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

G. A. Gambetta1, C. M. Manuck2, S. T. Drucker1, T. Shaghas1, K. Fort2, M. A. Matthews2, M. A. Walker2 and A. J. McElrone1,2,*

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), % aquaporin contribution to Lp, 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, and % aquaporin contribution to Lp, determined under both osmotic (Lp,osm) and hydrostatic (Lp,hyd) pressure gradients were consistently greater in high-vigour rootstocks. Interestingly, the % aquaporin contribution was nearly identical for Lp,osm and Lp,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. 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, 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 wine grape 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 NtAQP1 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 NtAQP1 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 V. berlandieri x V. riparia parentage) are commonly characterized as high vigour and drought resistant, rootstocks 5BB and SO4 (both with V. berlandieri x V. riparia parentage) are characterized as moderate vigour and drought intolerant, and rootstocks 101-14Mgt (V. riparia x V. riparia parentage) and 420A (V. berlandieri x 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 m$^2$) of polysocyanurate 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 –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(NO$_3$)$_2$, 3 mM KNO$_3$, 1.25 mM KH$_2$PO$_4$, 1.5 mM MgSO$_4$, 100 μM Na$_2$SiO$_3$, 40 μM H$_2$BO$_3$, 9 μM MnSO$_4$, 4 μM ZnSO$_4$, 4 μM CuSO$_4$, 0.10 μM H$_3$BO$_3$, and 100 μM NaFeDTPA. pH was adjusted to approximately 5.8 using
H₂PO₄ 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 grown 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 LP₀ included an intact root tip ensuring a measurement of the radial contribution to LP₀. 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 5.0ml 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 Castellarin 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 nM 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 3 s, 56 °C for 30 s, and 60 °C for 30 s. Both cDNA dilutions were run in duplicate. For rootstock studies gene transcripts were normalized to VvUbiquitin1 (TC23075, Institute for Genomic Research database) by comparing the cycle threshold (Cṭ) of the target gene with that of VvUbiquitin1 (Bogs et al., 2005) via the comparative Ct method. Gene expression was expressed as mean and SE calculated across all biological replicates. For the determination of VvUbiquitin1 variation expression was quantified absolutely using genomic DNA standards (Yin 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 isogene/allelic variants.

Fine-root LP₀

Hydraulic conductivity (LP₀) 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 OD, 200 series, Valco 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 (Pentron 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 LP₀, 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 LP₀ values were first obtained with the root submerged 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. LP₀ (m s⁻¹ MPa⁻¹) was calculated using the following equation: LP₀ = (Qv/P) × (1/A), where Qv 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 LP₀ 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 (r² = 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 diH$_2$O 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.

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). Vv$\text{PIP1-1}$ was the most prominently expressed aquaporin isogene with levels at least 3-fold greater than that of Vv$\text{PIP1-2:1-4}$ and at least 5-fold greater than any Vv$\text{PIP2}$ family member (Figs. 1 and 2). Vv$\text{PIP1-3:1-5}$ was expressed at extremely low levels in soil (Fig. 1A) and was undetectable in hydroponically grown roots (Fig. 2A).

**Table 1.** Leaf area and root weights of grape rootstocks tested in this study. Value are mean ± 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 ± 96$^b$ | 2111 ± 130$^a$ | 2609 ± 67$^b$ | 2574 ± 198$^a$ |
| Root dryweight (g) | 13.6 ± 1.1$^a$ | 12.6 ± 2.1$^b$ | 18.3 ± 2.2$^a$ | 23.2 ± 2.5$^b$ |
| Root freshweight (g) | 57.0 ± 3.4$^a$ | 56.0 ± 5.6$^b$ | 62.8 ± 7.4$^a$ | 72.2 ± 3.7$^b$ |

**Fig. 1.** Individual (greyscale) relative expression (relative to VvUbiquitin1) for Vv$\text{PIP1}$ (A) and Vv$\text{PIP2}$ (B) gene families in various rootstocks grown in soil media. Bars represent mean ± SE and different letters indicate significant differences between rootstocks for each isogene ($n = 3$; $P < 0.05$).
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 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 Lp, of the fine roots (see results below).

**Root Lp,**

Hydrostatically driven fine-root Lp, was originally measured on four rootstocks of varying vigour (Fig. 4). Average Lp,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. Lp,Hyd for all rootstocks was reduced in response to chemical inhibition, with the greatest
absolute reduction observed in 1103P and 110R. Differences in mean \( Lp_{Hyd} \) 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). \( Lp_{Hyd} \) was greater than \( Lp_{osm} \) 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 \( Lp_{osm} \) and \( Lp_{Hyd} \) were lower in the low-vigour rootstock 420A, but differences were not significantly different (\( P > 0.05 \)). The decrease in \( Lp_{osm} \) and \( Lp_{Hyd} \)
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 \( Lp \) 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 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. \( NtAQP1 \) 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 \( Lp \), 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 \( Lp \), due to aquaporin inhibition was correlated with \( Vv\text{PIP} \) 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 \( Lp \) 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 \( Lp \), found here are similar in magnitude to the 5–45% reductions in root-system \( Lp \), 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 \( Vv\text{PIP} \) 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 \( Vv\text{PIP} \) 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 also 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 (Schaffer, 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 (Eappen et al., 2005).

In this study, expression of several \( Vv\text{PIP2} \) 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 \( PIP1i \) isoforms, which are often hydraulically inactive/nonfunctional (Yamada et al., 1995; Chaumont et al., 2000; Dordas et al., 2000; Vandeleur et al., 2009). However, co-expression of particular \( PIP1i \) 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 \( Vv\text{PIPS} \) of grapevines (Vandeleur et al., 2009). Therefore, the current study suggests that differences in \( Vv\text{PIP1i} \) expression could play a large role in controlling hydraulic permeability through \( Vv\text{PIPS} \)-\( Vv\text{PIP2} \) interactions as suggested by Vandeleur et al. (2009). Fine-scale details of \( Vv\text{PIP} \) 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 \( Vv\text{PIPS} \) in controlling cellular water relations, particularly in the root tip under varying conditions.

This study found that \( Lp^{\text{Hyd}} \) was much greater than \( Lp^{\text{Osm}} \) 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, \( Lp^{\text{Hyd}} \) and \( Lp^{\text{Osm}} \) 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 \( Lp \), (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 $L_p$ (per root surface area or per biomass) appears much more variable. In the current study, root surface area-specific $L_p$ 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 $L_p$, with osmotic and hydrostatic pressure gradients.

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**References**

Aharon R, Shahak Y, Wininger S, Bendov R, Kapulnik Y, Galili G. 2003. Overexpression of a plasma membrane aquaporin in transgenic tobacco improves plant vigor under favorable growth conditions but not under drought or salt stress. *The Plant Cell* 15, 439–447.

Alleva K, Marquez M, Villarreal N, Mut P, Bustamante C, Bellati J, Martinez G, Civello M, Amodeo G. 2010. Cloning, functional characterization, and co-expression studies of a novel aquaporin (FaPIP2;1) of strawberry fruit. *Journal of Experimental Botany* 61, 3935–3945.

Alsina MM, Smart DR, Bauerle T, de Herralde F, Biel C, Stockert C, Negron C, Save R. 2011. Seasonal changes of whole root system conductance by a drought-tolerant grape root system. *Journal of Experimental Botany* 62, 99–109.

Atkinson CJ, Else MA, Taylor L, Dover CJ. 2003. Root and stem hydraulic conductivity as determinants of growth potential in grafted trees of apple (*Malus pumila* Mill.). *Journal of Experimental Botany* 54, 1221–1229.

Bogs J, Downey MO, Harvey JS, Ashton AR, Tanner GJ, Robinson SP. 2005. Proanthocyanidin synthesis and expression of genes encoding leucoanthocyanidin reductase and anthocyanidin reductase in developing grape berries and grapevine leaves. *Plant Physiology* 138, 652–663.

Candolfi-Vasconcelos MC, Candolfi MP, Koblet W. 1994. Retranslocation of carbon reserves from the woody storage tissues into the fruit as a response to defoliation stress during the ripening period in *Vitis vinifera* L. *Plant* 192, 567–573.

Castellarin SD, Matthews MA, Di Gaspero G, Gambetta GA. 2007. Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. *Planta* 227, 101–112.

Chaumont F, Barrieu F, Jung R, Chrispeels MJ. 2000. Plasma membrane intrinsic proteins from maize cluster in two sequence subgroups with differential aquaporin activity. *Plant Physiology* 122, 1025–1034.

Choat B, Gambetta GA, Shackel KA, Matthews MA. 2009. Vascular function in grape berries across development and its relevance to apparent hydraulic isolation. *Plant Physiology* 151, 1677–1687.

Chung HH, Kramer PJ. 1975. Absorption of water and $^{32}$P through suberized and unsuberized roots of loblolly pine. *Canadian Journal of Forest Research* 5, 229–235.

Clearwater MJ, Lowe RG, Hofstee BJ, Barclay C, Mandemaker AJ, Blattmann P. 2004. Hydraulic conductance and rootstock effects in grafted vines of kiwifruit. *Journal of Experimental Botany* 55, 1371–1382.

Cortell JM, Halbleib M, Gallagher AV, Righetti TL, Kennedy JA. 2005. Influence of vine vigor on grape (*Vitis vinifera* L. cv. Pinot noir) and wine proanthocyanidins. *Journal of Agricultural and Food Chemistry* 53, 5798–5808.

Cortell JM, Halbleib M, Gallagher AV, Righetti TL, Kennedy JA. 2007. Influence of vine vigor on grape (*Vitis vinifera* L. cv. Pinot Noir) anthocyanins. 1. Anthocyanin concentration and composition in fruit. *Journal of Agricultural and Food Chemistry* 55, 6575–6584.

Cortell JM, Sivertsen HK, Kennedy JA, Heymann H. 2008. Influence of vine vigor on Pinot Noir fruit composition, wine chemical analysis, and wine sensory attributes. *American Journal of Enology and Viticulture* 59, 1–10.

Dordas C, Chrispeels MJ, Brown PH. 2000. Permeability and channel-mediated transport of boric acid across membrane vesicles isolated from squash roots. *Plant Physiology* 124, 1349–1361.
Macfay JS, Johnson GA, Kramer PJ. 1990. Observation of a water-depletion region surrounding loblolly-pine roots by magnetic-resonance-imaging. Proceedings of the National Academy of Sciences, USA 87, 1203–1207.

Martre P, Morillon R, Barrieu F, North GB, Nobel PS, Chrisepeels MJ. 2002. Plasma membrane aquaporins play a significant role during recovery from water deficit. Plant Physiology 130, 2101–2110.

McElrone AJ, Bichler J, Pockman WT, Addington RN, Linder CR, Jackson RB. 2007. Aquaporin-mediated changes in hydraulic conductivity of deep tree roots accessed via caves. Plant, Cell and Environment 30, 1411–1421.

Myles S, Boyko AR, Owens CL, et al. 2011. Genetic structure and domestication history of the grape. Proceedings of the National Academy of Sciences, USA 108, 3530–3535.

Nardini A, Gasco A, Raimondo F, Gorton E, Lo Gullo MA, Caruso T, Salleo S. 2006. Is rootstock-induced dwarfing in olive an effect of reduced plant hydraulic efficiency? Tree Physiology 26, 1137–1144.

North GB, Nobel PS. 1991. Changes in hydraulic conductivity and anatomy caused by drying and rewetting roots of Agave deserti (Agavaceae). American Journal of Botany 78, 906–915.

Olmstead MA, Lang NS, Ewers FW, Owens SA. 2006a. Xylem vessel anatomy of sweet cherries grafted onto dwarfing and nondwarfing rootstocks. Journal of the American Society for Horticultural Science 131, 577–585.

Olmstead MA, Lang NS, Lang GA, Ewers FW, Owens SA. 2006b. Examining the vascular pathway of sweet cherries grafted onto dwarfing rootstocks. Hortsience 41, 674–679.

Otto B, Kaldenhoff R. 2000. Cell-specific expression of the mercury-insensitive plasma-membrane aquaporin NtAQP1 from Nicotiana tabacum. Planta 211, 167–172.

Pongrácz DP. 1983. Rootstocks for grape-vines. Cape Town, South Africa: David Philip Publisher.

Postaire O, Tournaire-Roux C, Gronin A, Boursiac Y, Morillon R, Schaffner AR, Maurel C. 2010. A PIP1 aquaporin contributes to hydrostatic pressure-induced water transport in both the root and rosette of Arabidopsis. Plant Physiology 152, 1418–1430.

Queen WH. 1967. Radial movement of water and 32P through suberized and unsuberized roots of grape. PhD dissertation, Duke University, Durham, NC, USA.

Sade N, Gebretsadik M, Seligmann R, Schwartz A, Wallach R, Mosheilon M. 2010. The role of tobacco aquaporin1 in improving water use efficiency, hydraulic conductivity, and yield production under salt stress. Plant Physiology 152, 245–254.

Schaffner AR. 1998. Aquaporin function, structure, and expression: are there more surprises to surface in water relations? Planta 204, 131–139.

Shelden MC, Howitt SM, Kaiser BN, Tyerman SD. 2009. Identification and functional characterisation of aquaporins in the grapevine, Vitis vinifera. Functional Plant Biology 36, 1065–1078.

Siefritz F, Tyree MT, Lovisolo C, Schubert A, Kaldenhoff R. 2002. PIP1 plasma membrane aquaporins in tobacco: from cellular effects to function in plants. The Plant Cell 14, 869–876.

Solarli LI, Johnson S, Dejong TM. 2006. Hydraulic conductance characteristics of peach (Prunus persica) trees on different rootstocks
Role of aquaporins in hydraulics of grapevine rootstocks

are related to biomass production and distribution. Tree Physiology 26, 1343–1350.

Steudle E. 2001. The cohesion-tension mechanism and the acquisition of water by plant roots. Annual Review of Plant Physiology and Plant Molecular Biology 52, 847–875.

Steudle E, Meshcheryakov AB. 1996. Hydraulic and osmotic properties of oak roots. Journal of Experimental Botany 47, 387–401.

Suga S, Murai M, Kuwagata T, Maeshima M. 2003. Differences in aquaporin levels among cell types of radish and measurement of osmotic water permeability of individual protoplasts. Plant and Cell Physiology 44, 277–286.

Vandeleur RK, Mayo G, Shelden MC, Gillham M, Kaiser BN, Tyerman SD. 2009. The role of plasma membrane intrinsic protein aquaporins in water transport through roots: diurnal and drought stress responses reveal different strategies between isohydric and anisohydric cultivars of grapevine. Plant Physiology 149, 445–460.

Wheatley MD, Tattersall EAR, Tillett RL, Cramer GR. 2009. An expanded clay pebble, continuous recirculating drip system for viable long-term hydroponic grapevine culture. American Journal of Enology and Viticulture 60, 542–549.

Yamada S, Katsuhara M, Kelly WB, Michalowski CB, Bohnert HJ. 1995. A family of transcripts encoding water channel proteins – tissue-specific expression in the common ice plant. The Plant Cell 7, 1129–1142.

Ye Q, Steudle E. 2006. Oxidative gating of water channels (aquaporins) in corn roots. Plant, Cell and Environment 29, 459–470.

Yun JJ, Heisler LE, Hwang II, et al. 2006. Genomic DNA functions as a universal external standard in quantitative real-time PCR. Nucleic Acids Research 34, 6718–6718.