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REVIEW PAPER

Aquaporins in seeds

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Abstract

The factual evidence for aquaporins, the constituents of water channels in cell membranes and operation of water channels is reviewed in developing, maturing and germinating seeds.

Keywords: aquaporin, cell elongation, plasmalemma, seed development, seed germination, seed maturation, tonoplast, vacuole, water channel, water transport

Recently developed studies of aquaporins, the proteins forming water channels in animal membranes, culminated in the Nobel Prize 'For the discovery of water channels' awarded to Peter Agre, an American scientist, in 2003. This new field of science has attracted the attention of plant physiologists dealing with the mechanisms of water transport. Previously, water transport was believed to occur by slow diffusion, and the possibility of the functioning of special water channels has been of obvious interest. A number of reviews concerning aquaporins have appeared recently (Maurel, 1997; Tyerman *et al.*, 1999; Chaumont *et al.*, 2001; Maurel *et al.*, 2008, 2009) demonstrating rapid penetration of water through water channels in the course of transcellular osmotic-driven movement. Water influx into the plant cell occurs through water channels formed by plasmalemma aquaporins (PIPs), whereas the delivery of excessive water to the vacuole depends on tonoplast aquaporins (TIPs). [According to universally accepted nomenclature, the PIP subfamily embraces plasmalemma intrinsic proteins, the TIP subfamily consists of tonoplast intrinsic proteins and the NIP subfamily includes nodulin-like intrinsic proteins. The first number indicates subtype, the second one indicates the number of the isoform. Previous names of TIPs were αTIP (TIP3;1), γTIP (TIP1) and δTIP (TIP2).] The rate of water transport along the channels exceeds the rate of water diffusion by at least tenfold. The efficiency of water transport depends on aquaporin content in the membranes and an opened or closed state of the channels, which is mainly regulated by phosphorylation/dephosphorylation, respectively (Maurel *et al.*, 2008).

Over the course of seed development, the earliest appearance of *PIP2* gene transcripts was shown in the ovules of *Solanum chacoense* at 48–60 h after pollination, i.e. just after fertilization (O'Brien *et al.*, 2002). During embryogenesis of *Pinus taeda* seeds, the expression of the *NIP1;1* gene was reported in the suspensors of both zygotic and somatic embryos (Ciavatta *et al.*, 2001). The NIP aquaporin belongs to the aqua-glyceroporins that are capable of transporting both water and glycerol.

In developing pea seeds, the expression of *PIP1;1*, *PIP2;1* and *TIP1;1* genes occurred in expanding cotyledons and in the seed coat, where expression of *NIP1;1* was also observed (Schuurmans *et al.*, 2003). PIP2;1 participates in water transport to a greater extent than NIP1;1 and TIP1;1. In the experiments of Zhou *et al.* (2007) with developing *Phaseolus vulgaris* seeds, an active expression of *PIP1;1*, *PIP2;2* and *PIP2;3* genes, as well as their water-channel activities, were shown in vascular parenchyma of seed-coat bundles, where water unloading from the phloem to the apoplast occurs. These data indicate participation of the water channel in the delivery of water, and possibly some organic compounds, into the developing seeds.

Much more attention was paid to tonoplast aquaporins in the course of vacuole transformation to protein storage vacuoles in maturing orthodox seeds. TIP3;1 is considered a marker of the tonoplast (Johnson *et al.*, 1989; Jauh *et al.*, 1999; Hunter *et al.*, 2007). It is specifically produced in protein-storing tissues of monocots, dicots and gymnosperms (Melroy and Herman, 1991; Höfte *et al.*, 1992; Inoue *et al.*, 1995; Oliviusson and Hakman, 1995; Herman and Larkins, 1999; Takahashi *et al.*, 2004; Willigen *et al.*, 2006;

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Li *et al.*, 2008). In the case of recalcitrant seeds, for example *Aesculus hippocastanum,* TIP3;1 proteins remained in the tonoplast of preserved vacuoles during maturation, shedding and germination (Obroucheva *et al.*, 2012). However, in maturing orthodox seeds, the vacuoles are transformed to protein-storage vacuoles as a result of the deposition of reserve proteins therein. TIP3;1 accumulated there when most of the storage proteins had already been delivered (Johnson *et al.*, 1989; Melroy and Herman, 1991). TIP3;1 proteins are synthesized in maturing seeds by the ribosomes on the endoplasmic reticulum and then transported in Golgi vesicles to the vacuole (Mäder and Chrispeels, 1984). This process was studied in more detail in pumpkin cotyledons (Inoue *et al.*, 1995). mRNA of the TIP3;1 precursor was detected at early cotyledon development, and this precursor, of size 29 kDa, gradually accumulated. Its conversion to the end product occurred by removal of the terminal 7 kDa peptide. The mature 22 kDa TIP3;1 protein accumulated in the tonoplast when the synthesis of precursor had ceased and its mRNA had disappeared. TIP3;1 aquaporins prevail in tonoplasts and form efficient water channels. Along with TIP3;1, other TIPs, namely TIP2 and TIP1;1, were found in pea seeds (Jauh *et al.*, 1999), and TIP2 was reported in mature horse chestnut seeds (Obroucheva *et al.*, 2012). The transcripts of *TIP3;1*, *TIP3;2* and *TIP5;1* genes have been identified in dry seeds of *Arabidopsis* (Willigen *et al.*, 2006), whereas in *Vicia faba minor* seeds the embryo axes contained *TIP3;1*, *TIP3;2*, *TIP1;1*, *TIP2;1* and *TIP2;2* transcripts (Novikova *et al.*, 2013).

Such TIP-formed water channels could provide water inflow to the enlarging vacuoles in maturing seeds and, later on, during vacuole transformation to protein-storage vacuoles, and they could participate in water outflow, resulting from water displacement by deposited reserve proteins. This possibility follows from the property of water channels allowing water to pass in both directions.

Information about PIPs during seed maturation is still lacking. Nevertheless, dry seeds are known to contain a number of aquaporins, apparently preformed in developing and maturing seeds. In dry *Arabidopsis* seeds, 11 PIP isoforms were found (Willigen *et al.*, 2006), as in dry rice seeds (Liu *et al.*, 2007). In the latter, the transcripts of some PIP genes were also present, mainly *PIP2;7* mRNAs, whereas mRNAs of PIP1;1, PIP1;2 and PIP2;1 were not abundant. At the same time, dry *Arabidopsis* seeds contained mRNAs of *TIP3;1*, *TIP3;2* and *TIP5;1* genes (Willigen *et al.*, 2006). *TIP1;1* and *TIP2;1* were absent from the embryos of *Arabidopsis* dry seeds, but were present in the seed coat (Gattolin *et al.*, 2011).

In the embryonic axes, where germination events will later develop, the presence of constitutive aquaporin genes was confirmed. In the axes of dry broad bean seeds, *PIP2;1*, *NIP1;1*, *TIP3;1; TIP3;2*, *TIP1;1*, *TIP2;1* and *TIP2;2* genes were identified (Novikova *et al.*, 2013). Preformed aquaporins *PIP1*, *PIP2*, *TIP3;1* and *TIP2* were detected in embryonic axes of horse chestnut seeds at shedding, during stratification and dormancy release (Obroucheva *et al.*, 2012). Therefore, the embryonic axes are equipped with the aquaporin machinery at the commencement of germination. Seed germination occurs by elongation of embryonic axis cells, triggered by water inflow during imbibition (Obroucheva, 2012). Aquaporin gene expression is low during seed imbibition and only starts when a hydration level of 50–55% (fresh weight) is achieved (Obroucheva, 2012). The main changes in aquaporin composition are initiated at radicle emergence and just after it, when the expression of *PIP* and *TIP* genes is strongly activated. *Arabidopsis* seeds have shown enhanced expression of *PIP1;1*, *PIP1;2, PIP1;4*, *PIP2;1*, *PIP2;2*, *PIP2;6* and *PIP2;7* genes (Willigen *et al.*, 2006). In germinating rice seeds, *PIP1;1* and *PIP1;3* genes were mostly transcribed (H.Y. Liu *et al.*, 2007; C. Liu *et al.*, 2013) whereas in the embryonic axes of broad bean seeds, only *PIP2;1* expression was observed. Along with *NIP1;1*, these genes demonstrated noticeably stable expression of their transcripts; *PIP1* transcripts were not identified (Novikova *et al.*, 2013). Apparently, these aquaporins form water channels, which, if opened, can provide sufficient water entry into embryo axis cells that are beginning to elongate.

Cell elongation is closely related to vacuole formation and enlargement because the vacuoles represent an osmotic compartment responsible for osmotically driven water inflow. Cell vacuolation precedes the beginning of elongation (Obroucheva, 2012; Novikova *et al.*, 2013). In orthodox seeds, vacuolation occurs in the course of restoration of protein-storage vacuoles in embryo cells. Due to the step-by-step proteolysis of reserve proteins deposited in protein-storage vacuoles, these are transformed back to vacuoles. In broad bean seeds, which are characterized by hypogeal germination, such vacuole biogenesis occurs mainly in the hypocotyl, which is the first to elongate (Novikova *et al.*, 2013). In root cells produced *de novo* by the root meristem after radicle emergence, vacuoles are formed from provacuoles that are developed in the lagoons of the endoplasmic reticulum. Both patterns of vacuole biogenesis in embryonic axes lead to vacuole fusion and enlargement prior to the beginning of cell elongation, and culminating in the formation of a central vacuole after radicle emergence, i.e. in rapidly elongating cells. Such vacuolar dynamics is closely related to TIP composition. First of all, it has been observed that the expression of *TIP3;1* and *TIP3;2* genes ceased after radicle emergence, and these proteins disappeared in parallel with the transformation of protein-storage vacuoles to vacuoles (Novikova *et al.*, 2013). Just after radicle emergence, the greatly increased expression of *TIP1;1*, *TIP2;1*, *TIP2;2* isoforms is closely related to accelerated water inflow to rapidly elongating cells.

This trend, which was analysed in embryo axes of broad bean seeds, is supported by observations in other seed species. The cessation of *TIP3* gene expression and progressive disappearance of these proteins after germination was demonstrated in *Arabidopsis* seeds (Willigen *et al.*, 2006; Hunter *et al.*, 2007; Gattolin *et al.*, 2011) and rice aleurone cells (Takahashi *et al.*, 2004; Li *et al.*, 2008). As protein-storage vacuoles were absent from horse chestnut embryonic axes, no TIP3;1 degradation occurred and TIP3;1 remained in the tonoplast at radicle emergence, along with TIP2 (Obroucheva *et al.*, 2012).

The active expression of *TIP1* subtype was correlated with cell growth (Ludevid *et al.*, 1992; Hunter *et al.*, 2007; Gattolin *et al.*, 2009). It started 2 d after radicle emergence (Takahashi *et al.*, 2004; Willigen *et al.*, 2006; Hunter *et al.*, 2007; Li *et al.*, 2008; Gattolin *et al.*, 2011), although TIP1;1 was previously absent from *Arabidopsis* embryos (Willigen *et al.*, 2006; Hunter *et al.*, 2007). The enhanced expression of *TIP1;1* at the transcript level and accumulation of TIP proteins in rapidly elongating cells (Novikova *et al.*, 2013) have confirmed the growth-related changes in TIP composition.

TIP2 isoforms are the next to be actively expressed in growing organs. Their expression is enhanced a bit later than the expression of TIPs. For example, in *Arabidopsis* embryos *TIP2* gene expression increased from 2 d after radicle emergence (Willigen *et al.*, 2006), after the enhancement of *TIP1;1* expression, although TIP2 proteins seemed to be virtually absent from the embryos (Gattolin *et al.*, 2011). In rice seedlings, *TIP2;2* expression increased on the fifth day after germination (Li *et al.*, 2008). According to Hunter *et al.* (2007), expression of *TIP2*s is typical of mature conductive tissues, i.e. of fully elongated cells. Turning back to our data (Novikova *et al.*, 2013), a sharp rise in *TIP2;1* and *TIP2;2* gene expression recorded in 2-cm long embryonic axes coincided with the promoted cell elongation in broad bean roots, and almost completed cell elongation in hypocotyls, which indicated a close relationship between TIP2 accumulation, accelerated water uptake, enlargement of central vacuoles and the transition of fully elongated cells to maturation.

Therefore, the changes in tonoplast aquaporin composition in embryonic axes are closely related to the patterns of vacuole biogenesis before the initiation of cell elongation. Subsequently, the contribution of vacuoles to cell elongation is due to the synthesis of TIP1 and TIP2 aquaporins and apparently to a greater presence of water channels in the tonoplasts. In all growing plant organs, cell proliferation is associated with plasmalemma aquaporins, whereas cell elongation is related to tonoplast aquaporins as well (Obroucheva and Sinkevich, 2010).

An adequate equipment of growing embryonic axis cells with aquaporins is a prerequisite, but still not an assurance, of water-channel participation in water transport, because the channels can be either open (active) or closed (inactive). For studying water-channel activity, the method developed for roots is suitable (Barrowclough *et al.*, 2000; Javot and Maurel, 2002). It consists of water-uptake measurements and treatments with mercurials, which block water channels, with subsequent recovery from mercury inhibition by reducing agents. This method evaluates the water permeability of both cell membranes, but primarily of the plasmalemma. Such experiments with horse chestnut (Obroucheva *et al.*, 2012) and broad bean (Novikova *et al.*, 2013) embryonic axes, both growing by cell elongation, have shown that at radicle emergence water channels are closed, whereas in growing axes they are open. In imbibing *Arabidopsis* seeds also, no inhibiting effect of mercury ions was observed until the onset of growth (Willigen *et al.*, 2006), apparently due to the closed state of water channels. Pea seeds, imbibed in the presence of mercury ions, did not display a change in the rate of water absorption (Veselova and Veselovsky, 2006), which indicates a closed state of water channels. This data can be interpreted in the following way: water uptake until radicle emergence occurs only by diffusion, without any participation of water channels, whereas aquaporin-mediated water inflow in embryo axes begins only when cell elongation is enhanced. According to Veselova and Veselovsky (2006), such slow diffusion of water during imbibition prevents seed hydration from occurring too rapidly, which could result in hypoxia. In ageing pea seeds, the water channels were open, which was evident from accelerated seed imbibition in the presence of mercurials; this effect was explained by damage to the dephosphorylation system and thus the inability to close the water channels (Veselova and Veselovsky, 2006).

The functioning of open water channels in growing embryo axes is closely related to the greatly enhanced water entry into rapidly elongating cells. Their operation occurs in parallel with continuing slow diffusion of water. Accelerated water inflow through water channels is driven by the accumulation of endogenous osmotic solutes in elongating cells due to sucrose import from storage tissues or organs. Therefore, although dry seeds contain the transcripts of aquaporin genes and aquaporins themselves, the operation of water channels starts only after radicle protrusion, to provide enhanced water inflow to growing seedling cells.

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Conflicts of interest

None

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