurements of flow in sucrose solutions of various osmotic strengths (0, 0.125, 0.25, 0.5 MPa). In some cases measurements were replicated on the same root using both sucrose and mannitol solutions of equal osmotic strengths with no difference in the resulting L_p . The root was allowed to equilibrate in each solution for at least 30 minutes and flows were stable. L_p was determined as the slope of the pressure–flow relationship across at least four different osmotic pressures (e.g. Fig. 5). Aquaporin inhibition was then carried out immediately on the same root as described above.

Rootstock and aquaporin inhibition effects on whole-vine transpiration

To measure the effects of rootstock on whole-vine transpiration, vines with a common Cabernet Sauvignon scion were grafted onto the four rootstocks described above. These vines were grown in 4.3 l pots under greenhouse conditions, watered to saturation once daily, fertilized with quarter-strength modified Hoagland's solution weekly.

Vines were watered to saturation at the start of each day with either diH₂O or a 35 mM H₂O₂ solution, allowed to drain, and sealed in plastic bags up to the base of the stem. Each bagged, potted plant was weighed every 2 h on a laboratory scale (EA150CE-1, Sartorius, Goettingen, Germany). Bags were removed after the final weighing of the day at 8 p.m. and all vines were watered with diH₂O. This protocol was repeated for multiple days, and midday water use for each rootstock was determined from this data. At the end of the experiment, leaf area was determined for each vine using a LI-3000C leaf area meter (Licor Biosciences, Lincoln, NE, USA), and midday transpiration rates of the whole vine were calculated on a leaf area basis.

Statistical analysis

All statistical analyses were carried out using SAS software (SAS Institute, <http://www.sas.com>). ANOVAs were carried out by both rootstock and treatment (control and inhibited) and Tukey's HSD was used for means separation when ANOVA results were significant.

Results

Rootstock effects on vine growth

Under the experimental conditions of this study, the vine growth parameters were consistent with commonly used vigour ratings for the study rootstocks (Table 1). Leaf area and/or root biomass were significantly greater in the high-vigour rootstocks (1103P and 110R) compared to those from rootstocks that are commonly considered low-to-moderate vigour (420A and 101-14Mgt) ($P < 0.05$; Table 1). These results are consistent with a recent

Table 1. Leaf area and root weights of grape rootstocks tested in this study. Value are mean \pm SE. Different superscript letters within a row indicate significant differences among rootstocks ($n = 12$; $P < 0.05$).

	Rootstock			
	420A	101-14Mgt	1103P	110R
Leaf area (cm ²)	2343 \pm 96 ^{a,b}	2111 \pm 130 ^a	2609 \pm 67 ^b	2574 \pm 198 ^b
Root dryweight (g)	13.6 \pm 1.1 ^a	12.6 \pm 2.1 ^a	18.3 \pm 2.2 ^{a,b}	23.2 \pm 2.5 ^b
Root freshweight (g)	57.0 \pm 3.4 ^a	56.0 \pm 5.6 ^a	62.8 \pm 7.4 ^{a,b}	72.2 \pm 3.7 ^b

summary of field rootstock trials from California; across five sites and three scion varieties pruning weights of scions grown on 1103P and 110R rootstocks were significantly greater by an average 43% (range of 25–70%) compared to 420A and 101-14Mgt rootstocks across sites ($P < 0.05$) (JA Wolpert, personal communication). There were also very few differences between rootstocks within a given vigour rating (i.e. 110R vs. 1103P) in these field trials.

Aquaporin gene expression

Under favourable growing conditions, expression of several aquaporin genes varied significantly between rootstocks and some were greater for higher-vigour rootstocks regardless of the growing media (Figs. 1 and 2). *VvPIP1-1* was the most prominently expressed aquaporin isogene with levels at least 3-fold greater than that of *VvPIP1-2:1-4* and at least 5-fold greater than any *VvPIP2* family member (Figs. 1 and 2). *VvPIP1-3:1-5* was expressed at extremely low levels in soil (Fig. 1A) and was undetectable in hydroponically grown roots (Fig. 2A).

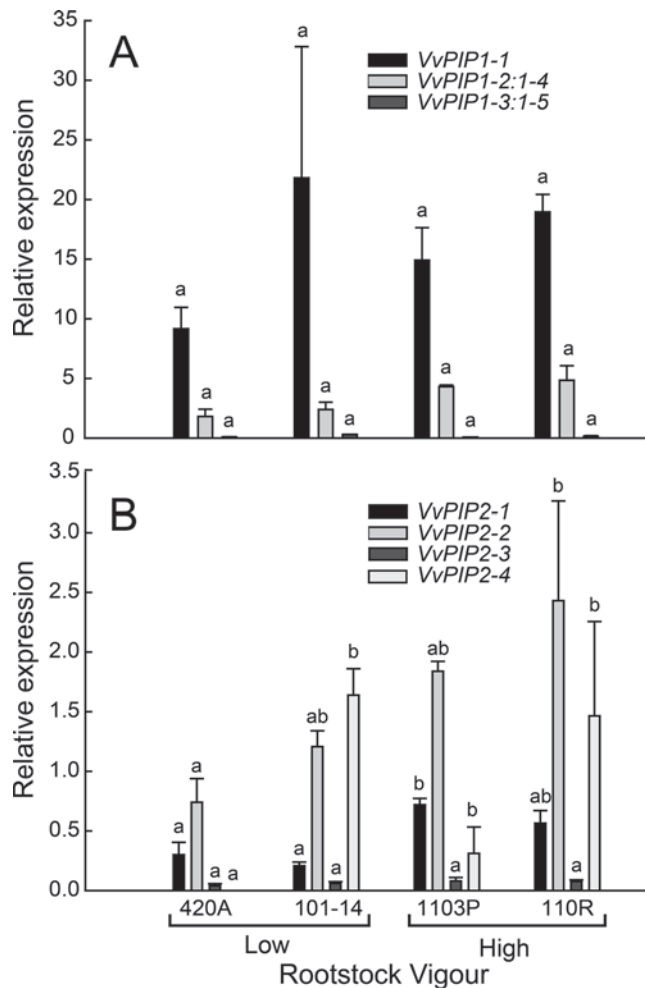


Fig. 1. Individual (greyscale) relative expression (relative to *VvUbiquitin1*) for *VvPIP1* (A) and *VvPIP2* (B) gene families in various rootstocks grown in soil media. Bars represent mean \pm SE and different letters indicate significant differences between rootstocks for each isogene ($n = 3$; $P < 0.05$).

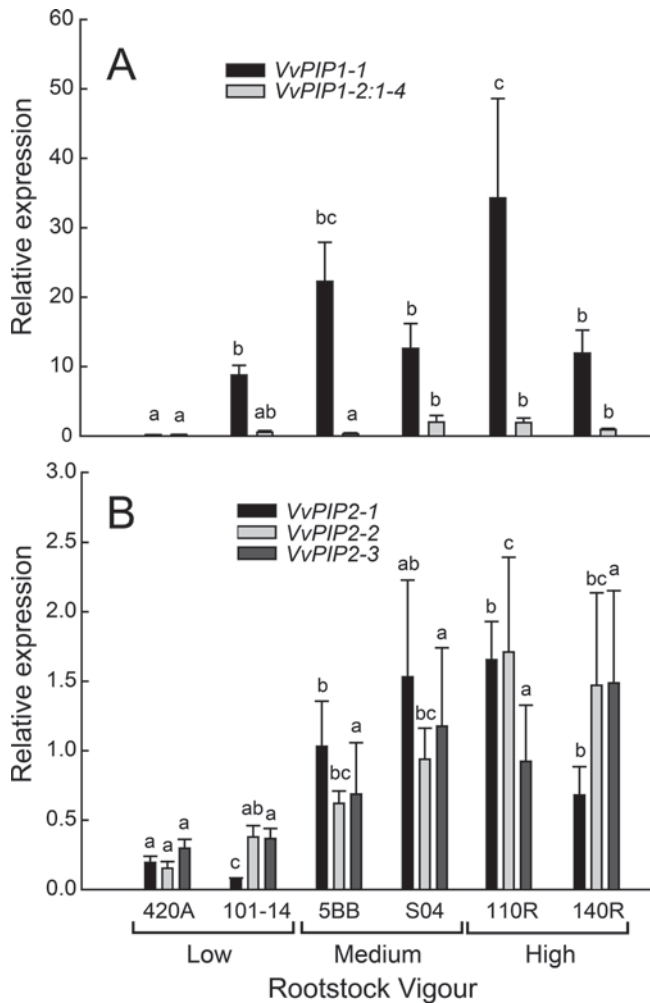


Fig. 2. Individual (grayscale) relative expression for the (A) *VvPIP1* and (B) *VvPIP2* gene families for various grapevine rootstocks grown in a re-circulating drip hydroponic system. Due to lack of adequate sample tissue at the time of sample repeat analysis, *VvPIP2-4* expression was not assessed in hydroponically grown roots. Bars represent mean \pm SE and different letters indicate significant differences between rootstocks for each isogene ($n = 4$; $P < 0.05$).

The expression of many *VvPIP2* family members were greater in high-vigour rootstocks (Figs. 1B and 2B). Expression of both *VvPIP2-1* and *VvPIP2-2* increased with vigour in both soil and hydroponics (Figs. 1B and 2B). *VvPIP2-3* followed this same general trend with increased expression in higher-vigour rootstocks when plants were grown in hydroponics (Fig. 2B), but in soil *VvPIP2-3* consistently had the lowest expression of all isogenes (Fig. 1B). *VvPIP2-4* was variably expressed in all rootstocks, but was undetected in the lowest-vigour rootstock 420A (Fig. 1B).

Root tips (apical 2 cm of the fine root) typically had greater levels of *VvPIP* expression than mature roots (10–20 cm back from the tip), and this pattern was even more dramatic in 110R compared to 420A (i.e. tips are more different than mature roots for 110R, whereas expression was much more consistent along the length of the root for 420A) (Fig. 3). Interestingly, there

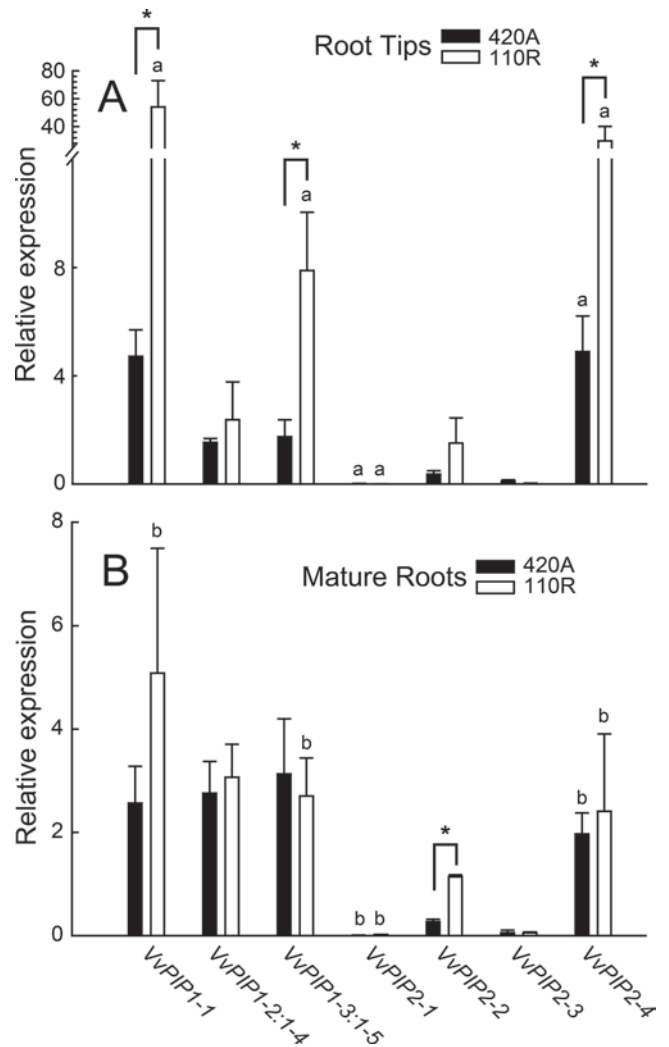


Fig. 3. Individual *VvPIP* isogene expression in root tips (A) and mature roots (B) of 420A (black) and 110R (white). Bars represent mean \pm SE and asterisks indicate significant differences between rootstocks for a given isogene ($n = 3-5$; $P < 0.05$). Bars labelled with different letters between panels A and B represent significant differences within a rootstock between root tips and mature roots for a given isogene ($n = 3-5$; $P < 0.05$).

were few significant differences between the rootstocks within the mature root zone, suggesting that any differential aquaporin physiology between rootstocks would be realized almost exclusively in the root tip. Even though aquaporin expression was dominant in the root tip, which represents a small portion of the total root absorptive area, aquaporins still contributed significantly to the L_{p_r} of the fine roots (see results below).

Root L_{p_r}

Hydrostatically driven fine-root L_{p_r} was originally measured on four rootstocks of varying vigour (Fig. 4). Average $L_{p_r}^{Hyd}$ of fine roots was approximately 60% greater in high-vigour rootstocks, although the differences were not significant ($P > 0.05$) due to high variability across roots. $L_{p_r}^{Hyd}$ for all rootstocks was reduced in response to chemical inhibition, with the greatest

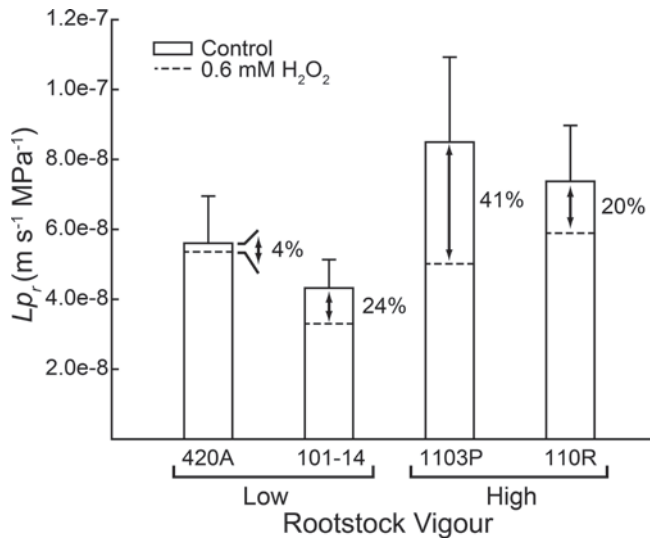


Fig. 4. Hydrostatic fine-root hydraulic conductivity (Lp_r^{Hyd}) across rootstocks. Bars represent mean native fine-root Lp_r^{Hyd} and dashed lines represent fine-root Lp_r^{Hyd} after inhibition. The percentage reduction in fine-root Lp_r caused by the inhibition is shown to the right of each bar. Values are mean \pm SE ($n \geq 7$).

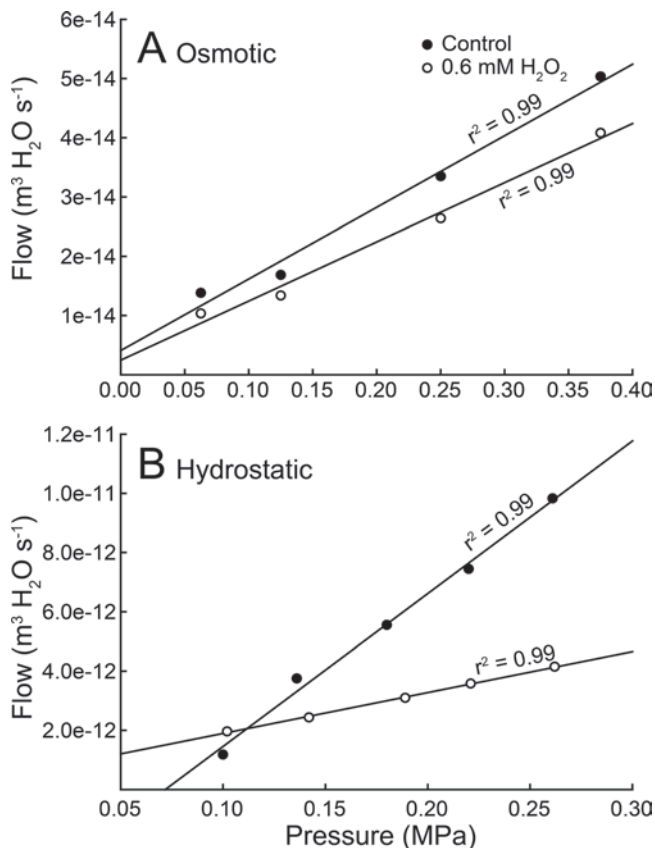


Fig. 5. Examples of osmotic (A) and hydrostatic (B) pressure–flow relationships: typical examples across a range of pressures for both control (filled circles) and 0.6mM H_2O_2 treated (open circles) 110R roots.

absolute reduction observed in 1103P and 110R. Differences in mean Lp_r^{Hyd} among high- and low-vigour rootstocks were reduced with aquaporin inhibition (Fig. 4A). 420A had the lowest level of inhibition, which corresponded with the consistently low expression levels of the VvPIP isogenes (Figs. 1 and 2).

Due to their consistently divergent patterns of VvPIP gene expression, rootstocks 420A and 110R were used in follow-up experiments to assess aquaporin contribution to fine-root Lp_r under both osmotic (Lp_r^{Osm}) and hydrostatic (Lp_r^{Hyd}) pressure gradients (Figs. 5 and 6). Pressure and flow were linearly related over a broad range of pressures for both osmotic and hydrostatic gradients, and aquaporin inhibition decreased Lp_r while the pressure–flow relationships remained linearly related (Fig. 5). Values of Lp_r and % aquaporin contribution to Lp_r were similar when a single pressure or range of pressures was used for the measurements (Figs. 4 and 6). Lp_r^{Hyd} was greater than Lp_r^{Osm} for both rootstocks, but the difference was only 2-fold greater for 420A, while it was ~5-fold greater for 110R (Fig. 6B; compare shaded regions to full bar). Both Lp_r^{Osm} and Lp_r^{Hyd} were lower in the low-vigour rootstock 420A, but differences were not significantly different ($P > 0.05$). The decrease in Lp_r^{Osm} and Lp_r^{Hyd}

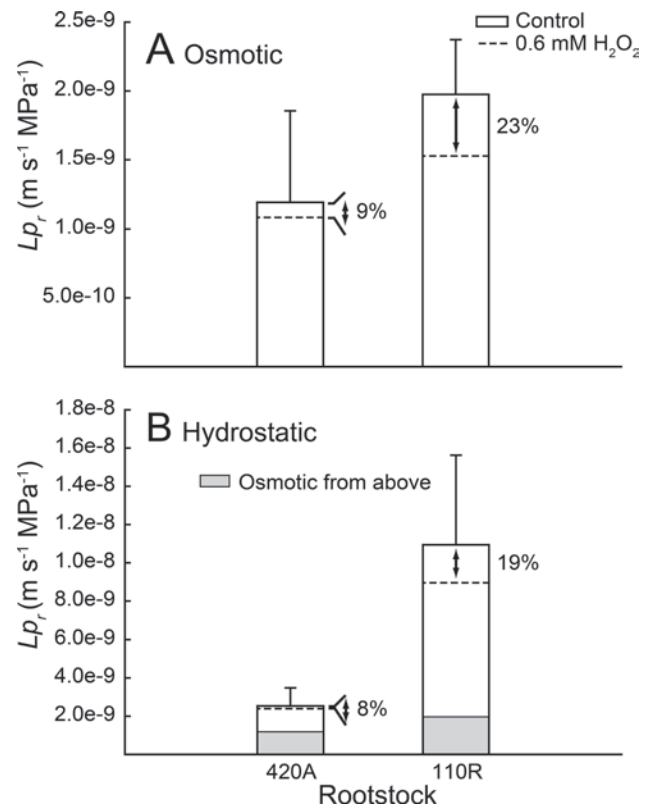


Fig. 6. Fine-root hydraulic conductivity (Lp_r) of 420A and 110R rootstocks for osmotically (A) or hydrostatically (B) produced pressure gradients. Bars represent mean native fine-root Lp_r (i.e. uninhibited) and dashed lines represent fine-root Lp_r after chemical inhibition. For hydrostatic data, the grey portion of the bar represents the osmotic data from (A) for reference. The percentage reduction in fine-root Lp_r caused by the inhibition is shown to the right of each bar. Values are mean \pm SE and different letters indicate significant differences between rootstocks ($n \geq 3$; $P < 0.05$).

due to aquaporin inhibition was equivalent within each rootstock (i.e. the % decrease was similar regardless of whether flow was driven osmotically or hydrostatically), but 110R roots exhibited a significantly greater reduction (~20%) in Lp_r , compared to ~9% in 420A ($P < 0.02$) under both conditions (Fig. 6).

Whole-plant relationships

Whole-vine water use was positively correlated with rootstock vigour; 110R had significantly greater transpiration per unit leaf area than 420A (Fig. 7). No significant effect of aquaporin chemical inhibition on transpiration was found for any of the rootstocks, but differences between the rootstocks disappeared when the root systems were treated with the inhibitor.

In order to relate differences in fine-root Lp_r to canopy water demands, this study calculated correlations among several parameters. Fine-root Lp_r was positively correlated with leaf area and whole-vine transpiration, respectively (Fig. 8). Fine-root Lp_r increased by 60% on average for high-vigour rootstocks compared to their low-vigour counterparts (Fig. 8A).

Discussion

The *Vitis* rootstocks with varying vigour classifications studied here exhibit significantly different patterns of VvPIP expression under favourable conditions in a variety of growth media; VvPIP expression was consistently greater in high-vigour rootstocks. Similarly, fine-root Lp_r and % aquaporin contribution to Lp_r , determined under both osmotic (Lp_r^{Osm}) and hydrostatic (Lp_r^{Hyd}) pressure gradients were consistently greater in high-vigour rootstocks. Leaf area-specific transpiration and total leaf area increased with rootstock vigour and were positively correlated

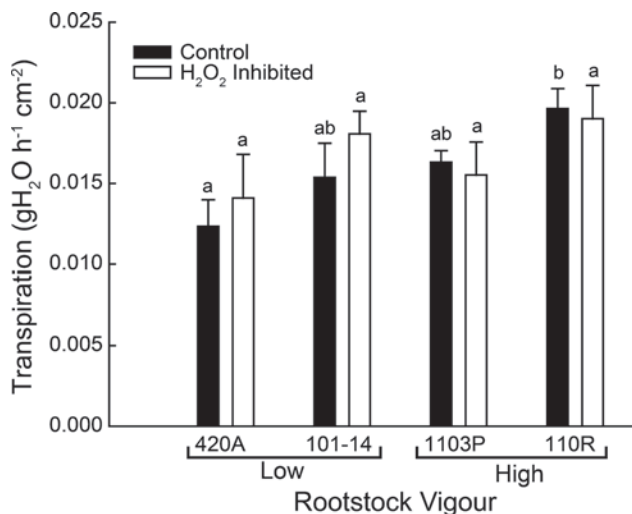


Fig. 7. Midday transpiration for whole vines grafted on to each of four rootstocks. Leaf area was determined for each vine at the end of the experiment and used to scale measurements per unit leaf area. Data represent the mean \pm SE and different letters indicate significant differences between rootstocks ($n \geq 5$; $P < 0.05$). There were no significant differences in transpiration between control and H₂O₂ inhibited ($P = 0.60$).

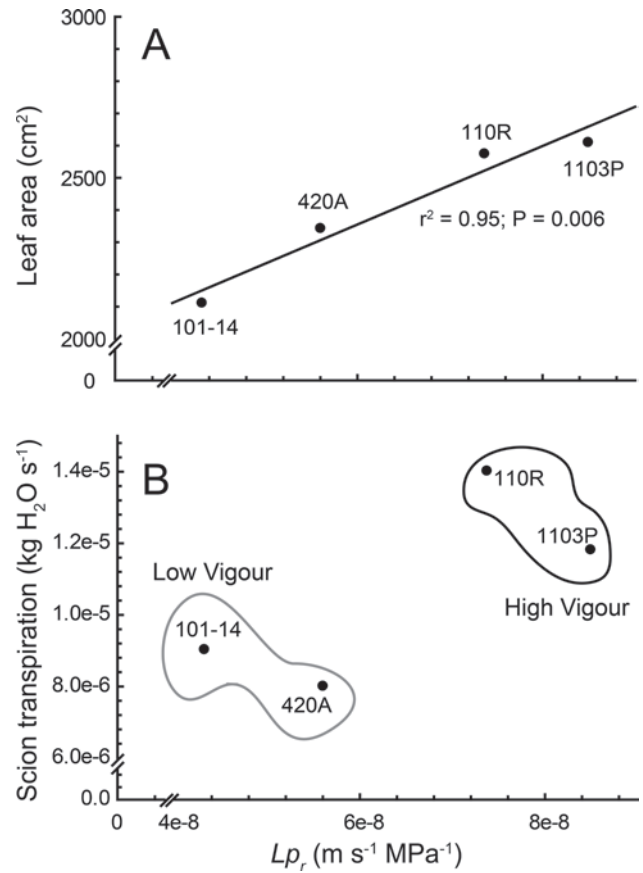


Fig. 8. Relationships of fine-root Lp_r^{Hyd} with leaf area (A) and whole-plant water use (B) across the four rootstocks.

with fine-root Lp_r . These results suggest that differences among rootstocks results in part from the differences in individual fine-root Lp_r (influenced by VvPIP gene expression and activity).

Variable aquaporin expression and activity across rootstocks

Since the C-C pathway plays a prominent role in radial water absorption across fine roots (Steudle, 2001), it follows that aquaporins would contribute to hydraulic differences among rootstocks. In this study, high-vigour rootstocks had greater expression of several VvPIP isogenes and an average Lp_r that was approximately 60% greater than low-vigour rootstocks, although Lp_r was so variable that differences were not significant. Evidence from multiple studies on transgenic systems is mixed regarding the linkage between aquaporin expression, root Lp_r , and vigour. NtAQPI antisense lines of tobacco exhibited reduced root Lp_r , yet no differences in plant growth (Siefritz *et al.*, 2002). Others found that constitutive overexpression of AtPIP1b in tobacco resulted in increased shoot biomass, but the role root hydraulics was not investigated (Aharon *et al.*, 2003). Several studies using *Arabidopsis* found that various aquaporin antisense and knockout lines exhibited decreases in Lp_r , with no change in shoot biomass (Kaldenhoff *et al.*, 1998; Javot and Maurel, 2002; Martre *et al.*, 2002). These studies and the results reported here suggest that varied aquaporin expression may be involved in altering the growth potential of the scion in ways

other than or in addition to directly altering hydraulic conductance consistently across the root system.

In this study, the % reduction in L_p , due to aquaporin inhibition was correlated with VvPIP expression and varied among rootstocks, ranging from just 4% in 420A to 40% in 1103P. This range suggests that in general the apoplastic pathway dominates radial transport in fine roots of grapevine under favourable conditions. The magnitude of aquaporin contribution to L_p , is highly variable across species, ranging from 20–90%, with higher values typically reported for herbaceous species (reviewed in Javot and Maurel, 2002). The reductions in L_p , found here are similar in magnitude to the 5–45% reductions in root-system L_p , when aquaporins were inhibited with mercuric chloride across several *Vitis* rootstocks under water deficit (Lovisolo et al., 2008). Studies in *Arabidopsis* utilizing antisense and mutant lines have found similar results with contributions ranging from 20–60% (Martre et al., 2002; Javot et al., 2003; Postaire et al., 2010).

Even though there were consistent differences in the expression of VvPIP genes in bulk fine-root tissue among rootstocks, the localized patterns of expression and varying permeability in different tissue and cell types likely play important roles in regulating water uptake (e.g. Knipfer et al., 2011). Large differences in VvPIP expression were found along the length of the root with greater expression in root tips. This finding is consistent with previous studies that reported peak mRNA and/or protein levels of PIPs in root tips of tobacco, grapevines, and barley, respectively (Otto and Kaldenhoff, 2000; Vandeleur et al., 2009; Knipfer et al., 2011). Despite historical literature demonstrating that unsuberized fine-root tips constitute a small proportion of total root system and that significant water uptake can occur in older suberized portions of roots (Kramer and Bullock, 1966; Chung and Kramer, 1975; Macfall et al., 1990), contemporary studies often imply that water uptake occurs only where fine-root tips proliferate. In fact, Queen (1967) measured the relative permeability of various portions of Concord grapevine root systems and found that the terminal end of the root (~8 cm) was 65–545-times less permeable to water than any other part of the current season roots (measured incrementally to ~20 cm back from the root tip and included partially suberized tissue). The current season roots were also only 5-times more permeable than the heavily suberized roots from previous growing seasons (Queen, 1967). Given the potential for substantial water uptake in older portions of grapevine roots, one would expect consistent PIP expression along the root length if aquaporins were playing a consistent role in increasing root permeability. Higher PIP expression and activity in root tips of woody plants may actually play an analogous role to patterns seen in barley roots, an herbaceous species (e.g. Knipfer et al., 2011). Instead of altering bulk permeability of the entire root-system, peak aquaporin expression in the differentiation and elongation zones of the root tip may play an important role in influencing the rate of growth and architecture of new root tissue. This is consistent with the hypothesis that the predominance of aquaporin localization in vascular tissues, and especially phloem tissues, suggests a role in source–sink relationships (Schaffner, 1998; Suga et al., 2003; Fraysse et al., 2005). Concentrated expression of aquaporins in the root tip could also enable the advancing tissue to better sense and

handle changing spatial and temporal conditions of the soil (Eapen et al., 2005).

In this study, expression of several VvPIP2 isogenes was significantly greater in high-vigour rootstocks when compared to the low-vigour rootstock 420A. However, there is overlap in expression patterns between the high-vigour rootstocks and 101-14, a low-vigour rootstock. Several studies on other plant species have shown that expression of PIP2 isoforms results in greater membrane water permeability in *Xenopus* oocytes compared with PIP1 isoforms, which are often hydraulically inactive/non-functional (Yamada et al., 1995; Chaumont et al., 2000; Dordas et al., 2000; Vandeleur et al., 2009). However, co-expression of particular PIP1 and PIP2 isoforms can increase membrane hydraulic permeability above levels measured with the expression of those genes alone (Fetter et al., 2004; Alleva et al., 2010); a similar pattern was found for VvPIPs of grapevines (Vandeleur et al., 2009). Therefore, the current study suggests that differences in VvPIP1 expression could play a large role in controlling hydraulic permeability through VvPIP1-VvPIP2 interactions as suggested by Vandeleur et al. (2009). Fine-scale details of VvPIP co-regulation may play a larger functional role under water deficit conditions when the contribution of the C–C pathway to radial transport increases due to the formation of apoplastic barriers (Vandeleur et al., 2009). More work is needed in this area to determine the interactive role of VvPIPs in controlling cellular water relations, particularly in the root tip under varying conditions.

This study found that L_p^{Hyd} was much greater than L_p^{Osm} for both low-vigour 420A and high-vigour 110R rootstocks. This finding is consistent with expectations based on the parallel pathways of the composite transport model (Steudle, 2001), where the apoplast has a lower resistance and would predominate during high flow conditions driving by transpiration. However, L_p^{Hyd} and L_p^{Osm} for a given rootstock exhibited similar %reductions in flow under aquaporin inhibition- implying there is still substantial flow through the C–C pathway even under a hydrostatic driving force. Roots provide a complex anatomical context for radial water movement, where the pathways exist in parallel in the cortex and stele but movement is presumably restricted to the C–C pathway through the endodermis (Knipfer and Fricke, 2010). In fine roots, including both growing and differentiated root portions, the anatomical context is further complicated since the growing root portions often lack a developed Casparian band or other apoplastic barriers.

Vigour and root hydraulic conductance

For several perennial crop species, altered scion vigour has been linked to differences in hydraulic parameters of the root system. The hydraulic capacity of a root system to deliver water to the scion can be brought about by increases in L_p , (per root surface area or per biomass), and/or whole-root-system surface area. Lovisolo et al. (2007) showed that lower whole-root-system hydraulic conductance of olive dwarfing rootstocks resulted primarily from decreased root-system biomass. Low whole-root-system hydraulic conductance found in low-vigour rootstocks of peach was associated with less fine-root surface area quantified as length per unit root dryweight (Solari et al., 2006). In a

recent study of drought resistance, [Alsina et al. \(2011\)](#) found that grapevines grafted onto 1103P rootstock (high vigour) exhibited greater whole-root-system hydraulic conductance compared to 101-14 (low vigour) resulting from continued growth at greater depth during the warmer and drier summer months.

Several studies suggest that whole-root-system hydraulic conductance is positively correlated with vigour ([Nardini et al., 2006](#); [Solari et al., 2006](#); [Clearwater et al., 2004](#); [Lovisolo et al., 2007](#)), but the correlation of fine-root Lp_r (per root surface area or per biomass) appears much more variable. In the current study, root surface area-specific Lp_r was positively correlated with leaf area and canopy water demands, a pattern similar to those demonstrated in deep fine roots accessed via caves ([McElrone et al., 2007](#)) and in apple using root hydraulic conductance per length ([Atkinson et al., 2003](#)). However, [Clearwater et al. \(2004\)](#) found a positive correlation between whole-root-system hydraulic conductance and vigour despite root hydraulic conductance per amount of leaf area being greatest in low-vigour rootstocks. The current analysis of results presented in [Solari et al. \(2006\)](#) demonstrated that when whole-root-system hydraulic conductance was scaled per unit root dryweight it was also greater for dwarfing rootstocks. Likewise, [Lovisolo et al. \(2007\)](#) found the same relationship in olive (using values scaled per unit root dryweight) and the authors concluded that higher aquaporin expression found in the dwarfing rootstock was responsible for the increased hydraulic conductance.

Supplementary material

Supplementary data are available at *JXB* online.

Supplementary Table S1. Primer pair sequences used in this study.

Supplementary Fig. S1. Experimental set-up for the determination of Lp_r with osmotic and hydrostatic pressure gradients.

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