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Rapid changes in root HvPIP2;2 aquaporins abundance and ABA concentration are required to enhance root hydraulic conductivity and maintain leaf water potential in response to increased evaporative demand

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Abstract. To address the involvement of abscisic acid (ABA) in regulating transpiration and root hydraulic conductivity (Lp_{Root}) and their relative importance for maintaining leaf hydration, the ABA-deficient barley mutant Az34 and its parental wild-type (WT) genotype (cv. Steptoe) were grown in hydroponics and exposed to changes in atmospheric vapour pressure deficit (VPD) imposed by air warming. WT plants were capable of maintaining leaf water potential (Ψ_L) that was likely due to increased Lp_{Root} enabling higher water flow from the roots, which increased in response to air warming. The increased Lp_{Root} and immunostaining for HvPIP2;2 aquaporins correlated with increased root ABA content of WT plants when exposed to increased air temperature. The failure of Az34 to maintain Ψ_L during air warming may be due to lower Lp_{Root} than WT plants, and an inability to respond to changes in air temperature. The correlation between root ABA content and Lp_{Root} was further supported by increased root hydraulic conductivity in both genotypes when treated with exogenous ABA (10^{-5} M). Thus the ability of the root system to rapidly regulate ABA levels (and thence aquaporin abundance and hydraulic conductivity) seems important to maintain leaf hydration.

Additional keywords: *Hordeum vulgare* L., absicisic acid, tissue hydration, water relations.

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Introduction

Maintaining tissue hydration is of pivotal importance for plant survival under a changing environment. This is achieved by fine regulation of leaf water relations, which is largely dependent on coordinated changes in stomatal and hydraulic conductivity (Meinzer 2002). Although both mechanisms are important for maintaining the balance between water uptake and losses, the former has attracted much more attention (Dodd, 2005; 2013 and references therein).

The discovery of the membrane located water channel proteins aquaporins, whose activity alters hydraulic conductivity (Maurel et al. 2008; Chaumont and Tyerman, 2014), led to an increase in research addressing the control of plant water uptake. The plant hormone abscisic acid (ABA), whose concentration increases in response to water deficit, can influence both stomatal (see ref. in review of Dodd 2005) and root and shoot hydraulic conductivity (Hose et al. 2000; Pantin et al. 2013), the latter effect being due to ABA-induced increase in activity of aquaporins (Parent et al. 2009). Thus the same hormone can induce opposite influences on water relations by either decreasing water flow due to stomatal closure, or increasing it by modulating hydraulic conductivity. The resulting effect may depend on the site of ABA accumulation in stressed plants: foliar ABA accumulation directly closes the stomata (McAdam et al 2016) and reduces transpiration by decreasing leaf hydraulic conductivity (Pantin et al. 2013), while root ABA accumulation increases hydraulic conductivity in a dose-dependent manner (Hose et al. 2000; Kudoyarova et al. 2011; Dodd 2013).

When plants experience a sudden increase in evaporative demand (eg. by warming the air that surrounds them), increased root ABA concentration was correlated with increased root hydraulic conductivity (Kudoyarova et al. 2011). However, using ABA-deficient or ABA-overproducing plants provides more specific evidence that ABA regulates root hydraulic conductivity and maintains leaf water relations. Genetic modification of ABA levels caused long lasting effects on plant hydraulic properties and aquaporin activity in maize (Parent et al. 2009) and tomato (Thompson et al. 2007) plants. However, the role of ABA in regulating plant water relations is likely to be most critical in response to abrupt step-changes in environmental conditions. Thus we compared leaf water relations, AQPs abundance and ABA content and localization in roots of the ABA deficient barley mutant (Az34) and its parental line cv. Steptoe in response to air heating (that increased evaporative demand). The goal of the work was to check the ability of the root system to rapidly regulate ABA levels (and thence hydraulic conductivity) and its importance to maintain leaf hydration.

Material and Methods

Seedlings of barley *Hordeum vulgare* L. (ABA deficient mutant *Az34* and its wild-type cv. *Steptoe*) were grown in 3-litre containers filled with 0.1 strength Hoagland-Arnon nutrient solution under illumination of 400 μmol m^{-2} s^{-1} from ZN and DNAT-400 fluorescent lamps, at a 14-h photoperiod (from 8:00 to 22:00), 24°C air temperature and 40 % relative air humidity (which corresponds to VPD of 2 kPa). When plants were 7-d-old and bearing one true leaf that was half-expanded, the air temperature was increased by 4° C from 24° C to 28° C (increase in VPD from 2 up to 2.6 kPa) and maintained at that level for 1 hour using a fan-heater, taking care not to direct the airflow directly on to the shoots. Experiments started at 11:00.

Transpiration was measured as a loss of weight during 15 min by 10 intact plants drawing water from 50 ml of nutrient solution in a container covered with aluminium foil to minimise surface evaporation. Stomatal conductance was determined with a porometer Model AP4, Delta T Devices, United Kingdom).

Leaf water potential (Ψ_L) of tissue discs of 7 mm diameter were punched from mature leaves, placed immediately on clean sample holders and wrapped in aluminium foil to minimize water losses. After 16 discs had been collected (approximately 15 min), they were unwrapped and then loaded into C52 chambers (Wescor Inc., Logan, UT, USA), incubated for 2 h then voltages were read with a microvoltmeter (model HR-33T-R; Wescor Inc., Logan, UT, USA). Voltages were converted into water potentials based on calibration with salt solutions of known osmotic potential.

Xylem sap flow from detached root systems was measured according to Carvajal et al. (1996) with modifications described by Veselov et al. (2008) and Vysotskaya et al. (2004). Applying the method for measuring Lp_{Root} in plants after air heating is described in detail by Kudoyarova et al. (2011). In short, the aerial parts of the plant were removed leaving a cylinder of leaf bases. These were connected to thin pre-weighed capillaries by means of silicon tubing. Xylem sap flow was measured in this way at 20°C for all plants. After 1 h, the capillary containing osmotically-driven xylem sap was disconnected from the root system and weighed. The procedure was started after transpiration had stabilized following air heating (normally after 40 min). Xylem sap flow was measured in this way for all plants (either control i.e. kept at 24 °C all the time or exposed to 28 °C for about 40 min). In some cases ABA (10^{-5} M) was added to the nutrient solution of control Az34 and Steptoe plants 15 min before the start of sap collection and was present in the solution during xylem sap collection. Bleeding sap from each capillary was diluted five times to provide sufficient sample for measurement of osmotic potential using a freezing point depression osmometer (Osmomat 030, Germany). In preliminary experiments, proportionality of the effect of dilution on the obtained values was checked. Root hydraulic conductivity, Lp_{Root} was calculated according to equation: $Lp_{Root} = J/((\Psi_s - \Psi_x) \times FW)$ where J is

the bleeding sap flow rate and (Ψ_s -Ψ_x,) the difference in osmotic pressure between xylem sap and root medium and FW is the root fresh weight: a root solute reflection coefficient of 1.0 was used (Knipfer and Fricke 2010). Because roots were dipped in 0.1 strength Hoagland-Arnon nutrient solution with near-zero osmolality, the gradient of osmotic pressure was equal to Ψ_x .

To inhibit AQP activity, hydroxyl radicals (*OH) were produced through the Fenton reaction ($Fe^{2+}+H_2O_2= Fe^{3+}+OH^-+*OH$) by mixing equal volumes of 6 mM H_2O_2 and 6 mM FeSO4 (Ye and Steudle 2006).

Excised roots might have lower Lr as shown by Vandeleur et al (2014) since the measured values of hydraulic conductivity are the result of osmotically induced flow rather than hydrostatic induced flow. However, since osmotically driven flow depends on aquaporins, our measurements seems appropriate within the context of the research problem posed

ABA was immunoassayed as previously described (Vysotskaya et al. 2009) in the roots of control plants (continuously kept at 24 ^oC) and exposed to air heating (after transpiration had stabilised about 40 min after the start of experiment). Aqueous residues of ethanol extracts were diluted with distilled water, acidified with HCl to pH 2.5 and partitioned twice with peroxide-free diethyl ether (ratio of organic to aqueous phases was 1:3). Subsequently hormones were transferred from the organic phase into 1% sodium hydrocarbonate (pH 7-8, ratio of organic to aqueous phases was 3:1), re-extracted with diethyl ether after acidification to pH 2.5, methylated with diazomethane and immunoassayed using antibodies to ABA (Veselov et al. 1992). ABA recovery calculated in model experiments was about 80%. Reducing the amount of extractant, based on the calculated distribution of ABA in organic solvents, increased the selectivity of hormone recovery and the reliability of immunoassay. The reliability of the immunoassay for ABA was enabled by both specificity of antibodies and purification of hormones according to a modified scheme of solvent partitioning (Veselov et al. 1992).

For immunolocalization of AQPs, root sections were harvested from control Steptoe and Az34 plants. Root tip segments 3-5 mm in length were fixed in 4% carbodiimide (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, Sigma, United States) for 4 h as described earlier (Sharipova et al. 2016). Tissues were infiltrated with carbodiimide under vacuum during the first 30 min of fixation. After dehydration in ethanol solutions of increasing grades (up to 96%), samples were embedded in the methacrylate resin (JB-4, Electron Microscopy Sciences, United States) as recommended by manufacturers. Histological sections (1.5 μm thickness) were cut with the rotation microtome (HM 325, MICROM Laborgerate, Germany) and placed on slides. Immunolocalization was performed as described earlier (Sharipova et al. 2016). Sections were treated with 0.1 M Na-phosphate buffer (pH 7.3) containing 0.2% gelatin and 0.05% Tween 20 (PGT) for 30 min. Rabbit anti-ABA serum (20 μl), and diluted with PGT at the ratio of 1 : 80,

was poured on some sections. To check specificity of immunostaining, other sections were treated with non-immune serum at similar dilution. Sections were covered with 50 µl 0.1 M phosphate buffer (pH 7.2–7.4) with 0.2% gelatine and 0.05% Tween 20 (PGT) and incubated for 30 min in a moist chamber. Serum and gold conjugates were diluted with PGT. Sections washed with distilled water were incubated with immune serum to HvPIP2;1, HvPIP2;2 and HvPIP2;5 aquaporins for 2 h in a moist chamber. Polyclonal antibodies for HvPIP2s were raised in rabbits against synthetic oligopeptides (Medical & Biological Laboratories Co., Japan) corresponding to the amino acid sequences in the N- region of HvPIP2;1 (Katsuhara et al. 2002), HvPIP2;2 (Horie et al. 2011), and HvPIP2;5 (Sharipova et al., 2016). Control sections were treated with rabbit nonimmune serum. To visualize serum binding with aquaporins, sections were treated with gold conjugate (BBInt, United Kingdom) for 1 h in a moist chamber. After three washes with PT samples were incubated with silver enhancer (BBInt, United Kingdom) for 15–20 min in dark and examined under a light microscope. Excess silver was removed with distilled water. Preparations were visualized under an Axio Imager.A1 light microscope (Carl Zeiss Jena, Germany) equipped with an AxioCam MRc5 digital camera (Carl Zeiss Jena, Germany).

Intensity of immunostaining of plasmalemma aquaporins was estimated from 8-bit grayscale images using ImageJ software (v.1.48, National Institutes of Health). Staining values, obtained by determining the pixel intensity, were averaged for each root section (about 160 circles per image of one root section). Intensity of root section staining was measured by using the “Freehand Selections” Tool of the same software by selecting the entire area of root sections and measuring mean pixel intensities within the region of interest. Images were taken from 9 independent sections per genotype or temperature-treatment. Intensity of staining was expressed in arbitrary units, maximal staining of circles within root section images was taken as 100 %, while minimal staining was 0 %.

Significant differences between treatments were determined by employing an analysis of variance (ANOVA) using the Excel software. The least squares difference (LSD) test was performed to discriminate significant ($p<0.05$) treatment differences.

Results

Transpiration of Az34 plants was initially 45% higher than in Steptoe plants (Fig. 1). Air heating increased transpiration rate of Steptoe and Az34 plants by 39% and 25% respectively with Steptoe plants ultimately transpiring at the same rate as Az34 plants under control conditions.

Stomatal conductance of Steptoe and Az34 plants was about 55 and 70 mmol m^{-2} s^{-1}, respectively (statistically different at $p<0.05$, $n=10$) and did not change significantly with the air warming.

Leaf water potential (Ψ_L) was measured after transpiration stabilized about 40 min after the start of air heating. Leaf water potential of Az34 was 0.32 MPa lower than that of parental cv. Steptoe under control conditions, and decreased by another 0.23 MPa with the increase in air temperature, while it did not significantly change in Steptoe plants (Table 1).

Xylem sap flowed from detached WT roots about 2 times faster than in Az34 (Fig. 2a). Air heating increased the flow rate from WT roots by about 1.5 times but did not influence that of Az34. Adding ABA to the nutrient solution of Az34 plants increased xylem sap flow rate 2.6-fold. The increase in Steptoe was of less magnitude (only 1.6-fold), but also statistically significant. Since the driving force for osmotically driven flow of xylem sap was the same in both genotypes and did not change significantly with air heating (Table 1), a similar pattern of Lp_{Root} was detected in the plants: lower level in Az34 plants, increase in Steptoe with the air heating and no response to air heating in Az34 plants (Fig. 2b). Adding ABA to the nutrient solution increased Lp_{Root} of both Az34 and Steptoe plants. Thus ABA treatment increased Lp_{Root} of Steptoe plants kept at control temperature to the level of heated Steptoe plants, while this exogenous hormone increased Lp_{Root} of Az34 plants to the level of Steptoe control plants. Inhibiting AQP activity by producing reactive hydroxyl radicals during the Fenton reaction decreased hydraulic conductivity of both genotypes, however the extent of decline was greater in the plants under air warming suggesting that AQPs contribute to the increased hydraulic conductivity under this treatment (Table 1).

In Steptoe plants changes in transpiration induced by air warming strongly correlated with the increase in hydraulic conductivity (r=0.87), while in the case of Az34 the correlation was moderate (r=0.56).

Bulk root ABA concentration of Steptoe plants was ~50% higher than in Az34 plants and further increased with air heating (Fig. 3). No significant changes in ABA content were detected in the roots of Az34 plants following air warming.

Air warming increased immunostaining for HvPIP2;2 aquaporins in the roots of Steptoe (Fig. 4, Table 3), but no such effect was detected in Az34. Increased air temperature did not affect the abundance of HvPIP2;1 or HvPIP2;5 aquaporins in either Az34 or Steptoe roots.

Discussion

Previous experiments have addressed long-term effects (days to weeks) of ABA deficiency on leaf elongation and stomatal conductance of barley plants exposed to dry or compacted soil (Mulholland et al. 1996; Martin-Vertedor and Dodd, 2011). In accordance with these earlier reports, leaf water potential (Ψ_L) was lower in Az34 than WT plants (Table 1), likely due to the

higher transpiration rate of Az34 plants (Fig. 1). The latter effect is apparently explained by ABA's ability to close stomata and its reduced level in ABA deficient Az34 plants (Mulholland et al. 1996; Martin-Vertedor and Dodd, 2011). Although air warming increased transpiration of Steptoe plants almost to the level of Az34 plants (measured before air warming – Fig. 1), Ψ_L of Steptoe was not decreased by this treatment (Table 1). This suggests that the lower Ψ_L of Az34 was not entirely due to altered stomatal behaviour.

Previously, air warming increased transpiration of wheat plants several-fold (Kudoyarova et al. 2011), which was due to increased stomatal conductance. Transpiration increased to a lesser extent (20-30% - Fig. 1) in both barley genotypes (due to the absence of changes in stomatal conductance) and caused a drop in leaf water potential in Az34 plants but no effect in Steptoe (Table 1). This suggests that elevated transpiration of Steptoe plants was balanced by higher water flow from the roots, which was supported experimentally by measuring xylem sap flow from the roots (Fig. 2). While air warming increased xylem flow in Steptoe plants, there was no change in Az34 plants, suggesting impaired functionality of the ABA-deficient barley roots.

Experiments with both exogenous ABA application to roots (Hose et al. 2000), and transgenic ABA-overproducing plants (Thompson et al. 2007) have shown that increased ABA concentrations result in increased root hydraulic conductance. In agreement, hydraulic conductance of both genotypes was increased by exogenous ABA in the present experiments (Fig. 2). Consequently the increased hydraulic conductivity and abundance of PIP2;2 detected in Steptoe roots under air warming and the lack of response in Az34 is likely related to the increased root ABA concentration of the former and to the unchanged ABA levels of the latter (Fig. 3). ABA involvement in modulating aquaporin abundance in barley plants is supported by experiments demonstrating increased PIPs abundance in ABA treated roots of Az34 and Steptoe plants (Sharipova et al., 2016).

Perturbed water relations are characteristic of ABA deficient plants, and most frequently explained by their failure to control stomatal conductance (Neil and Horgan 1985; Makela et al. 2003). ABA is important in this respect under conditions that require stomatal closure to maintain leaf water status. On the contrary, adaptation to increased air temperature demands maintaining high transpiration rates to allow plant cooling (Reynolds et al., 1998). Under high evaporative demand, increased root hydraulic conductance may serve as the main mechanism increasing water flow from the roots thereby maintaining increased transpiration (Tardieu et al. 2010). Previous experiments with inhibition of phloem transport have shown that under air warming, root ABA accumulation was mainly the outcome of increased export from the shoots

(Kudoyarova et al., 2011). Thus ABA-controlled changes in (root) hydraulic conductivity is also of great importance for maintaining water balance of the plants.

Molecular genetic approaches allow manipulation of ABA level (e.g. transgenic plants overproducing ABA - Thompson et al. 2007) but negative effects of ABA on plant productivity may be expected since crop yield is often positively related to transpiration (Collins et al. 2008; Blum, 2015). However experiments with tomato plants overproducing ABA showed that increased ABA levels may improve water supply to the shoot, thereby maintaining water status when evaporative demand is high (Thompson et al. 2007). Thus ABA may act as a growth-promoter via its effect on aquaporin activities, which is expected to have a greater influence under high evaporative demand (Tardieu et al. 2010). Our results confirm these suggestions by showing that sufficient ABA is necessary to adequately control root hydraulic conductivity (Lp_{Root}) in barley following a step-change in VPD under air warming.

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335

336 **Table 1. Leaf water potential (Ψ_l) and ~~gradient of osmotic potential ($\Delta\Psi$)~~ osmotic pressure**
337 **of xylem sap (Ψ_x) collected prior to and 40 min after the start of air warming**
338 Statistically different values (n=10) are labeled with different letters (LSD-test $p \leq 0.05$)
339

Genotype	Treatment time (min)	Ψ_l (MPa)	Ψ_x
Steptoe	0	-0.57 ± 0.08 [a]	0.32 ± 0.01 [a]
	40	-0.43 ± 0.05 [a]	0.25 ± 0.03 [a]
Az34	0	-0.89 ± 0.06 [b]	0.35 ± 0.06 [a]
	40	-1.12 ± 0.09 [c]	0.34 ± 0.02 [a]

340

341

Table 2. Effect of inhibiting AQP activity by producing reactive hydroxyl radicals during the Fenton reaction on root hydraulic conductance (mg h^{-1} g^{-1} root fresh weight MPa^{-1}) of roots excised from the barley plants prior to and 40 min after the start of air warming. Significantly different means for each variable are labelled with different letters (n=5, LSD test).

Genotype, treatment	Control	Increased air temperature
Steptoe, - Fenton	320 ± 41^{c}	590 ± 61^{d}
Az34, - Fenton	130 ± 19^{ab}	170 ± 21^{b}
Steptoe, +Fenton	165 ± 22^{b}	280 ± 31^{c}
Az34, +Fenton	82 ± 9^{a}	110 ± 16^{ab}

Table 3. Intensity of staining for HvPIP2 aquaporins of control and treated of ABA deficient (Az34) mutant and parental cv. (Steptoe)

Means ± SE, arbitrary units, maximal staining of circles within section images was taken for 100 %, while minimal staining was 0 %. Significantly different means for each variable are labelled with different letters (n=9, LSD test)

Staining for	Steptoe		Az34	
	Control	Increased air temperature	Control	Increased air temperature
HvPIP2;1	25+7 [a]	29+4 [a]	29 ± 8 [a]	31 ± 14 [a]
HvPIP2;2	20 ± 6 [a]	71+9 [b]	31 ± 7 [a]	25± 12 [a]
HvPIP2;5	69+9 [a]	57+7 [a]	59 ± 11 [a]	49 ± 15 [a]

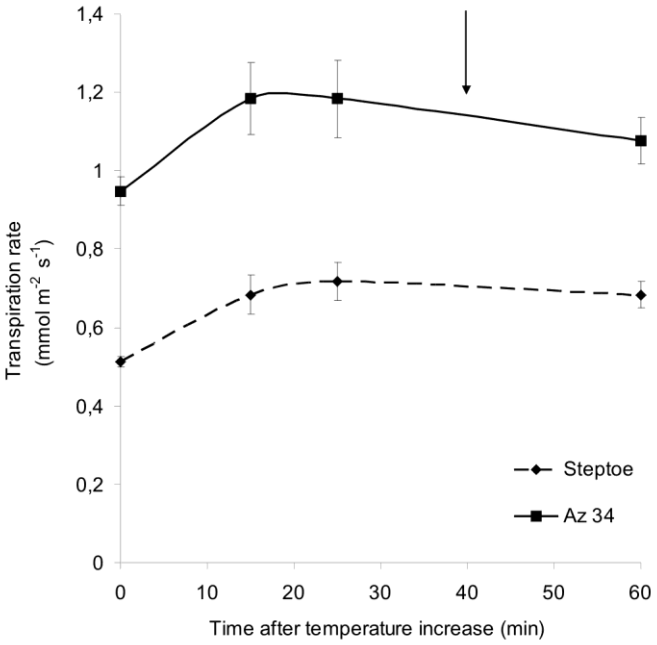
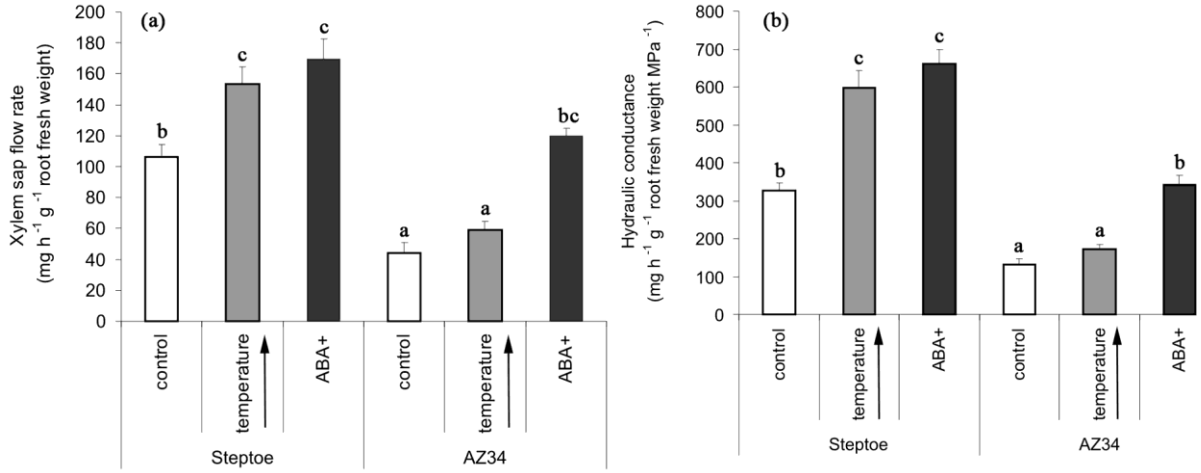


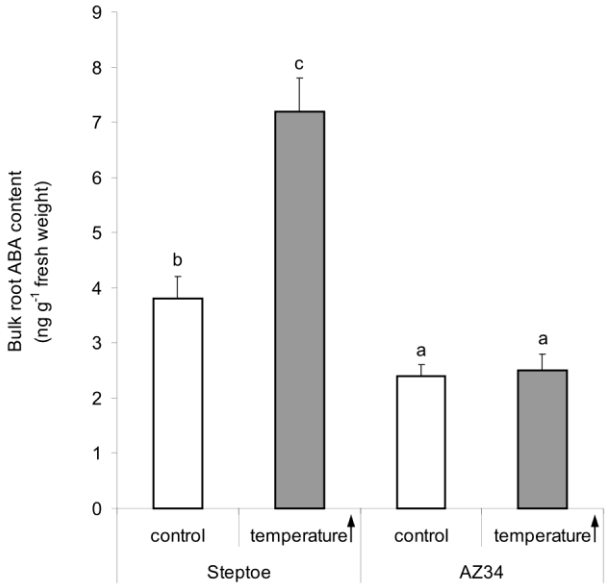
Fig. 1. Effect of air warming on transpiration (normalized to leaf area) of Steptoe and Az34 plants. Arrow indicates sampling time for ABA assay, root excision for hydraulic conductivity measurements and tissue fixation for immunolocalization. Data are means ±SE of 10 plants.



364

365 **Fig. 2.** Xylem sap flow (a), and root hydraulic conductivity (b) of Steptoe and Az34 plants
366 measured in control plants exposed to 24 ^{o}C and 40 min after the start of temperature increase
367 (temp). ABA (10^{-5} M) was added to the nutrient solution of control Steptoe and Az34 plants 20
368 min before the start and was present in the nutrient solution during the time of xylem sap
369 collection. Statistically different values ($P<0.05$) are labeled with different letters

370



371

372 **Fig. 3.** Bulk root ABA content (mean values ± SE, n=5) of Steptoe and Az34 plants measured in
373 control plants exposed to 24 ^{o}C and 40 min after the start of temperature increase (temp).
374 Statistically different values ($P<0.05$) are labeled with different letters

375

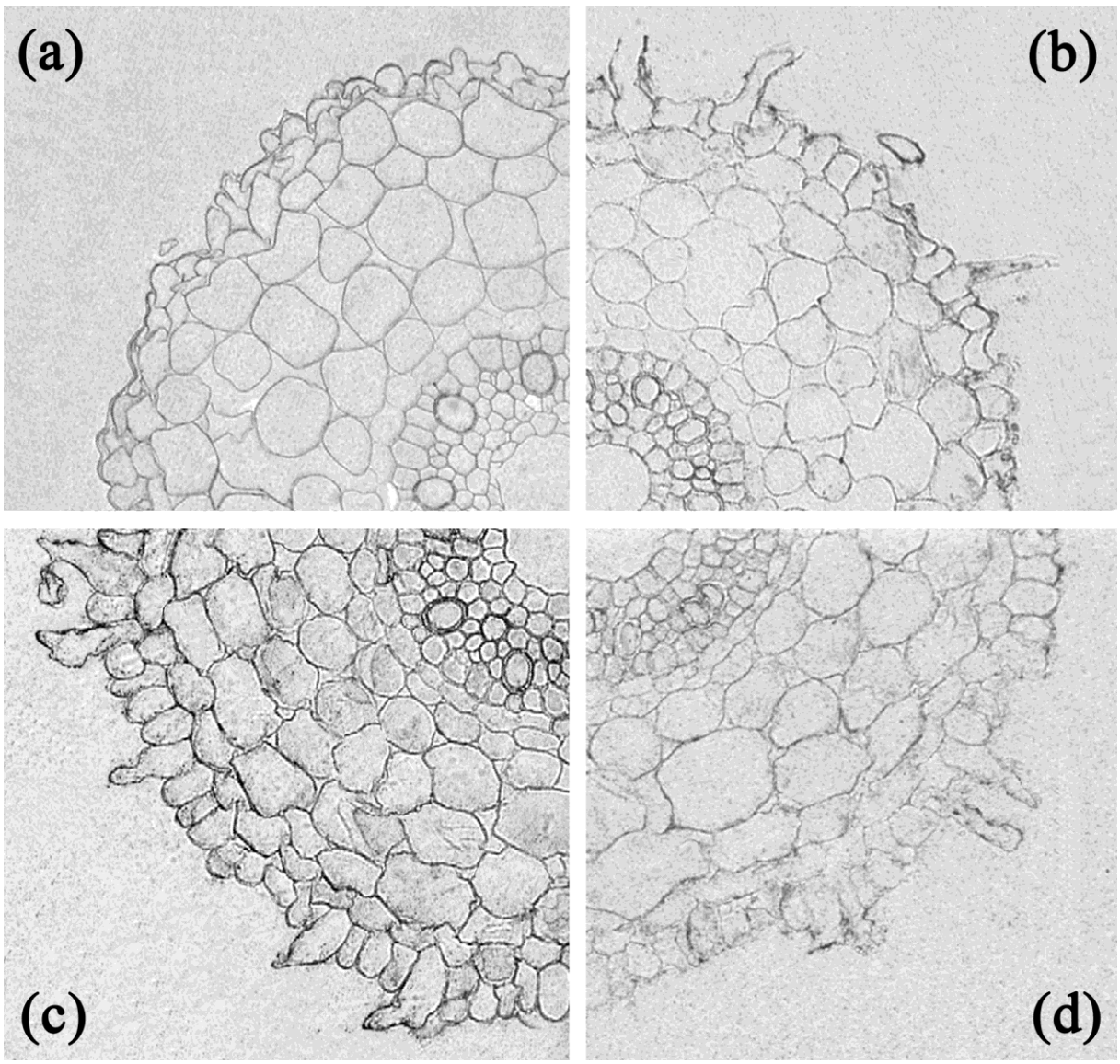


Fig. 4. Immunohistochemical localization of HvPIP2;2 AQPs in root sections (3-5 mm from the tip where root hairs appeared) of Steptoe (a,c) and Az34 (b,d) plants. a,b – control plants; c,d – plants exposed to air warming for 40 min.

Table 1. Leaf water potential (Ψ_l) and ~~gradient of osmotic potential ($\Delta\Psi$)~~ osmotic pressure of xylem sap (Ψ_x) collected prior to and 40 min after the start of air warming
Statistically different values (n=10) are labeled with different letters (LSD-test $p \leq 0.05$)

Genotype	Treatment time (min)	Ψ_l (MPa)	Ψ_x
Steptoe	0	-0.57 ± 0.08 [a]	0.32 ± 0.01 [a]
	40	-0.43 ± 0.05 [a]	0.25 ± 0.03 [a]
Az34	0	-0.89 ± 0.06 [b]	0.35 ± 0.06 [a]
	40	-1.12 ± 0.09 [c]	0.34 ± 0.02 [a]

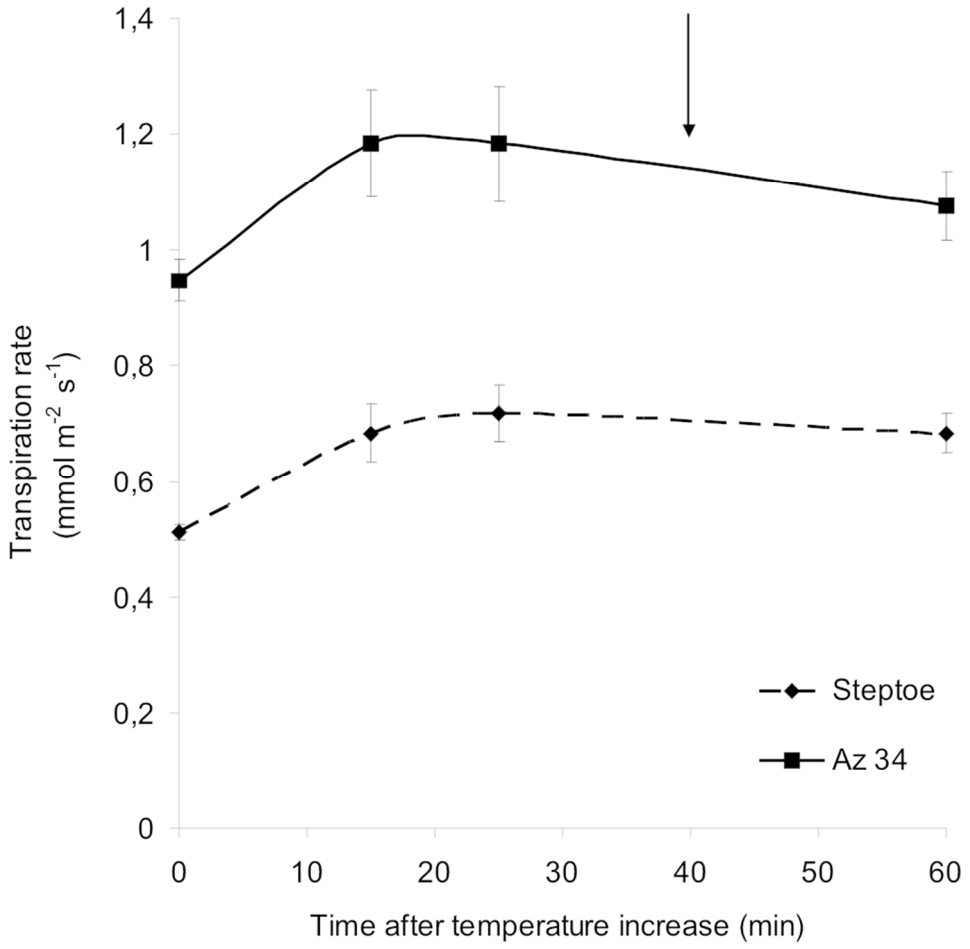
Table 2. Effect of inhibiting AQP activity by producing reactive hydroxyl radicals during the Fenton reaction on root hydraulic conductance (mg h^{-1} g^{-1} root fresh weight MPa^{-1}) of roots excised from the barley plants prior to and 40 min after the start of air warming. Significantly different means for each variable are labelled with different letters (n=5, LSD test).

Genotype, treatment	Control	Increased air temperature
Steptoe, - Fenton	320 ± 41^{c}	590 ± 61^{d}
Az34, - Fenton	130 ± 19^{ab}	170 ± 21^{b}
Steptoe, +Fenton	165 ± 22^{b}	280 ± 31^{c}
Az34, +Fenton	82 ± 9^{a}	110 ± 16^{ab}

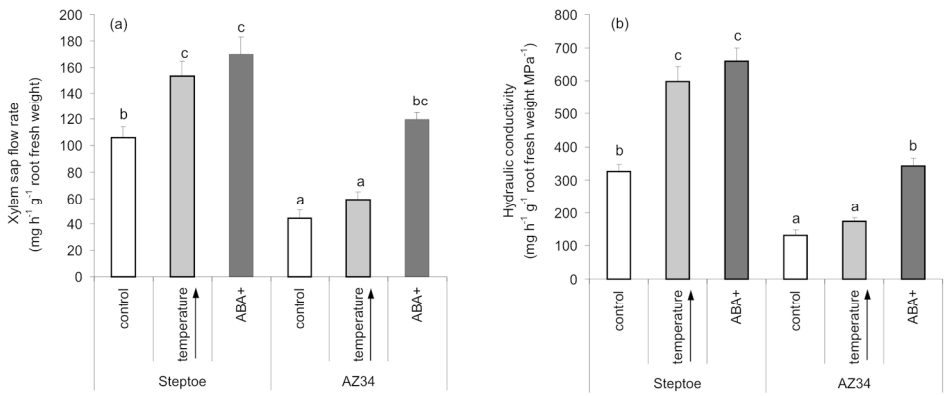
Table 3. Intensity of staining for HvPIP2 aquaporins of control and treated of ABA deficient (Az34) mutant and parental cv. (Steptoe)

Means ± SE, arbitrary units, maximal staining of circles within section images was taken for 100 %, while minimal staining was 0 %. Significantly different means for each variable are labelled with different letters (n=9, LSD test)

Staining for	Steptoe		Az34	
	Control	Increased air temperature	Control	Increased air temperature
HvPIP2;1	25+7 [a]	29+4 [a]	29 ± 8 [a]	31 ± 14 [a]
HvPIP2;2	20 ± 6 [a]	71+9 [b]	31 ± 7 [a]	25± 12 [a]
HvPIP2;5	69+9 [a]	57+7 [a]	59 ± 11 [a]	49 ± 15 [a]



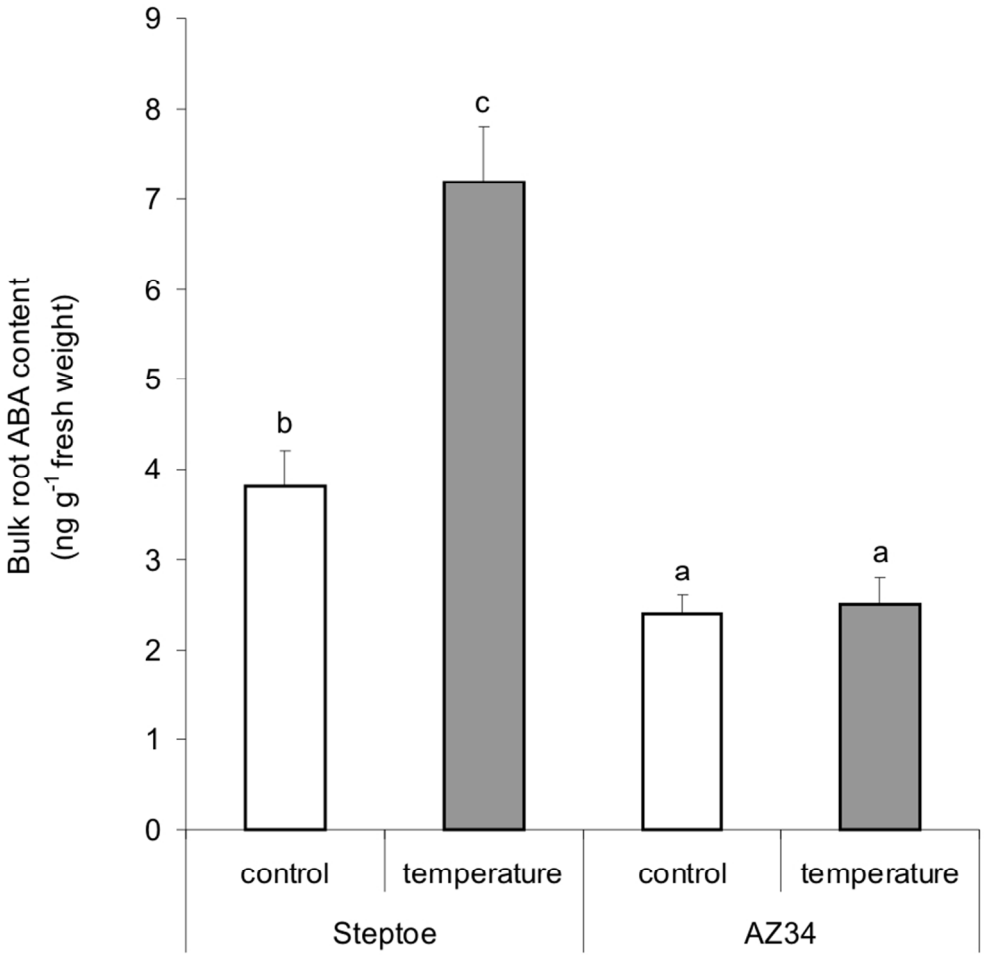
Effect of air warming on transpiration (normalized to leaf area) of Steptoe and Az34 plants. Arrow indicates sampling time for ABA assay, root excision for hydraulic conductivity measurements and tissue fixation for immunolocalization. Data are means ±SE of 10 plants.
Fig. 1.
89x87mm (300 x 300 DPI)



Xylem sap flow (a), and root hydraulic conductivity (b) of Steptoe and Az34 plants measured in control plants exposed to 24 oC and 40 min after the start of temperature increase (temp). ABA (10-5 M) was added to the nutrient solution of control Steptoe and Az34 plants 20 min before the start and was present in the nutrient solution during the time of xylem sap collection. Statistically different values ($P<0.05$) are labeled with different letters

Fig. 2.

162x64mm (300 x 300 DPI)



Bulk root ABA content (mean values ± SE, n=5) of Steptoe and Az34 plants measured in control plants exposed to 24 oC and 40 min after the start of temperature increase (temp). Statistically different values ($P<0.05$) are labeled with different letters

Fig 3

79x78mm (300 x 300 DPI)