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ORIGINAL ARTICLE

Over-expression of PIP2;5 aquaporin alleviates gas exchange and growth inhibition in poplars exposed to mild osmotic stress with polyethylene glycol

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Abstract The effects of mild osmotic stress conditions on aquaporin-mediated water transport are not well understood. In the present study, mild osmotic stress treatments with 20 and 50 g L^{-1} polyethylene glycol 6000 (PEG) in Hoagland's mineral solution were applied for 3 weeks under controlled environmental conditions to transgenic *Populus tremula* × *Populus alba* plants constitutively over-expressing a *Populus PIP2;5* aquaporin and compared with the wild-type plants. The PEG treatments resulted in growth reductions and triggered changes in net photosynthesis, transpiration, stomatal conductance and root hydraulic conductivity in the wild-type plants. However, height growth, leaf area, gas exchange, and root hydraulic conductivity were less affected by the PEG treatments in *PIP2;5*-over-expressing poplar lines. These results suggest that water transport across the PIP2;5 aquaporin is an important process contributing to tolerance of mild osmotic stress in poplar. Greater membrane abundance of PIP2;5 was most likely the factor that was responsible for higher root hydraulic conductivity leading to improved plant water flux and, consequently, greater gas exchange and growth rates under mild osmotic stress conditions. The results also provide evidence for the functional significance of PIP2;5 aquaporin in water transport and its strong link to growth processes in poplar.

Keywords Aquaporins · Gas exchange · Growth · Hydraulic conductivity · Osmotic stress · PIP2;5

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Introduction

Tree water balance may be altered by various abiotic and biotic factors that affect the processes of water uptake, transport, and (or) loss. Fine water balance must be maintained in the soil–tree–air continuum for CO_2 uptake through the stomata without excessive transpirational water loss. Therefore, efficient water transporting system in trees is essential for timely delivery of water to the stomata. When trees are subjected to water-deficit stress, down-regulation of water flux may be beneficial to reflect the reduced water availability and prevent xylem cavitation and excessive water efflux from cells. Dynamic control of plant water fluxes can be partly achieved through the regulation of hydraulic conductivity in roots, especially in the radial water flow pathway where water is transported to the xylem and passes across cell membranes (Wan and Zwiazek 2001). This cell-to-cell water transport is facilitated and regulated by aquaporins (AQPs) (Baiges et al. 2002; Maurel et al. 2008). Therefore, understanding the processes that affect and regulate the function of AQPs is essential to understand effective water balance maintenance strategies in trees exposed to environmental stresses.

AQPs are classified into five subfamilies including plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), small basic intrinsic proteins (SIPs), nodulin 26-like intrinsic proteins (NIPs) and X intrinsic proteins (XIPs) (Gupta and Sankararamakrishnan

2009; Maurel et al. 2008, 2015). Among these five groups, PIPs and TIPs are likely the principal AQPs that are involved in a dynamic regulation of cell-to-cell water transport in plants exposed to stress (Laur and Hacke 2013). However, the processes regulating transmembrane water transport in plants subjected to water-deficit stress are little understood (Heinen et al. 2009). Similarly, the roles of different PIP1 and PIP2 isoforms in drought stress responses are unclear. The PIP1 and PIP2 subgroups differ not only in the length of their N and C termini and in amino acid residues, but also in their capacity for water movement (Chaumont et al. 2000). When expressed in *Xenopus laevis* oocytes, PIP2, but not PIP1, increases the water permeability of the oocyte membranes, but when co-expressed, PIP1 and PIP2 interact to act as a water channel (Fetter et al. 2004). In *Arabidopsis* roots, *PIP1;3, PIP1;4, PIP2;1,* and *PIP2;5* genes were up-regulated, whereas *PIP1;5, PIP2;2, PIP2;3,* and *PIP2;4* were down-regulated under water-deficit stress conditions (Jang et al. 2004) suggesting that different PIP isoforms may be involved in stress responses. Transgenic banana plants constitutively over-expressing *MusaPIP1;2* were more resistant to water-deficit stress (Sreedharan et al. 2013). Also, over-expression of *RWC3* of the PIP1 subgroup in rice (*Oryza sativa* L. spp *japonica*) resulted in increased resistance to polyethylene glycol (PEG)-induced osmotic stress through the enhancement of root hydraulic conductivity (Lian et al. 2004). It has been previously suggested that AQPs could be used to regulate plant responses to water-deficit stress and different strategies may be required depending on stress intensity (Siemens and Zwiazek 2004), especially since AQPs may also be involved in the transport of other small molecules including CO_2 (Uehlein et al. 2003) and O_2 (Zwiazek et al. 2016). Similar to low root temperature (Lee et al. 2012; Ranganathan et al. 2016), an increase in hydraulic conductivity of the roots through the increase in AQP-mediated water flow may be helpful to plants during the initial stages of drought or osmotic stress when this stress is just beginning to be perceived by the plant, or under mild drought or osmotic stress events.

In the present study, we tested the hypothesis that over-expression of PIP2;5, a major water-conductive and drought-responsive poplar AQP (Almeida-Rodriguez et al. 2010), would help alleviate the effects of mild osmotic stress in transgenic plants. Therefore, we generated two hybrid poplar (*Populus tremula* × *alba*) lines over-expressing *PtdPIP2;5* (Ranganathan et al. 2016) and subjected the plants to mild osmotic stress with PEG 6000. We used relatively low concentrations of PEG (Caruso et al. 2008) to maintain strictly controlled levels of mild osmotic stress for up to 3 weeks. We examined the root water transport, gas exchange and transcript abundance of PIPs in two *PtdPIP2;5*-over-expression lines (*PtdPIP2;5ox1* and *PtdPIP2;5ox2*) to 2 and 5% PEG 6000 in modified Hoagland's solution and compared them to the wild-type plants. Based upon predictions made in our earlier studies of drought responses (Siemens and Zwiazek 2004), we expected that over-expression of this major water-conducting AQP in poplar would enhance root water transport under mild osmotic stress, and that transgenic plants would maintain higher growth rates compared with the wild-type plants under stress conditions.

Materials and methods

Plant material

PIP2;5, a major water-conducting and drought-responsive poplar AQP (Almeida-Rodriguez et al. 2010), was selected to produce transgenic plants of *Populus tremula* × *alba* (INRA Clone 717 1B4 obtained from INRA-Versailles, France). The generation and initial characterization of these transgenic plants have been earlier reported (Ranganathan et al. 2016). Of the transformed lines, two lines (31—*PIP2;5ox1* and 121—*PIP2;5ox2*) that exhibited the highest expression of *PtdPIP2;5* in leaves were selected to produce rooted cuttings for the study (Ranganathan et al. 2016).

The plants produced from rooted cuttings were grown in controlled-environment growth room. Environmental conditions in the growth room were: 24/18 °C (day/night) temperature, 65 ± 10% relative humidity, and 16-h photoperiod with 350 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) at the top of the plants (full-spectrum fluorescent bulbs, F96T8/TL835/HO, Markham, ON, Canada). Prior to commencing polyethylene glycol (PEG) experiments, the plants were watered daily and fertilized weekly with 15:30:15 N–P–K (nitrogen–phosphorus–potassium) commercial fertilizer until they reached a height of 30–40 cm and 3–6 mm stem diameter.

PEG experiments

Each PEG treatment was replicated in three 30-L hydroponic containers. Each container housed 18 plants: six plants of each transgenic line (*PIP2;5 ox1* and *PIP2;5 ox2*) and of the wild type. The containers were filled with modified Hoagland's solution (Zhang et al. 2013). A split plot design was used with randomized blocks within each plot. An aeration pump (Model 9.5 950GPH, Danner MFG Inc., New York, USA) was immersed in the solution to maintain continuous aeration, resulting in dissolved O_2 values of >5 mg L^{-1} in the presence of plants. The hydroponic units were cleaned once per week to prevent algal and bacterial build-up. All containers had spouts

installed into their sides to facilitate drainage, aeration, and circulation of the nutrient solution via PVC tubing.

To establish the hydroponic system, roots of poplar plants were gently washed with distilled water and immersed into the culture solution in each container. Plants were held in place with foam plugs fitted into holes within Styrofoam boards that floated on the top of the solution culture. After 3 weeks in hydroponic culture, the plants were subjected to the following treatments: 0, (control), 20, and 50 g L^{-1} PEG 6000 (Sigma-Aldrich, Taufkirchen, Germany) by gradually adding PEG to the nutrient solution over 36 h. The mineral solutions with or without PEG were replaced every week. We used PEG concentrations commonly used for screening of osmotic resistance for plants in tissue culture (Biswas et al. 2002; Bidabadi et al. 2012) to induce relatively mild stress conditions for up to 3 weeks. Calculated osmotic potential of the 20 and 50 g L^{-1} PEG solutions was 0.02 and 0.05 MPa, respectively (Michel and Kaufmann 1973).

Height and weight measurements

Plant heights were measured every week for each plant, taken from the root collar to the shoot tip. The dry weight determinations of roots and shoots of individual plants were obtained by drying the plants in an oven at 85 °C for 72 h. The relative shoot height growth (RSHR) was calculated as: RSHR = [(Final height − Initial height)/Initial height] × 100 (Hoffmann and Poorter 2002).

Leaf expansion measurements

Leaf expansion was determined by measuring over time the lengths and widths of developing (youngest) leaves in control and PEG-treated plants (Lu et al. 2010). The same leaf was measured every week for 3 weeks. Width and length values were used to calculate actual leaf area values from the previously determined linear regression between leaf length (L) × width (W) and projected leaf area (A): $A = 0.6712\ L \times W$ ($r^2 = 0.99$, $n = 20$). For the regression, 20 leaves from top, bottom and middle parts of one wild-type replicate were randomly picked and length and width of each leaf was measured. Each leaf was scanned and the leaf area was measured using MIPAR, image analysis software (MathWorks, Natick, MA, USA).

Gas exchange measurements

After 3 weeks of PEG 6000 treatments, stomatal conductance (g_s), net photosynthesis (P_n), and transpiration (E) rates were measured in the mornings on the second fully expanded leaf of each plant with a portable open-flow photosynthesis system (LI-6400, Li-Cor Inc., Lincoln, NE, USA) equipped with an incorporated auxiliary LI-6400B red/blue LED light source. Light intensity in the leaf chamber was 350 μmol m^{-2} s^{-2} and reference CO_2 was set at constant 386 μmol mol^{-1} using the 6400-01 CO_2 mixer. The data were logged every 30 s during a 5-min period (Voicu et al. 2008).

Root hydraulic conductivity (L_p)

The root hydraulic conductance (K_r) was measured by attaching excised root system to the high-pressure flow meter (HPFM, Dynamax Inc, Houston, TX, USA) using compression couplings. Water was then forced inside stems at increasing pressure. The computer-recorded water flow rate and the applied pressure were used to compute K_r (Apostol et al. 2004). Root volumes were measured for each root system using volume displacement of water in a graduated cylinder. L_p was determined after dividing K_r by root volume (Kamaluddin and Zwiazek 2003).

Statistical analyses

Statistical analyses were performed using the R v2.15.3 statistical software (R Development Core Team 2011) at $\alpha = 0.05$ confidence level. The data sets were checked for parametric assumptions of normality (Shapiro–Wilk and Kolmogorov–Smirnov tests) and homogeneity of variances (Bartlett's test). Box plots were used for identifying outliers from the data set that were removed before the statistical analyses. When necessary to meet the assumptions of normality and homogeneity of variance, data were transformed, either by log transformation or square root transformation. The data were analyzed using ANOVA. Tukey's multiple comparison test was used to determine significant differences at $p \leq 0.05$.

Results

Relative shoot height growth rates, dry weights, and leaf areas

Relative shoot height growth rate (RSHG) was reduced with increasing concentrations of PEG (Fig. 1a) After 3 weeks of treatment with 20 and 50 g L^{-1} PEG, RSHG reduction was significantly greater in the wild-type plants compared with the transgenic plants and there was no significant difference in RSHG between the two *PIP2;5* over-expression lines (Fig. 1a). Total plant dry weight showed a decreasing trend with the increasing concentration of PEG in the nutrient solution (Fig. 1b). When subjected to 20 and 50 g L^{-1} PEG for 3 weeks, both transgenic lines showed significantly higher total plant dry weights compared to the wild-type plants (Fig. 1b). Similar

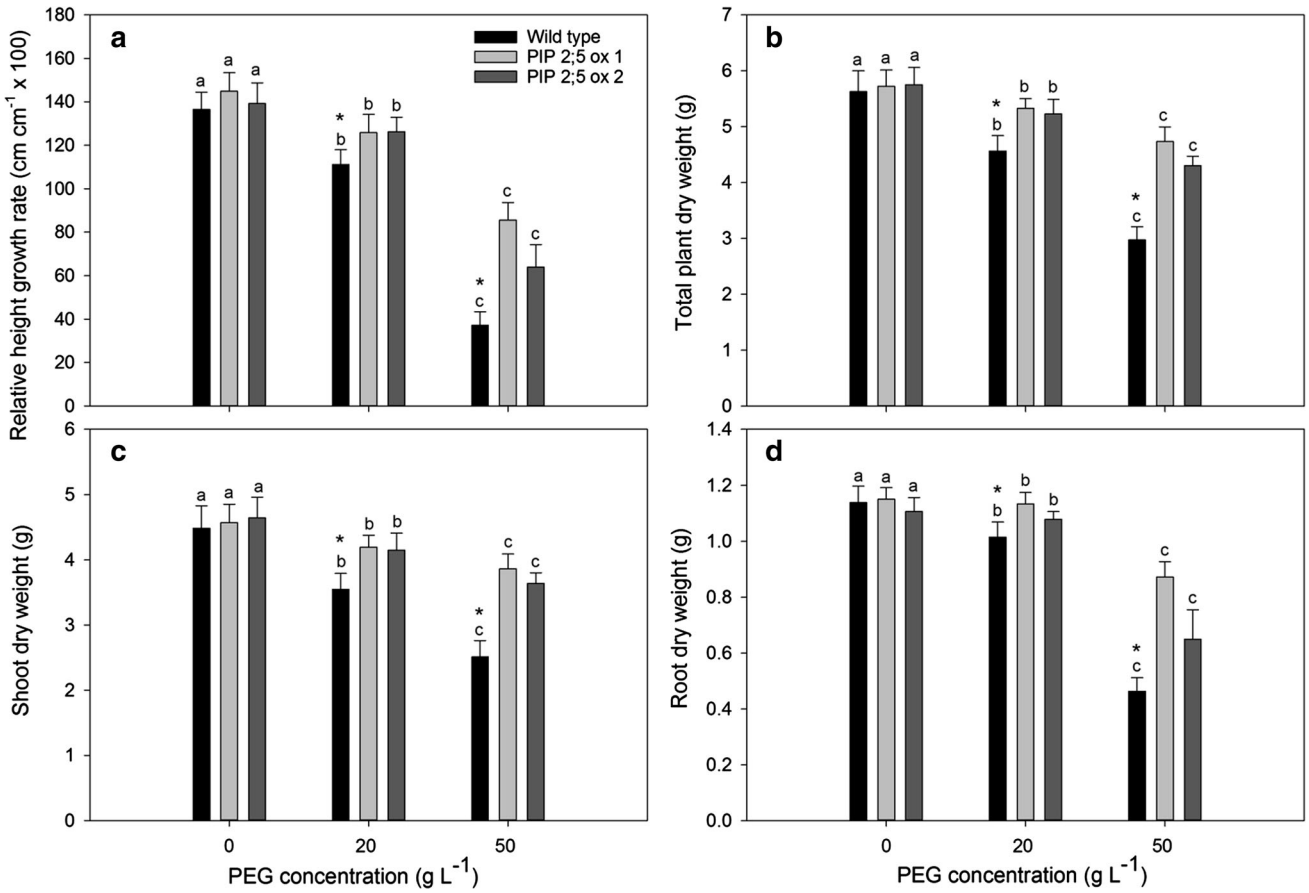

Fig. 1 Relative shoot height growth (RSHG) (**a**), total dry weights (**b**), shoot dry weights (**c**), and root dry weights (**d**) of two *PIP2;5ox* lines and wild-type poplar plants subjected to 0 (control), 20 and 50 g L^{-1} PEG 6000 treatments for 3 weeks in mineral nutrient solution culture. Mean ± SE ($n = 6$) are shown. *Letters* significant differences ($P \leq 0.05$) between PEG treatments within a genotype, while *asterisks* significant differences ($P \leq 0.05$) among genotypes at a given PEG treatment, determined by Tukey's multiple comparison test

trends were also observed for shoot dry weights (Fig. 1c) and root dry weights (Fig. 1d).

In control (no PEG added) plants, leaf areas were similar in the wild-type and the two *PIP2;5ox* lines (Fig. 2a). However, when the plants were exposed to 20 and 50 g L^{-1} PEG for 3 weeks in nutrient solution, leaf area values in the wild-type plants were significantly lower compared with the over-expression lines (Fig. 2b, c).

Gas exchange

Net photosynthetic rates in transgenic and wild-type plants were lower compared to the control after 3 weeks in 20 and 50 g L^{-1} PEG (Fig. 3a). There were no significant differences in net photosynthesis between plants of the wild-type and *PIP2;5ox* lines when they were exposed to 0 g L^{-1} PEG (i.e., control, Fig. 3a). However, the *PIP2;5ox* plants that were subjected to 20 g L^{-1} PEG treatment had significantly higher net photosynthesis compared to the wild-type plants; there was no significant difference in the net photosynthetic rates between the two *PIP2;5ox* lines (Fig. 3a). Similar responses of net photosynthesis were observed in plants subjected to 50 g L^{-1} PEG (Fig. 3a).

Transpiration rates (Fig. 3b) and stomatal conductance (Fig. 3c) responded to PEG treatments similar to net photosynthesis. Transgenic lines showed significantly higher transpiration rates and stomatal conductance than the wild-type plants when they were exposed to 20 and 50 g L^{-1} PEG treatment (Fig. 3b, c).

Water use efficiency (WUE) values for the transgenic and wild-type poplars significantly increased with increasing PEG concentrations (Fig. 3d). However, there were no significant differences in the WUE values between the wild-type and transgenic lines within any of the PEG concentrations (Fig. 3d).

Root hydraulic conductivity

PEG treatments significantly reduced the root hydraulic conductivity (L_p) in all poplar lines (Fig. 4). There were no

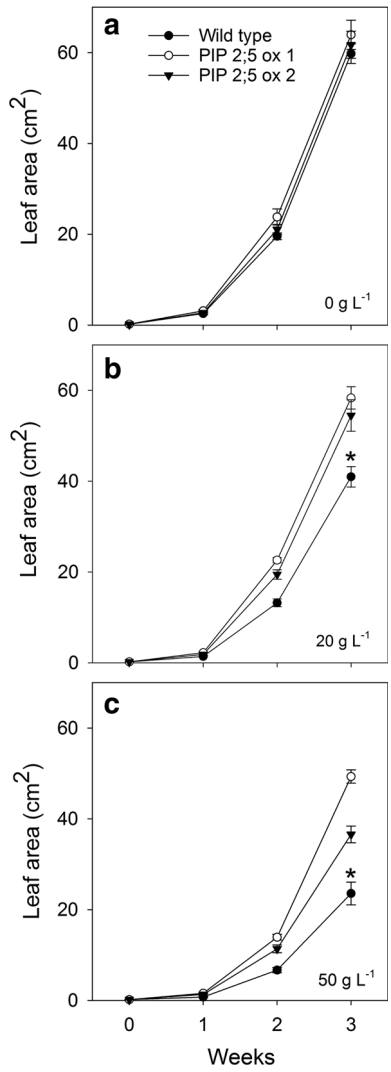

Fig. 2 Areas of developing leaves measured over time in the two *PIP2;5ox* lines and wild-type poplar plants subjected for 3 weeks to 0 (control) (**a**), 20 (**b**), and 50 g L^{-1} (**c**) of PEG 6000 treatments in mineral nutrient solution culture. Mean ± SE ($n = 6$) are shown. *Asterisks* significant differences ($P \leq 0.05$) among genotypes at a given PEG treatment (Tukey's multiple comparison test)

significant differences in L_p between the wild type and *PIP2;5ox* lines when exposed to 0 g L^{-1} PEG (Fig. 4). However, when the plants were treated with 20 and 50 g L^{-1} PEG for 3 weeks, transgenic line *PIP2;5 ox1* showed significantly higher L_p values compared with the wild-type plants (Fig. 4). There were no significant differences in L_p between the *PIP2;5 ox2* and wild-type plants in both PEG treatments.

Discussion

PEG induces osmotic stress in plants and triggers plant responses similar to those produced by natural drought (Dhanda et al. 2004; Lawlor 1970; Mujtaba et al. 2005). We used relatively low concentrations of PEG 6000 to trigger partial stomatal closure in plants and maintain stress conditions for an extended period of time. A similar range of PEG concentrations has been used to induce osmotic stress in tissue culture (Biswas et al. 2002; Bidabadi et al. 2012). Our results show that plants of the *PIP2;5ox* lines were more resistant to PEG treatments compared with the wild-type plants in terms of height growth, dry weights, and leaf size. Differences that we observed in osmotic stress responses between the two transgenic lines were likely due to the differences in levels of transgene expression, as previously reported (Ranganathan et al. 2016).

Water-deficit stress is a common factor affecting tree growth and can be induced by drought and many other environmental stresses including salinity, high and low temperature, and low air humidity (Almeida-Rodriguez et al. 2010; Greenway and Munns 1980; Lee et al. 2012; Ranganathan et al. 2016). Growth reductions observed in these mildly PEG-stressed plants were likely the result of altered balance between water uptake and water loss leading to cell turgor reduction and stomatal closure (Siemens and Zwiazek 2004). Improved water uptake in plants over-expressing AQPs under stress conditions could potentially improve water balance and consequently growth rates. However, over-expression of AQPs can also alter plant growth patterns. In transgenic tobacco plants over-expressing *AtPIP1b*, root-to-shoot fresh weight ratios were reduced compared with the wild-type plants, likely reflecting a more efficient root water uptake due to the increased abundance of AQPs (Cui et al. 2005). Interestingly, *Vicia faba* plants over-expressing *VfPIP1* had longer lateral and primary roots than controls which enhanced their root hydraulic conductivity (Cui et al. 2005). The over-expression of durum wheat *TdPIP1;1* in tobacco plants also made plants more tolerant to abiotic stresses by increasing root growth and leaf areas (Ayadi et al. 2011). Root growth is directly related to the whole plant water transport efficiency and the phenotypic changes produced by over-expression of AQPs are the key factors that influence stress tolerance. In our study, the net photosynthesis and transpiration rates were significantly higher in transgenic poplars compared with the wild-type plants subjected to 20 and 50 g L^{-1} PEG treatments. In osmotically stressed plants, gas exchange capacity was likely reduced due to the slow water delivery to the leaves, resulting in the reduction of stomatal opening. Since plant water balance is the net result of the rates of water uptake, transport and loss from the plant, hydraulic conductivity of the plant tissues plays an important role in this process (Siemens and Zwiazek 2004; Voicu and Zwiazek 2004; Cui et al. 2005). In most plants, the greatest resistance to water transport is in the root radial pathway and changes in

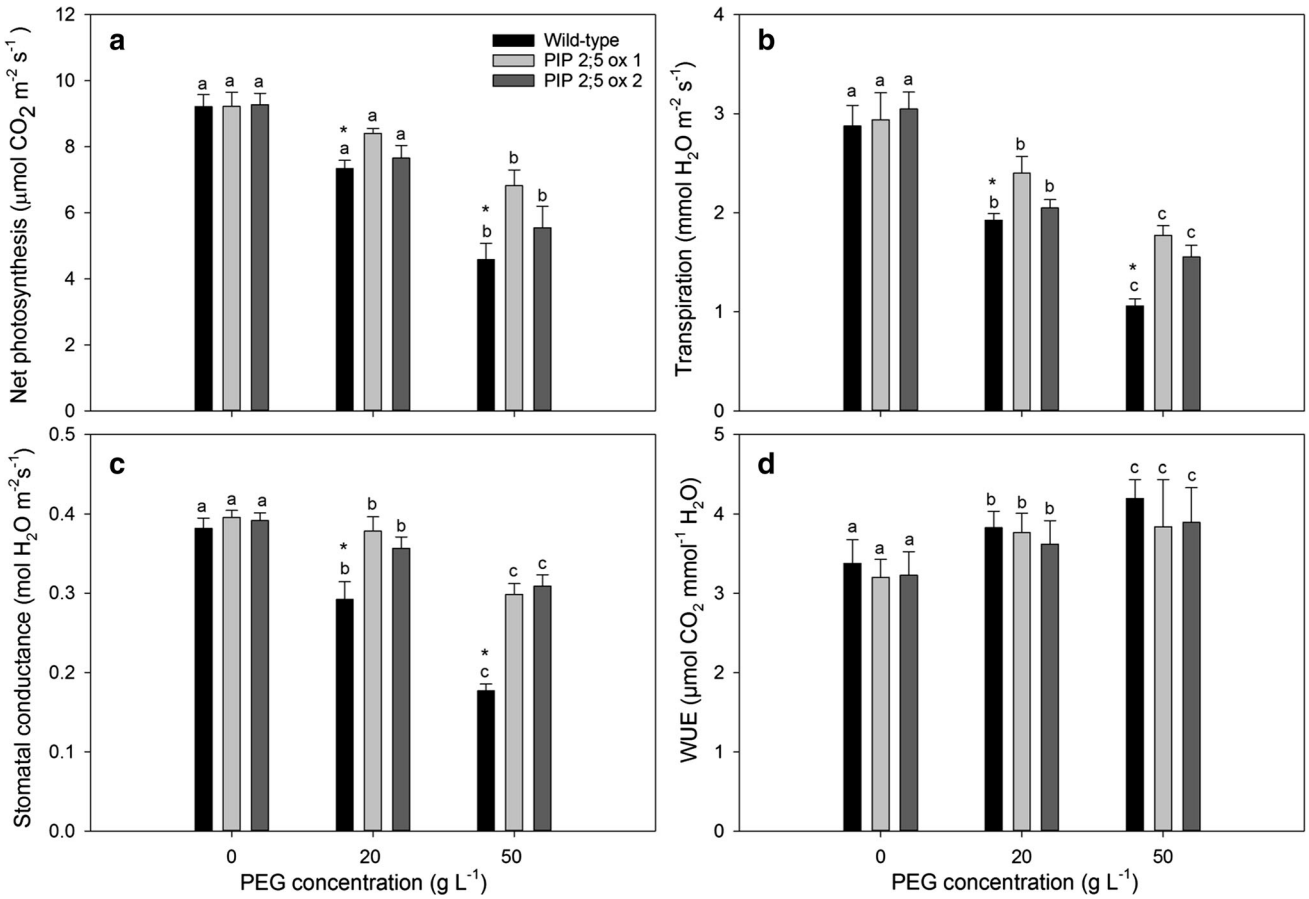

Fig. 3 Net photosynthetic rates (**a**), transpiration rates (**b**), stomatal conductance (**c**), and water use efficiency (**d**) in two *PIP2;5ox* lines and wild-type poplar plants subjected for 3 weeks to 0 (control), 20, and 50 g L^{-1} of PEG 6000 treatments in mineral nutrient solution culture. Mean ± SE ($n = 6$) are shown. *Letters* significant differences ($P \leq 0.05$) between PEG treatment concentrations within a genotype, while *asterisks* significant differences ($P \leq 0.05$) among genotypes at a given PEG treatment concentration, determined by the Tukey's multiple comparison test

root AQP activity and abundance can be effectively used to regulate plant water fluxes (Steudle and Peterson 1988). The increase in the abundance of AQPs could be an effective stress resistance strategy in plants exposed to mild water-deficit stress conditions such as those in this study imposed by PEG. It has been previously reported that plants subjected to mild water-deficit stress may respond with increased L_p to increase water delivery rate to the transpirational areas, while the conditions of severe water-deficit stress commonly decrease L_p to preserve water (Siemens and Zwiazek 2004).

Changes in leaf and root biomass are often reflected by changes in root and leaf hydraulic conductivity (Liu et al. 2014). Similarly, a compensation effect may result in the production of a smaller root system in transgenic plants over-expressing AQPs (Aharon et al. 2003; Vandeleur et al. 2009) that could have also affected their water uptake capacity under stress conditions. In our study, L_p was significantly less sensitive to PEG treatments in the *PIP2;5ox1* line compared with wild-type plants, while the differences in L_p between the *PIP2;5ox2* line and wild-type plants was not statistically significant. In transgenic lowland rice (*Oryza sativa* L.) plants over-expressing *RWC3* AQP, increased resistance to osmotic stress was achieved through higher root L_p and lower leaf water potential Lian et al. 2004). The responses to over-expression may depend on the stress resistance strategy of plants. When anisohydric grapevine plants were subjected to drought, *PIP1* expression increased in roots, resulting in an increase in the cortical cell hydraulic conductivity (Vandeleur et al. 2009). In trembling aspen, root hydraulic conductivity responded with an increase or a decrease depending on the severity of water-deficit stress (Siemens and Zwiazek 2004).

In our study, stomatal conductance was reduced in all poplar lines subjected to PEG treatments. However, the transgenic lines maintained higher stomatal conductance compared with the wild-type poplars when exposed to 50 g L^{-1} PEG for 3 weeks. This demonstrates a direct link between gas exchange responses and AQP function in plants exposed to osmotic stress. Reductions in plant

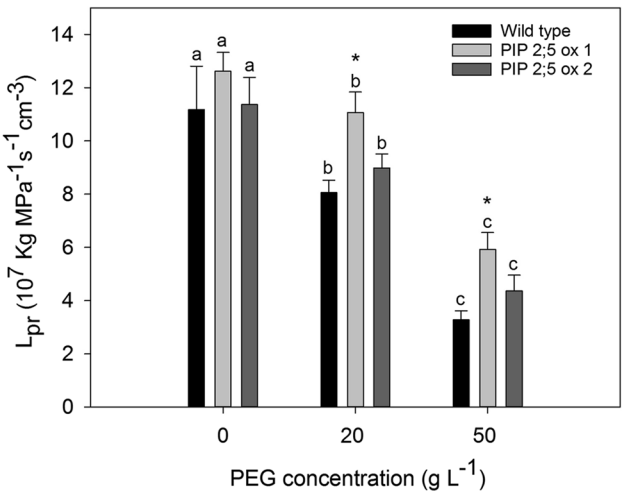

Fig. 4 Root hydraulic conductivity (L_p) of the two *PIP2;5ox* lines and wild-type poplar plants subjected for 3 weeks to 0 (control), 20, and 50 g L^{-1} of PEG 6000 treatments in mineral nutrient solution culture. Mean ± SE ($n = 6$) are shown. *Letters* significant differences ($P \leq 0.05$) between PEG treatment concentrations within a genotype, while *asterisks* significant differences ($P \leq 0.05$) among genotypes at a given PEG treatment concentration, determined by Tukey's multiple comparison test

growth due to water-deficit stress are commonly associated with decreases in photosynthetic activities due to decreased CO_2 uptake and irreversible inactivation of PSI and PSII (Allakhverdiev et al. 2000) Photosynthetic responses of plants over-expressing AQPs are not well understood. Net photosynthesis in tobacco plants over-expressing *Mesembryanthemum crystallinum* AQP was higher compared with the wild-type plants under well-watered conditions and when exposed to prolonged soil water-deficit stress (Kawase et al. 2013). Similarly, over-expression of the barley AQP gene *HvPIP2;1* enhanced internal CO_2 conductance and CO_2 assimilation in rice (Tsuchihira et al. 2010). However, in both *McMIPB* and *HvPIP2;1* over-expressing plants, a reduced shoot/root biomass ratio was observed under stress. AQP over-expression may affect gas exchange responses by altering morphological characteristics of the plants (Hanba et al. 2004). In transgenic tobacco plants over-expressing *AtPIP1b*, stomatal density increased and enhanced transpiration (Zhou et al. 2012). Similar to transgenic *Arabidopsis* plants over-expressing *NtAQP1* (Cui et al. 2005), we did not observe changes in stomatal density or distribution.

In our study, WUE significantly increased in response to PEG treatments in all poplar lines. This demonstrates that the stress resistance strategy resulting in the observed increases in WUE was not a function of PIP2;5 expression levels. Increasing WUE, atmospheric carbon gain and the improvement of plant water retention by absorption are important for the development of plant tolerance to abiotic stresses (Tsuchihira et al. 2010). When plants detect water-deficit conditions, molecular signaling pathways are triggered that result in changes in growth patterns, gas exchange reduction, osmoregulation and adjustments of water use efficiency (Bogeat-Triboulot et al. 2007; Barbieri et al. 2012).

The objective of the PEG treatments was to induce mild osmotic stress levels that trigger partial stomatal closure and reduce net photosynthesis and growth without producing in plants severe water-deficit symptoms of turgor loss and wilting. The notion that growth regulation under drought stress conditions is independent of hydraulics, and occurs even when water potential is not affected, has been confirmed by some studies (Claeys and Inzé 2013). Our results show that the aquaporin-mediated water transport is the limiting factor to gas exchange under mild water-deficit stress conditions and that the over-expression of PIP2;5 aquaporin can alleviate growth inhibition. This corroborates the earlier findings that demonstrated an increase in root hydraulic conductivity to be among the initial responses of *Populus tremuloides* to mild drought stress (Siemens and Zwiazek 2004). Due to the dynamic responses of plants to increasing levels of water-deficit stress, the effects of PIP2;5 overexpression that are described in this study are limited to mild stress and are likely to differ from those that are triggered by severe drought stress where a different suite of plant responses is needed for plant survival (Siemens and Zwiazek 2004).

Conclusions

Height growth, leaf size, and gas exchange were less affected by the PEG-induced stress in poplar lines over-expressing *PIP2;5* AQP compared with wild-type plants. A combination of characteristics that likely included more efficient water transport helped maintain higher stomatal conductance that contributed to improved PEG stress resistance in these poplar lines. The ability of a particular AQP isoform to confer resistance to osmotic stress may depend on its contribution to the plant control of water loss by transpiration as well as its ability to maintain CO_2 assimilation and water transport properties. The responses of the wild-type and *PIP2;5* overexpression plants demonstrate that root hydraulic conductivity is among the primary limiting factors to the gas exchange and growth of plants under mild water-deficit stress conditions.

Author contribution statement RK performed the experiment. WEK provided training and assistance with the transgenic plants used for these experiments. RK, AE and MV wrote the manuscript. JJZ and JEKC supervised RK and WEK, edited the MS and funded the project.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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