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Dynamics of the contents and distribution of ABA, auxins and aquaporins in developing caryopses of an ABA-deficient barley mutant and its parental cultivar

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Research Paper

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Abstract

Dynamics of abscisic acid (ABA) and indole-3-acetic acid (IAA) contents were followed in developing barley caryopses of the ABA-deficient mutant AZ34 and its parental cultivar Steptoe. Distribution of these hormones and HvPIP2 aquaporins (AQPs) was studied with the help of immunohistochemical methods in the roots and coleorhiza of developing embryos. In Steptoe, maturation of the caryopsis was accompanied by vast accumulation of ABA, while this hormone accumulated more slowly in the caryopsis of AZ34 and its content was lower than in Steptoe. Accumulation of ABA was accompanied by a decline in IAA level in the developing caryopsis, the process being delayed in AZ34 in accordance with the slower accumulation of ABA. ABA accumulated to high levels in the coleorhiza cells of Steptoe, while the effect was absent in AZ34. The high level of ABA was likely to be important for maintaining the barrier function of the coleorhiza, preventing germination of seminal roots and enabling seed dormancy, while the absence of ABA accumulation in coleorhiza of AZ34 may be responsible for the initiation of root germination inside the caryopsis. The abundance of HvPIP2 AQPs in the seminal roots was higher at the beginning of maturation of Steptoe caryopsis and declined afterwards, while the levels of APQs increased later in AZ34 in accordance with the delay in ABA accumulation. These results suggest the importance of ABA accumulation in coleorhiza for preventing precocious growth of seminal roots, and suggest regulation of IAA and aquaporin levels by this hormone during maturation of embryos.

Introduction

Hormones play an important role in the regulation of seed development (Locascio *et al.*, 2014; Doll *et al.*, 2017; Tuan *et al.*, 2018), although more attention is paid to seed dormancy release and germination (Kucera *et al.*, 2005). Abscisic acid (ABA) controls seed dormancy and induces synthesis of dehydrin proteins, protecting tissues of embryos against dehydration damage during seed maturation (Son *et al.*, 2016). However, an important aspect of ABA action during seed maturation that has not received enough attention is the capacity of this hormone to regulate the expression of water channel aquaporins as has been previously demonstrated in seedlings, particularly when grown in stressful environments (Kaldenhoff *et al.*, 2008). Involvement of aquaporins (AQPs) in seed germination has also been studied and discussed (Obroucheva, 2013, and references therein); however, knowledge about their importance for seed development is limited with more attention being paid to TIP AQPs (tonoplast intrinsic proteins) than to PIP AQPs (plasma membrane intrinsic proteins). Changes in expression of AQP genes during seed development are likely to be important for the control of tissue hydration (Shiota *et al.*, 2006). Expression of genes coding for PIP AQPs was studied in the cotyledons of developing and germinating pea seeds (Schuurmans *et al.*, 2003) and cellular localization of PIP expression was detected in seed coats of bean (Zhou *et al.*, 2007). As far as we know, embryo AQPs have been studied only during somatic embryogenesis *in vitro* (Ciavatta *et al.*, 2001). In all cases, AQPs were studied in developing seeds of dicots and not monocots.

A further important aspect of ABA action in developing seeds is its interaction with other hormones. In particular, the interaction of ABA with auxins is likely to be implicated in the regulation of seed development. The necessity of auxins for seed development is suggested by their high level in seeds and involvement in embryogenesis (Locascio *et al.*, 2014). Interaction of auxins and ABA is manifested by the ability of auxins to influence ABA signalling during

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the transition to dormancy in *Arabidopsis* (Liu *et al.*, 2013). However, the reverse effect of ABA on auxins during seed development has not received sufficient attention, even though ABA is known to influence auxin metabolism in seedlings (Seo *et al.*, 2009). The study of hormonal dynamics in developing seeds has mostly been limited to whole seed or endosperm assays (Lur and Setter, 1993; Yang *et al.*, 2003; Liu *et al.*, 2010). Their content in developing embryos has been infrequently detected (Hess *et al.*, 2002) and is mostly performed with biosensors enabling detection of individual hormones in transgenic plants (mostly dicotyledonous) transformed with the help of hormone-sensitive reporter constructions (e.g. Friml *et al.*, 2003). Meanwhile it is important to study the distribution patterns of multiple interacting hormones within the embryo tissues in order to reveal the role of individual and interacting hormones.

This goal can be achieved by taking immunohistochemical approaches using specific antibodies to hormones (Forestan *et al.*, 2010). Previously, we have detected auxins and cytokinins with the help of immunolocalization during the induction of morphogenesis in wheat callus (Seldimirova *et al.*, 2016). Immunohistochemical localization methods allow comparisons of the distribution of hormones and presumably hormone-regulated proteins between cells. Thus, with the help of antibodies we have previously shown overlap in the accumulation of HvPIP2 aquaporins and ABA in the root epidermal cells of barley plants treated with ABA (Sharipova *et al.*, 2016).

The use of ABA-deficient mutant plants is a convenient approach for studying the involvement of this hormone in any processes. Comparisons of the ABA-deficient barley mutant AZ34 with its parental cultivar allowed us to confirm the importance of the accumulation of ABA for increased aquaporin levels in the roots and increased water flow to the shoots necessary for compensation of high transpiration following air warming (Veselov *et al.*, 2018). Thus, using AZ34 is appropriate for studying the role of ABA in the regulation of AQP levels in the cells of developing barley embryos.

The goal of our research was to study the dynamics of ABA and auxins in developing caryopses and the distribution of these hormones in embryos of the ABA-deficient barley mutant AZ34 and its parental cultivar during their maturation, and to compare the abundance of hormones with the levels of HvPIP2 aquaporins in the cells with the help of immunohistochemical techniques. The results of this research are expected to improve knowledge about the involvement of hormones and AQPs in the control of seed development in general, and to detect the potential linkage between hormone levels and AQPs.

Materials and methods

Plant growth

Barley plants (*Hordeum vulgare* L., ABA-deficient mutant AZ34 and its wild-type cv. Steptoe) were grown in an experimental field of the Institute (54°59′ N, 55°61′ E, 91 m above sea level). The soil was a leached chernozem of South wooded steppes of Bashkortostan. To ensure the development of whole grains containing germ (embryos), plants were artificially pollinated.

Histological analysis

Samples for histological analysis were taken daily during grain development after artificial pollination (days after pollination: DAP). Caryopses at the end of grain filling (20th DAP) and ripening (whole grain stage, 30th DAP) were fixed under vacuum for 4 h with 4% 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide prepared in 0.1 M phosphate buffer (pH 7.3). Subsequent post-fixation was performed in a mixture of 4% paraformaldehyde and 0.1% glutaraldehyde overnight. Fixation agents were then washed out with phosphate buffer; the caryopses were dehydrated in an ethanol series and embedded in hydrophilic methylacrylate resin JB-4. Semi-thin sections (3 μm thick) were cut with a rotation microtome (HM 325, MICROM) and stained using 0.2% (w/v) Toluidine Blue solution (Belami Fine Chemicals) in 0.1 M phosphate buffer (Lynn, 1965) for 30 s. The sections were observed and photographed using a light microscope (Carl Zeiss Axio Imager A1). Image documentation was conducted using a digital camera (Carl Zeiss AxioCam MRc5 with Axio Vision 4.7 software). Histological analysis of embryo development was related to the stages of grain development established visually with the help of a stereo microscope (Carl Zeiss Technival 2).

Immunoenzyme assay of IAA and ABA

The content of endogenous hormones was determined by immuno-enzymatic solid-phase assay. Plant material was homogenized and hormones were extracted in 80% ethanol at 4°C overnight. The homogenate was filtered and the liquid phase evaporated to aqueous residue. Extractions of IAA and ABA were performed with a modified solvent partitioning scheme based on the distribution constants of hormones in organic solvents, which permits a drastic reduction in the amount of extractant used, thereby providing increased specificity and rapidity of the immunoassay (Veselov *et al.*, 1992; Kudoyarova *et al.*, 2011; Vysotskaya *et al.*, 2018). In short, after adjusting the pH to 2.5 with HCl the extract was partitioned two times with diethyl ether, with a ratio of organic to aqueous phases of 5:1. Then IAA and ABA were transferred from the organic phase into 1% sodium hydrocarbonate (organic phase/aqueous ratio 3:1). After readjusting the pH of the aqueous phase to 2, re-extraction with ether gave the secondary ether extract, which was methylated with diazomethane and evaporated to dryness. The content of IAA and ABA in dry residues dissolved in 80% ethanol was immunoassayed as previously described (Veselov *et al.*, 1992) using specific antibodies to IAA and ABA.

Immunohistochemical localization of hormones and aquaporins

To prevent washing out of hormones during the process of dehydration, IAA and ABA present in the caryopses were fixed under vacuum for 4 h with the help of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide as described above. Subsequent post-fixation, dehydration, embedding and section preparation was performed as described above. We used specific antibodies, previously used for immunolocalization of IAA (Seldimirova *et al.*, 2016), ABA (Sharipova *et al.*, 2016) and aquaporins (Veselov *et al.*, 2018). Polyclonal antibodies for HvPIP2s were raised in rabbits against synthetic oligopeptides (Medical & Biological Laboratories Co., Tokyo, Japan) corresponding to the amino acid sequences in the N-region of HvPIP2;2 (Horie *et al.*, 2011), HvPIP2;3 (TKLGSSASFGRS) and HvPIP2;5 (Sharipova *et al.*, 2016). Antibodies raised against amino acid sequences in the N-region of HvPIP2;3 also recognize the homologous region of HvPIP2;4 due to similarity between them [only three amino acids being

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different between sequences of HvPIP2;3 and HvPIP2;4 (TKLGSSASFGRS and TKFGSSASFGSR, correspondingly)]. Prepared sections were treated for 30 min with 0.1 M Na-phosphate buffer (pH 7.3) containing 0.2% gelatin and 0.05% Tween 20 (PGT), washed with distilled water and incubated for 2 h in a moist chamber at room temperature with immune rabbit anti-ABA, anti-IAA or anti-HvPIP2 sera (20 µl) diluted with PGT. To visualize antibodies bound to ABA, IAA or AQPs, sections were treated for 1 h in a moist chamber with goat antibodies raised against rabbit immunoglobulin labelled with colloidal gold (in PGT; Aurion, Hatfield, PA, USA). After three washes with phosphate buffer (PB), samples were post-fixed in 2% glutaraldehyde in PB for 5 min. The sections were then washed with distilled water, and incubated with silver enhancer (Aurion) for 30 min. Excess silver was removed with distilled water and sections were examined under a light microscope (Carl Zeiss, Jena, Germany) equipped with an AxioCam MRc5 digital camera (Carl Zeiss). Intensity of immunostaining for IAA and ABA was estimated as described previously (Sharipova *et al.*, 2016; Veselov *et al.*, 2018) from 8-bit greyscale images using ImageJ software (version 1.48, National Institutes of Health). Staining values were averaged for areas of coleorhiza and different regions of seminal roots by using the 'freehand selections' tool of the same software and measuring mean pixel intensities within the region of interest. Images were taken from 20 independent sections per treatment. Intensity of staining was expressed in arbitrary units: maximal staining was taken as 100% and minimal staining was 0%.

In the present experiments, specificity of immunolocalization was confirmed by decreased immunostaining of the sections of ABA-deficient mutant (negative control). Specificity and reliability of immunostaining was also confirmed by earlier experiments, where increased immunostaining was detected in the plants treated with exogenous hormones (positive control; Sharipova *et al.*, 2016).

Statistical analysis

Data were expressed as means ± SE, which were calculated in all treatments using MS Excel. Significant differences between means were analysed by *t*-test.

Results

Stages of caryopsis and grain development

The following stages of barley embryo development were identified: zygote (1 DAP), blastomerization (2–5 DAP), organogenesis (6–18 DAP), formed embryo (19–20 DAP), embryo maturation (20–27 DAP), mature embryo (28–30 DAP). Grain development passed the setting stage (0–9 DAP), milk (10–13 DAP), dough (14–25 DAP) and full ripened grain (26–35 DAP).

Dynamics of ABA and IAA content

The dynamics of ABA and IAA content was determined in caryopses assayed at different time points covering the developmental stages indicated above. The initial contents of IAA were similar in caryopses of both genotypes (about 140–150 ng per g fresh weight) at zygote and blastomerization (Fig. 1a). In the grains of Steptoe, this level of IAA remained until the 14 DAP (organogenesis), then increased sharply reaching a maximum at 21 DAP (start of maturation) and subsequently decreased to the initial level by 35 DAP (full ripened grain). In caryopses of AZ34,

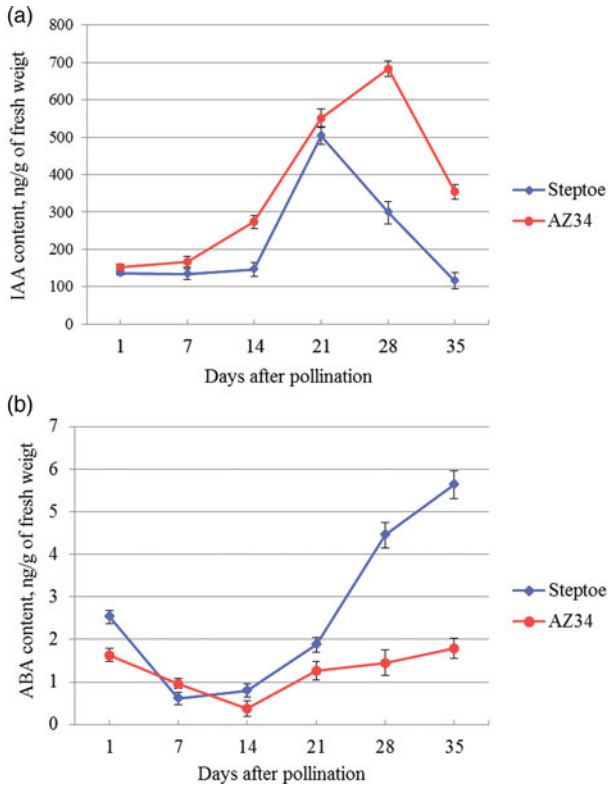

Fig. 1. IAA (a) and ABA (b) content in developing barley caryopsis of the ABA-deficient mutant AZ34 and cv. Steptoe.

IAA content began to increase earlier (starting from 7 DAP) reaching maximal values (about 700 ng per g fresh weight) at 28 DAP (mature embryo), when this indicator was higher than in Steptoe. At 35 DAP, IAA content in caryopses was two times higher (about 350 ng per g fresh weight) than its initial level.

The ABA content was higher in caryopses of Steptoe than in AZ34 at the zygote stage (Fig. 1b; about 2.5 and 1.6 ng per g fresh weight, correspondingly). In the course of subsequent development, ABA content decreased and reached a minimum at 7 DAP in caryopses of Steptoe (about 0.6 ng per g fresh weight) and at 14 DAP in AZ34 caryopses (about 0.4 ng per g fresh weight). A gradual increase in the level of ABA was detected in Steptoe from 7 to 21 DAP followed by a sharp 2.5-fold increase in ABA content at 35 DAP compared with its initial level. In caryopses of AZ34, the increase in ABA content began later (starting from 14 DAP) and only by 35 DAP ABA reached its initial level (about 1.7 ng per g fresh weight).

Morphology of barley caryopsis at the stages of formed and mature embryos

Formed embryos (20 DAP) of both genotypes had similar histological characteristics (Fig. 2). Comparison of the histology of mature embryos (30 DAP) of both genotypes (Fig. 3) showed an interesting pattern where not one, but several seminal roots were located inside the coleorhiza of both genotypes. Nevertheless, there was some difference in morphology of coleorhiza between the genotypes. In the embryo of Steptoe, the coleorhiza had the typical cereal structure: large root protecting sheath consisting of numerous cell layers (Fig. 3a). The coleorhiza of AZ34 is

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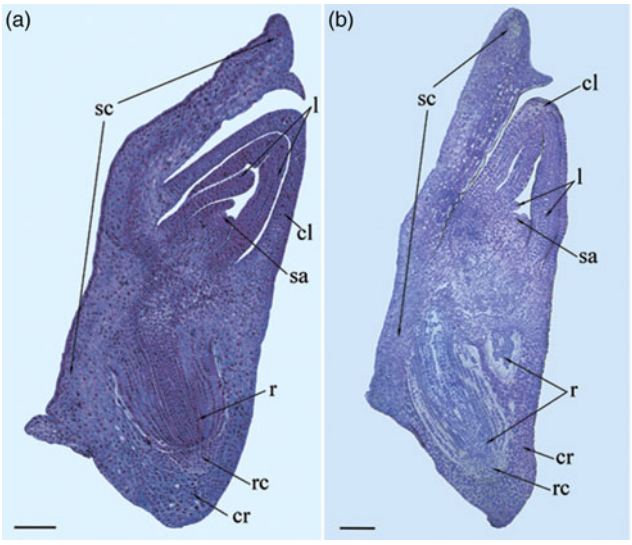

Fig. 2. Median longitudinal sections of barley embryos of ABA-deficient mutant AZ34 (a) and cv. Steptoe (b) at 20 DAP. cl, coleoptile; cr, coleorhiza; l, leaves; r, root; rc, root cap; sa, shoot apex; sc, scutellum. Scale bars: 200 μm.

thinned, especially at the site of future root breakthrough and consists of few cell layers (Fig. 3b) or merely a pellicle consisting of cell membrane residues (Fig. 3c). In some cases, a complete seminal root breakthrough of the coleorhiza was detected (Fig. 3d). Thus, despite the outward morphological similarity of Steptoe and AZ34 grains, the structure of the lower part of the mature embryo at the site of coleorhiza was significantly different in these genotypes. Also, AZ34 typically had a greater embryo size than in Steptoe (Figs 2 and 3). Fully mature grains of ABA-deficient mutant were capable of germination immediately after their harvest, while Steptoe grains remained dormant for several months unless stratified.

Immunohistochemical staining for ABA, IAA and HvPIP2 AQPs

Figure 1 shows the amounts of IAA and ABA in the entire caryopsis at the early stage of caryopsis development when separation of different parts of the caryopsis was not possible. To reveal interaction of IAA and ABA and their relation to abundance of AQPs during maturation of embryos immune-histochemical methodology was used. We sampled caryopses at 20 DAP (stage of formed embryo with all organs typical for cereals, where cell division has stopped and there is a slight size increase by cell expansion; caryopsis at dough stage and desiccation started) and at 30 DAP (mature embryo, where growth has stopped and embryo ready for dormancy; grain is fully ripened with maximal dry matter content).

Immunostaining of the embryo for ABA detected both similarities and differences in abundance and distribution of this hormone between embryo organs (Fig. 4). As histological analysis revealed peculiar properties of seminal root development in the ABA-deficient mutant, we focused on this part of the germ. Staining for ABA of coleorhiza and seminal root cells was detected both in AZ34 and Steptoe. Nevertheless, in AZ34 the intensity of staining of coleorhiza and different root parts was similar (Fig. 4a), while the coleorhiza cells of Steptoe were more intensively stained than the cells of seminal roots (Fig. 4b).

Semi-quantitative analysis of staining with the help of the ImageJ program showed significantly higher levels of ABA in the cells of coleorhizae and roots of Steptoe than in AZ34 on the 20th DAP (Fig. 5a). Nevertheless, the difference between the two genotypes decreased with embryo maturation due to an increase in intensity of immunostaining of the root meristem and cap in AZ34 (Fig. 5b). At both stages of embryo development studied in our experiments, the difference between genotypes in immunostaining for ABA was most striking in coleorhiza, where its intensity was about two times higher in the mature embryo of Steptoe than in AZ34 (Fig. 5b).

Immunohistochemical detection of auxins revealed uniform intensity of staining of the cells of root and coleorhiza of either AZ34 or Steptoe (Fig. 6). Estimation of staining intensity showed significantly higher levels of IAA in the root meristem and cap of Steptoe than of AZ34 detected at 20 DAP (Fig. 7a). This pattern changed at 30 DAP owing to an increase in auxin staining in the seminal roots of AZ34, most noticeable in root meristem (Fig. 7b). In the formed embryos (20 DAP), cells of the coleorhiza and all parts of the roots of Steptoe were more intensively stained for HvPIP2;2 APQs (Fig. 9a). However, in mature embryos the pattern was reversed and levels of staining were higher in AZ34 than in Steptoe due to increased HvPIP2;2 abundance in AZ34 (Figs 8 and 9b). More intensive staining for HvPIP2;3 and HvPIP2;5 AQPs was also detected in the root cap and meristem of AZ34 compared with Steptoe (Supplementary Figs S1 and S2).

Discussion

The presence of several seminal roots detected by us in the barley embryos is in agreement with other data showing that, unlike other cereals such as wheat, barley embryos may have not one, but two to three seminal roots (Luxová, 1986). A characteristic feature of AZ34 mature embryos was, unlike its parental genotype whose seminal root was surrounded by several intact cell layers of coleorhiza, a thinned out coleorhiza accompanied by root movement towards the grain surface. An absence of dormancy and tendency for pre-harvest sprouting is a characteristic feature of some ABA-deficient mutants including rice (Shu *et al.*, 2016). However, this effect was less pronounced in the AZ34 embryo than in that of ABA-deficient rice, and although the seminal roots precociously germinated, they did not emerge from the spike before harvest, perhaps explained by the ABA deficiency being not as great in AZ34 as in the rice mutant.

The ABA content in the grains of AZ34 was several times lower than in that of Steptoe (Fig. 1b) in accordance with earlier reports showing low basal levels of ABA in the AZ34 barley mutant (Walker-Simmons *et al.*, 1989; Ulferts *et al.*, 2015). Agreement of our data with those obtained with the help of HPLC-MS (Ulferts *et al.*, 2015) provides confirmation of the reliability of the quantitative immunoassay used in our experiments. Nevertheless, weak accumulation of ABA still occurred in AZ34 grains starting from 21 DAP. In general, the dynamics of ABA accumulation in barley grains of both genotypes was similar in that this hormone was present at the beginning of caryopsis development, then its level dropped and subsequently increased. The presence of two peaks in ABA content is in accordance with data obtained for developing grains of maize (Hess *et al.*, 2002).

Quantitative hormone data were in some cases supported by the results of the immunohistochemical technique, although complete similarity between the two approaches used cannot be expected, as in the former case hormone content was measured

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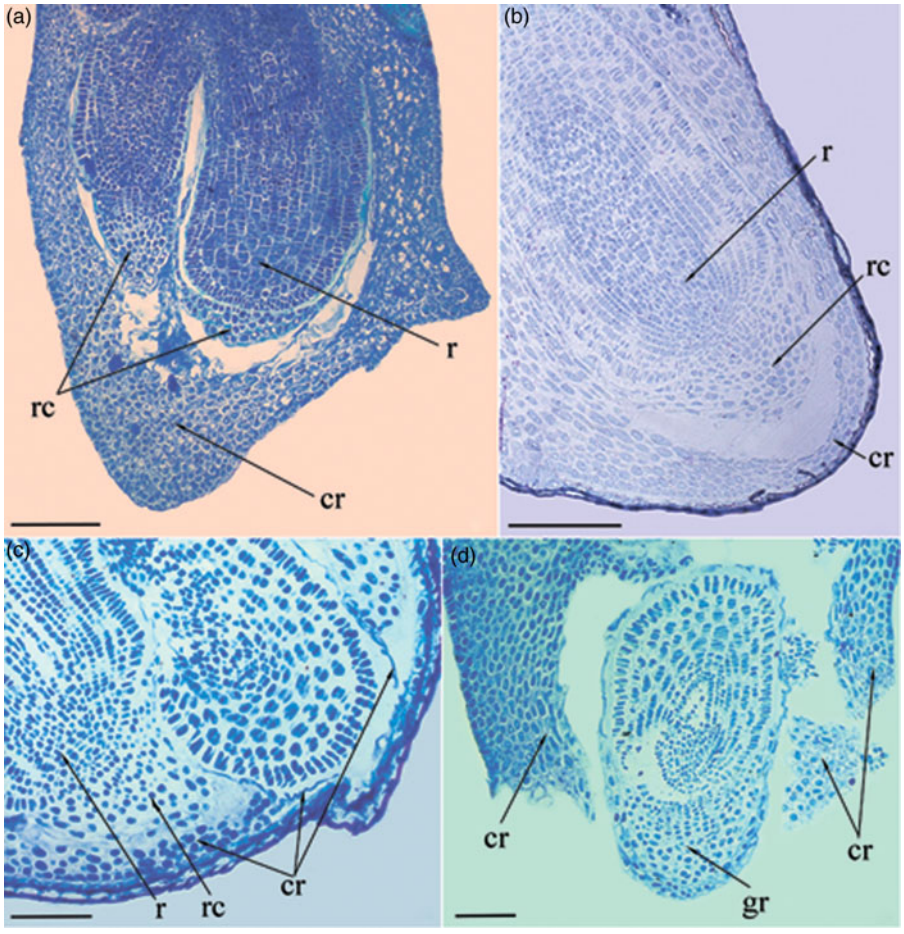

Fig. 3. Structural peculiarities of coleorhiza of Steptoe (a) and AZ34 (b–d) embryos at 30 DAP. cr, coleorhiza; gr, germinating root; r, root; rc, root cap. Scale bars: panels a and b, 200 μm; panels c and d, 100 μm.

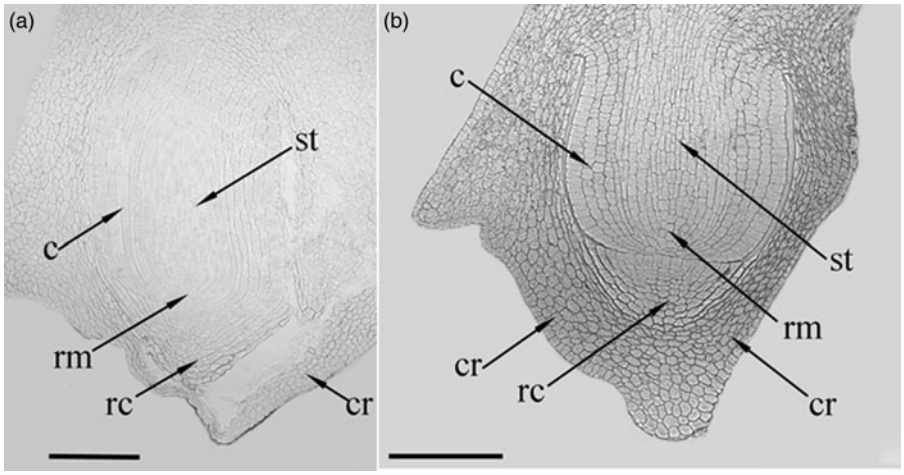

Fig. 4. Immunolocalization of ABA in coleorhiza and roots of AZ34 (a) and Steptoe (b) embryos at 30 DAP. c, cortex; cr, coleorhiza; rm, root meristem; rc, root cap; st, stele. Scale bars: 200 μm.

in the whole caryopsis, while immunolocalization allowed detection of hormones in the cells of different parts of the embryo. Higher levels of ABA were detected in the cells of roots and coleorhizae of Steptoe embryos with immunohistochemical staining (Fig. 4). This was most noticeable in coleorhiza, where, unlike AZ34, high intensity of immunostaining for ABA was detected at the stage of mature embryo. Coleorhiza is implicated in the control of seed dormancy serving as a barrier that prevents precocious germination of seminal roots. ABA is suggested to play an important role in the fulfilment of barrier function by coleorhiza. It was shown that, after induction of the germination, processes associated with ABA metabolism and a decline in its content resulted in destruction of the coleorhizae and emergence of the seminal root (Barrero *et al.*, 2009). We are the first to show how ABA accumulates in the coleorhizae during embryo maturation. The fact that low ABA levels in coleorhizae of the ABA-deficient barley mutant was accompanied by a decline in grain dormancy and a tendency for root germination confirms

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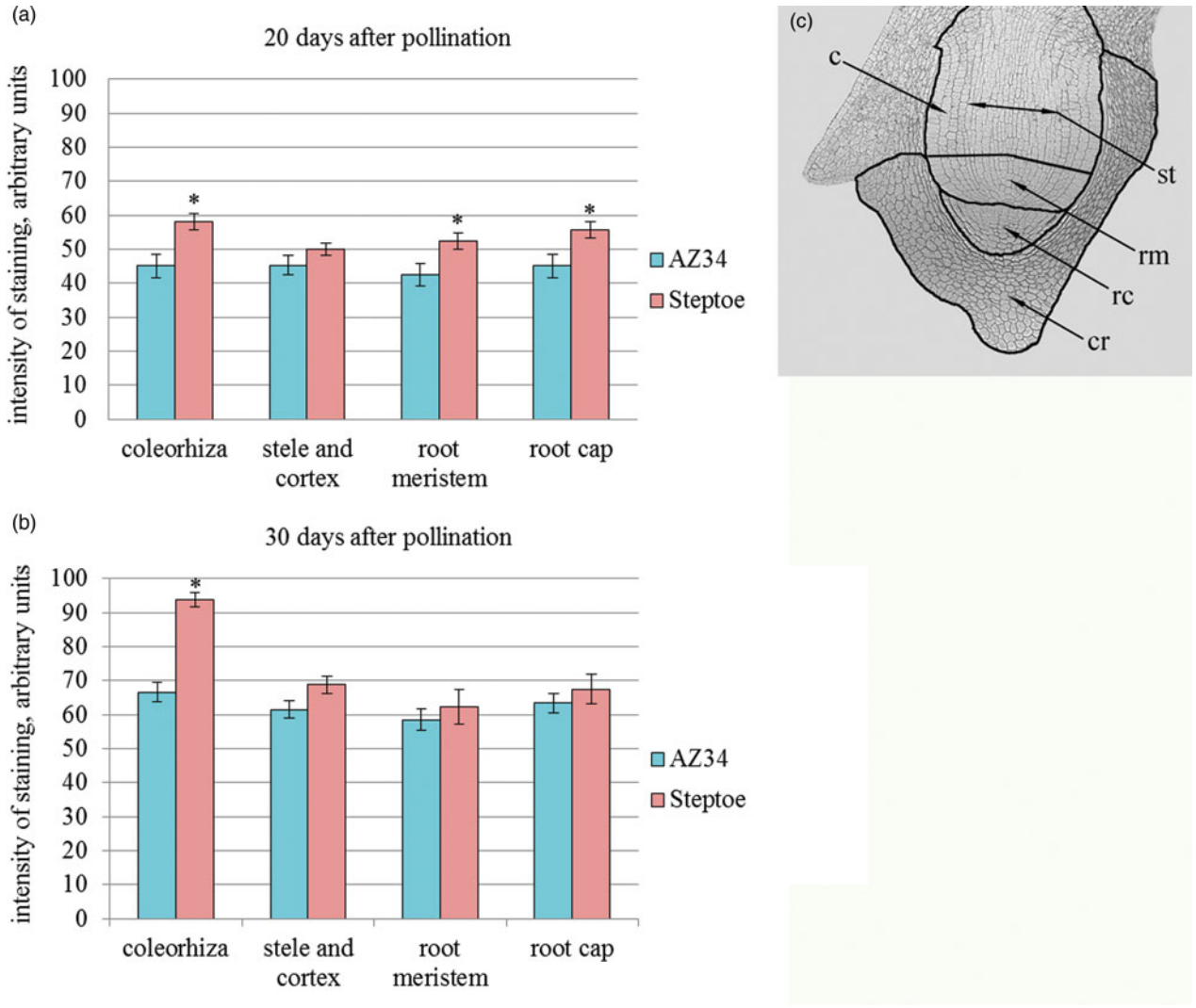

Fig. 5. Intensity of staining for ABA (means ± SE, arbitrary units, maximal staining taken as 100%, minimal as 0%) of AZ34 and Steptoe embryo organs at 20 DAP (a) and 30 DAP (b). Means significantly higher in one genotype than in the other are marked with an asterisk ($n = 20$, $P \leq 0.05$, *t*-test). Panel c shows a schematic image of zones selected for detection of staining values with ImageJ software; cr, coleorhiza; rm, root meristem; rc, root cap; st and c, stele and cortext.

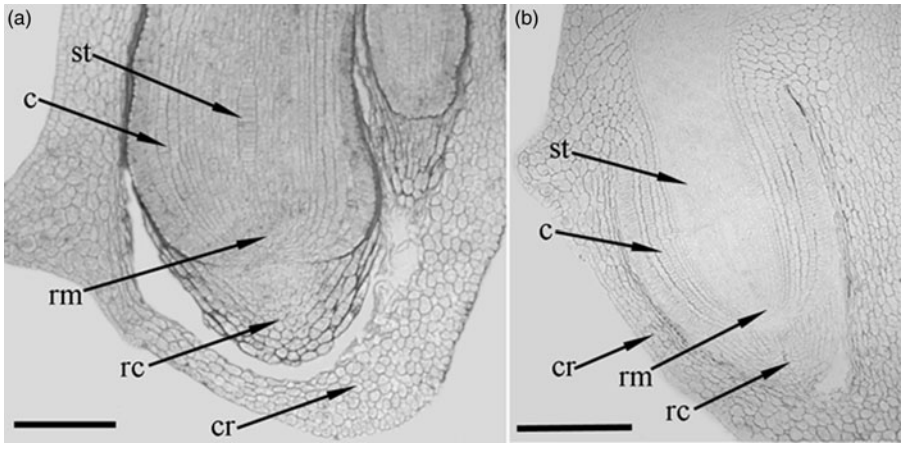

Fig. 6. Immunolocalization of IAA in coleorhiza and roots of AZ34 (a) and Steptoe (b) embryos at 30 DAP. c, cortex; cr, coleorhiza; rm, root meristem; rc, root cap; st, stele. Scale bar: 200 μm.

that ABA accumulation in coleorhiza is functionally important not only for grain germination, but also for their maturation. Another feature of AZ34 is its larger embryo compared with Steptoe, which is in accordance with reports on the embryo size of *aba2-1*, an ABA-deficient *Arabidopsis* mutant, regenerating *in vitro* (Cheng *et al.*, 2014).

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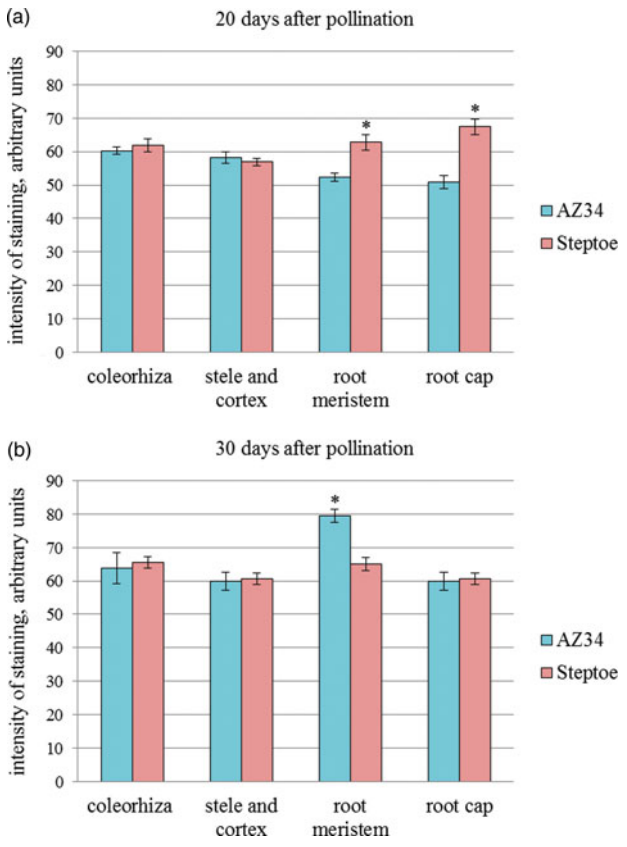

Fig. 7. Intensity of staining for IAA (means ± SE, arbitrary units, maximal staining taken as 100%, minimal as 0%) of AZ34 and Steptoe embryo organs at 20 DAP (a) and 30 DAP (b). Means significantly higher in one genotype than in the other are marked with an asterisk ($n = 20$, $P \leq 0.05$, *t*-test).

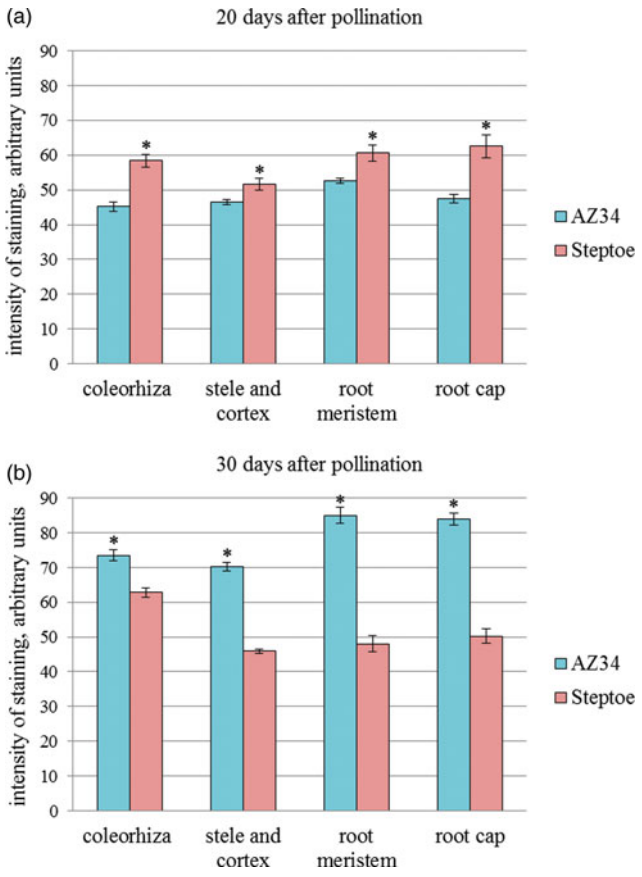

Fig. 9. Intensity of staining for HvPIP2;2 AQPs (means ± SE, arbitrary units, maximal staining taken as 100%, minimal as 0%) of AZ34 and Steptoe embryo organs at 20 DAP (a) and 30 DAP (b). Means significantly higher in one genotype than in the other are marked with an asterisk ($n = 20$, $P \leq 0.05$, *t*-test).

One of the goals of the present research was to reveal the effects of decreased ABA levels on auxin content in the mutant grains. Comparison of the dynamics of these hormones in the developing grains of the two genotypes showed that a lower level of ABA accumulation was accompanied by slower decline in IAA in AZ34 compared with Steptoe (Fig. 1). ABA is known to decrease the level of auxins in plants by activation of IAA-oxidase (Li *et al.*, 2014) and up-regulation of *GH3* gene expression, enabling a decline in the content of free IAA by its conjugation (Seo *et al.*, 2009). High expression of some

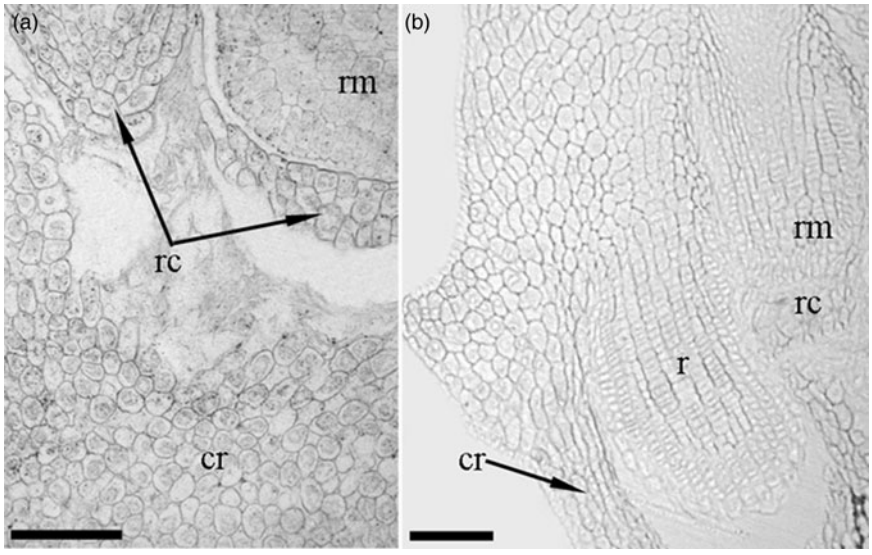

Fig. 8. Immunolocalization of PIP2 AQPs in coleorhiza and roots of AZ34 (a) and Steptoe (b) embryos at 30 DAP. cr, coleorhiza; r, root; rc, root cap; rm, root meristem. Scale bar: 50 μm.

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GH3 genes was also detected during the development of rice grains (Fu *et al.*, 2011), suggesting that the apparent link between the declining auxin levels and accumulation of ABA detected by us may be caused by ABA activating processes that enable the decline in embryo auxins. The relationship between ABA and IAA content in the cells of embryos was less clear than in the total caryopsis. Still, the immunohistochemical assay revealed that the most striking difference between the two genotypes was in the higher auxin content in the root meristem of the ABA-deficient mutant (Fig. 7b). This may be related to the accumulation of ABA in the coleorhiza of Steptoe and absence of such an accumulation in AZ34 during the process of maturation. Although the precise sites of accumulation of ABA in Steptoe and IAA in AZ34 did not coincide, diffusion of ABA from the coleorhizae influencing IAA content in the roots could be a possible explanation. Auxins have been shown to accumulate in the tips of emerging lateral roots (Benková *et al.*, 2003). These data suggest that accumulation of IAA in the root meristem cells of AZ34 embryos could contribute to activation of elongation of the seminal roots, near breaching of the barrier created by coleorhiza cells.

Aquaporins in germinating seeds have received plenty of attention, though are less studied during seed development. One report showed that in developing tomato seeds, the expression of genes coding for PIPs initially increased and then decreased, contributing to water flow out of the seeds during the start of desiccation and keeping them dry at the mature state (Shiota *et al.*, 2006). This pattern is similar to that revealed by us in the grains of barley during their maturation, when the levels of HvPIP2;2 (Fig. 9), HvPIP2;3 and HvPIP2;5 (supplemental figures) were initially higher in Steptoe root than in AZ34, and then declined. In AZ34, an opposite response was observed and the level of AQPs in the roots increased with maturation of caryopsis. The difference in the dynamics of AQPs between the two genotypes may be related to normal development of Steptoe caryopsis and the disturbance in grain maturation detected in the ABA-deficient mutant. ABA is known to increase expression of AQP genes (Kaldenhoff *et al.*, 2008) and promoters of seed-specific vacuolar aquaporins (TIP3;1 and TIP3;2) were activated in the presence of ABA in Arabidopsis protoplasts (Mao and Sun, 2015). Furthermore, a simultaneous increase in abundance of both HvPIP2;2 and ABA was detected in the epidermal cells of roots treated with exogenous ABA (Sharipova *et al.*, 2016). These data suggest that higher levels of ABA detected in the root cells of Steptoe embryos at 20 DAP could contribute to the increased levels of AQPs detected at this stage. The delay in ABA accumulation in AZ34, detected only at 30 DAP, could explain the later increase in AQP level in this ABA-deficient barley mutant.

There is little and contradictory data concerning the effects of auxins on AQPs. Tonoplast intrinsic AQPs of *Panax ginseng* (PgTIP1) were down-regulated when cells were treated with auxin (Lin *et al.*, 2007) and most AQP genes were repressed during lateral root formation and by exogenous auxin treatment (Péret *et al.*, 2012). In contrast, IAA induced *LeAQP2* expression in tomato (Werner *et al.*, 2001). Thus, the effects of auxins on AQP expression may be species or age specific, and further experiments are needed before the difference between the barley plants in PIP AQPs detected in our experiments may be related to the auxins content in the plants of the two genotypes.

Conclusions

Comparison of the dynamics of IAA and ABA in developing barley grains and distribution of hormones and AQPs in the seminal roots of the ABA-deficient barley mutant and its parental cultivar implied an important role for ABA in the control of grain maturation. High levels of ABA detected in coleorhizae of Steptoe embryos contributed to a more effective fulfilment of the barrier function by the coleorhizae and maintaining the seed's dormancy. Lower ABA accumulation in the seeds of AZ34 was related to a slower decline in the level of auxins. Differences between the genotypes in accumulation of auxins were also detected in the seminal roots, whereas higher levels of IAA were most clearly revealed in the root meristem of AZ34, which was likely to contribute to pre-harvest elongation of the seminal roots of this ABA-deficient mutant. A delay of the increase in AQP levels was also detected in the ABA-deficient mutant which was in accordance with a slower accumulation of ABA.

Supplementary Material. To view Supplementary Material for this article, please visit: https://doi.org/10.1017/S0960258519000229

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