Acta Agrophysica, 2004, 4(1), 141-148

SOME ASPECTS OF AQUAPORIN MEDIATED WATER TRANSPORT IN PEA SEEDS

Grażyna Plenzler

Department of Physics, University of Agriculture
ul. Wojska Polskiego 38/42, 60-637 Poznań
e-mail:gplenz@au.poznan.pl

A b s t r a c t. Aquaporins are membrane water channels that play fundamental roles in controlling the water contents of cells. An increasing number of aquaporins has been identified on both the vacuolar (tonoplast) and plasma membranes of plant cells. Direct or indirect regulation of aquaporin activity appears to be a mechanism by which plants can control cellular and tissue water movement and adapt to a constantly changing environment. Recent studies have concentrated on the explanation of this mechanism. This paper reports a study, which tends towards recognition of aquaporins role in water flow during pea seeds germination in osmotic stress conditions. It has been observed that the water uptake decreases in the presence of $HgCl_2$, in the germinating medium. That indicates the aquaporins participation in the effect observed because mercuric chloride is known as an inhibitor of water channels. For the seeds germinating in the stress conditions the uptake/loss of water has been found not to be affected by the presence of $HgCl_2$. This result is consistent with the model proposed earlier for cytosolic osmoregulation of water transport activity by aquaporins phosphorylation and dephosphorylation.

K e y w o r d s: membranes, aquaporins (water channels), germination, pea seeds, osmoregulation

INTRODUCTION

Water is indispensable for plant growth and development. There are three possible routes for water flow in living tissues: (i) a roundabout apoplastic route, through the porous cell walls and intercellular space, (ii) the symplastic path (across plasmodesmata) and (iii) the transcellular route, through the cytoplasm, vacuole, and associated membranes [8]. However, because it has not been experimentally possible to distinguish between the symplastic and transcellular contributions to flow, they are collectively determined as the cell-to-cell movement of water [12]. The actual route the water flow takes in a plant appears to depend upon the plant species, developmental state, or the tissue observed. Water

penetration across the cell membrane is achieved by three distinct mechanisms: (i) simple diffusion, (ii) bulk water transport and (iii) aquaporins or water channels which provide a rapid regulated and selective transmembrane route for water movement. Simple diffusion allows the water penetration across the cell membrane. Since the cell membranes are not very permeable to water, there is a relatively high-energy barrier to diffusion. Water penetration by simple diffusion is therefore a slow and temperature dependent process and is not efficient. In bulk transport, water can be passively transported in association with the actively transported ions and other solutes even against an osmotic gradient in some cases.

The plant aquaporins belong to the large MIP (major intrinsic proteins) family of transmembrane channels. An increasing number of aquaporins has been identified on both the vacuolar and plasma membranes of plant cells, with molecular masses of between 26 and 30 kD. The aquaporin polypeptide typically contains six membrane-spanning α helices, with the N- and C- termini both located on the cytoplasmic side of the membrane. For ten years, since when the first aquaporin from plants was cloned and functionally expressed [7], there has been a growing interest in the molecular biology of these proteins and their participation in water penetration across plant membranes.

Water-stress regulated aquaporins might contribute to the tolerance of plants to drought or salinity, but it is not clear whether they are involved in adjusting the overall water transport of the plant, locally facilitate water mobilization during drought [2], or support in adjustment of desiccated tissues to a rapid rehydration after drought [7].

In order to explain some of these problems in the study presented, the water uptake was investigated in osmotic stress conditions realised by the seed germination in media of low water potentials. In these conditions some of the plant reactions (e.g. photoinhibition of white clover and radish seeds germination [10,11]) are revealed or run with greater or lower intensity.

MATERIALS AND METHODS

The pea seeds (cv. Sześciotygodniowy, purchased in 2002 from CN-Poland) were previously selected with great care to have approximately the same mass and volume. Twenty pea seeds in triplicate were exposed to preliminary imbibition on three layers of blotting paper, soaked with distilled water or 1 mM $HgCl_2$ and 1 mM $AgNO_3$ solution in Petri dishes, at 294 K, in darkness. They were subsequently transferred onto a bed saturated with the PEG-6000 solution of different concentration. The osmotic pressure of PEG solutions was adjusted using the formula of Michel and Kaufmann [9]. Germination media containing PEG were routinely replaced every 24h in order to secure a constant osmotic pressure. The water uptake was determined gravimetrically at restricted light access.

Cell expansion can be described by the following mathematical model:

$$G = 1/V\,dV/dt = (mL/(m+L))\,(GP-\Pi_o) \quad (1)$$

which is a modification of the equation first derived by Lockhart [6], where G represents the steady-state relative volume growth rate (water uptake), m – the cell-wall extensibility (cell-wall yielding coefficient), L – the hydraulic conductance, GP – germination potential, and Π_0 osmotic pressure of the internal medium, respectively. The term $mL/(m+L)$ is often referred to as the growth coefficient. The term (GP – Π_o) is the driving force of cell expansion. In another expression $GP = \Delta\Pi - Y$ where $\Delta\Pi$ is the difference in osmotic pressure between the tissue and the medium and Y – is the yield threshold, which is the turgor pressure value that must be exceeded for the cell expansion.

Steady-state water uptake kinetics of seeds incubated in a series of osmotic test solutions were used for constructing curves relating water uptake rates to the osmotic pressure of the external medium. Both k_G and GP may be estimated from these curves as the slope and the intersection with the base line, respectively. The water flux rates as a function of osmotic pressure were determined by incubating seed batches in osmotic test solutions which were adjusted to defined values of osmotic pressure.

RESULTS AND DISCUSSION

Results of gravimetric measurements on the time course of water uptake in pea seeds imbibed on blotting paper soaked with distilled H_2O or 1mM $HgCl_2$ solution are presented in Figure 1. A decrease in the rate of water uptake is shown in seeds treated with mercuric chloride in comparison with the control sample. This result suggests that the observed effect is caused by a slower rate of water uptake resulting from aquaporins inhibition. That effect does not occur for pea seeds treated with $AgNO_3$ (Fig. 2). Pea seeds contain about 8-9% of water in the air-dry state. Most of the water is bound to macromolecules, so that little is available for metabolic reactions. Cellular membranes are disorganised in this state so water channels cannot function. During early phase of imbibition, cell membranes reorganise and aquaporins become able to perform their function. That is why the initial course of peas mass changes with time for the two samples studied is almost the same till a certain time (45h) and after that it grows faster for the sample without $HgCl_2$ (Fig. 1).

Heavy metal ions, such as Hg^{2+}, are known to inhibit water transport activity of some mammalian aquaporins [13], a majority of plant tonoplast [8], and plant plasma membrane aquaporins [5,7,8]. However, it was shown that some plasma membrane aquaporins were mercury insensitive [3]. Mercury inhibition is thought to occur via oxidation of cysteine residue proximal to the aqueous pore, and

subsequent occlusion of the latter by the large mercury ion [1,3]. Mercury inhibition could be reversed in all cases by reducing agents, suggesting that the blocker did not alter the intratissue organisation. The remaining part of the water flux that cannot be inhibited by $HgCl_2$ can be attributed to mercury-insensitive aquaporins and to passive diffusion across the lipid bilayer, and in plant tissue to the apoplastic water route.

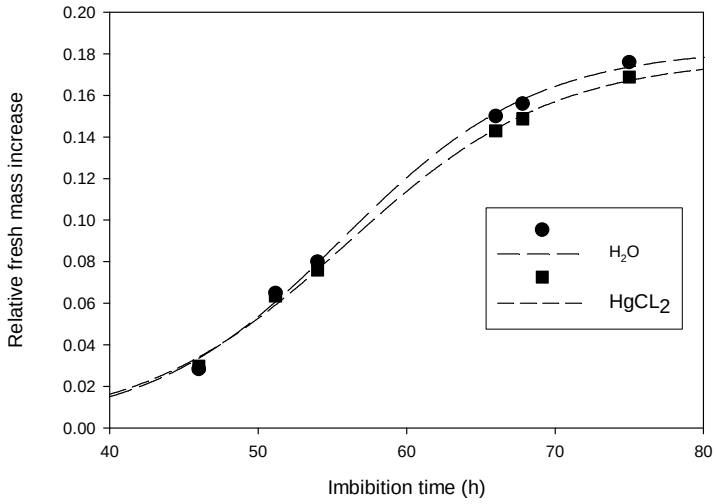

Fig. 1. Effect of $HgCl_2$ on water uptake kinetics in germinating pea seeds

The water transport in seeds in hypertonic conditions, expressed as time dependent water loss, is demonstrated in Figure 3. As can be seen, the water loss by seeds is not influenced by 1mM $HgCl_2$ solution as compared with the control sample. This result is consistent with the earlier proposed model for cytosolic osmo-regulation at the single cell level involving regulation of water transport activity of plasma membrane aquaporins by phosphorylation and dephosphorylation. This permeability is controlled by phosphorylation of serine residue attached to the cytoplasmatic part of aquaporins. This is a calcium-dependent protein-kinase process. In high osmotic potential cells the aquaporins are in phosphorylated state, which facilitates the water movement through the cell membranes. As the potential decreases, the aquaporins in membranes undergo phosphorylation and their permeability decreases. Because the tonoplast water channels are open, the water transport to cytoplasm is possible and water content is protected.

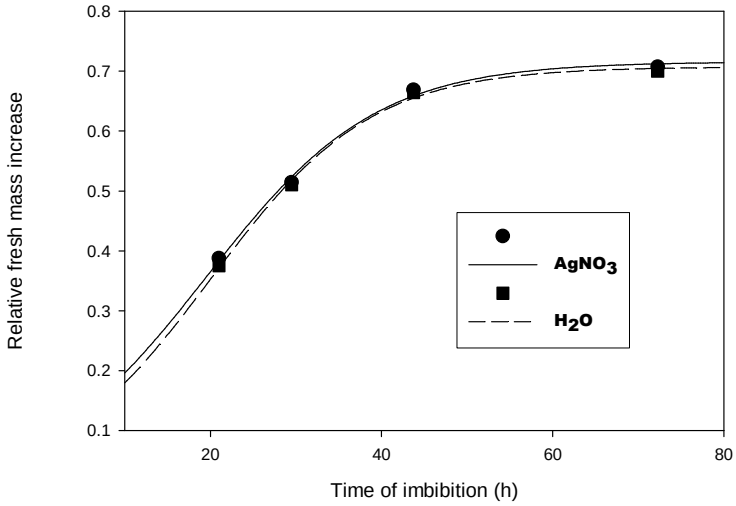

Fig. 2. Effect of $AgNO_3$ on water uptake kinetics in germinating pea seeds. The seeds were preimbibed for 16 h and subsequently transferred to a bed soaked with 1mM nitrate solution

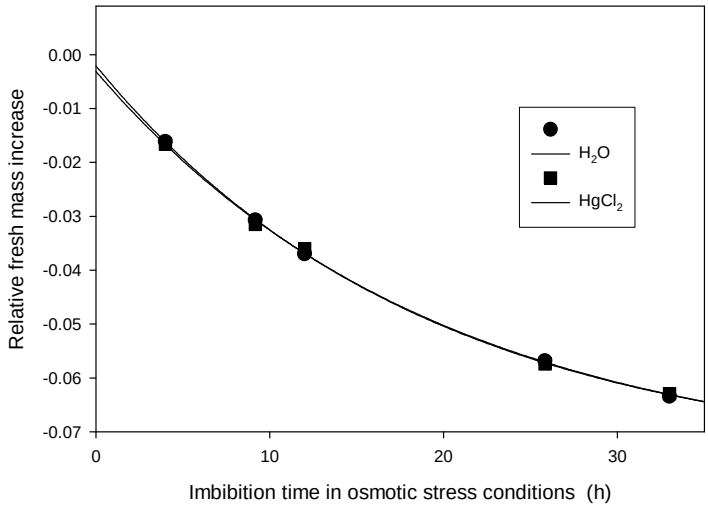

Fig. 3. Effect of $HgCl_2$ on water loss kinetics in pea seeds germinated in osmotic stress conditions. The seeds were preimbibed for 24h

The growth of a plant cell is primary driven by the uptake of water into the cytoplasm and vacuole of the plant cell. The expanding vacuole presses against the cell wall, which leads to the elastic strain of the wall. The volume growth rate of plant cell depends on the difference in the osmotic pressure between the cell internal and external medium and on the mechanical properties of the cell walls. In order to analyse whether the blocking of water channels influences the growth parameters of the germinating seeds, the Lockhart equation describing the cell volumetric growth has been used. Figure 4 shows that the presence of $HgCl_2$ does not cause changes in the relative mass rate vs. osmotic pressure curve, especially in low water potential in blotting paper. In these conditions the plant intracellular osmoregulation takes place and the presence of 1mM $HgCl_2$ solution is not substantial. The calculated value for potential growth and growth coefficient are 12.3 MPa and 5.6 10^{-5} MPa^{-1} h^{-1}.

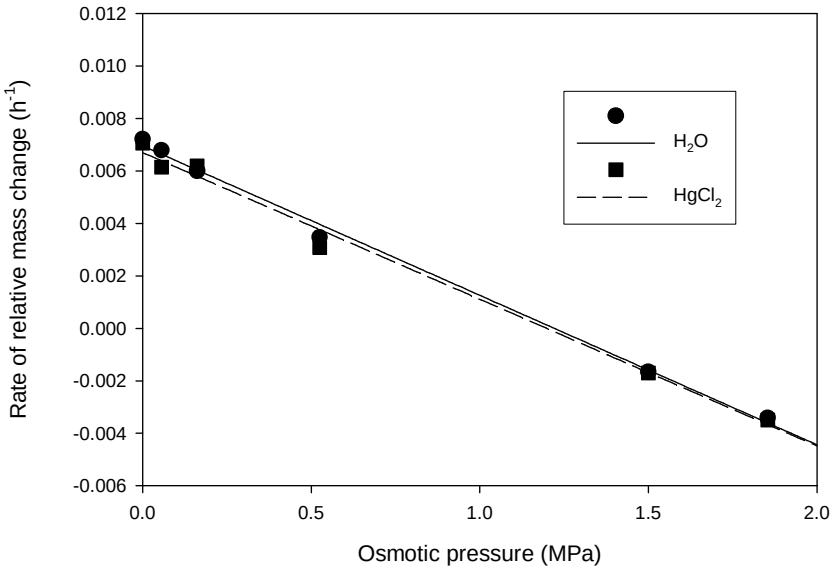

Fig. 4. Effect of osmotic pressure on the rate of water uptake/loss in pea seeds. The seeds after preimbibition for 42h in the presence or in absence of $HgCl_2$ were transferred to a bed soaked with PEG solution at different concentration levels

Although the equation is only the first approximation of the plant cell growth, it is convenient to use for an assessment of the effects of growth-controlling agents such as osmotic conditions in the germinating medium.

CONCLUSIONS

1. The water channels in germinating pea seeds are present.
2. When the seeds germinate in the osmotic stress conditions, $HgCl_2$ does not influence the water loss.
3. The observed effect supports the hypothesis of cytosolic osmoregulation of water status in the seed cell.

REFERENCES

1. **Agre P., Bonhivers M., Borgnia M. J.:** The aquaporins blueprints for cellular plumbing systems. J. Biol. Chem., 273, 14659-14662, 1998.
2. **Chrispeels M. J., Maurel C.:** Aquaporins: the molecular basis of facilitated water movement through living plant cells? Plant Physiol., 105, 9-13, 1994.
3. **Daniels M. J., Mirkov E. T., Chrispeels M. J.:** The plasma membrane of *Arabidopsis thaliana* contains a mercury insensitive aquaporin that is a homolog of the tonoplast water channel protein TIP. Plant Physiol., 106, 1325-1333, 1994.
4. **Daniels M. J., Chaumont F., Mirkov T. E., Chrspeels M. J.:** Characterization of a new vacuolar membrane aquaporin sensitive to mercury at a unique site. Plant Cell, 8, 587-599, 1996.
5. **Kammerloher W., Fischer U., Piecottka G. P.,Schäffner A. R.:** Water channels in the plant plasma membrane cloned by immunoselection from a mammalian expression system. Plant J., 6, 187-199, 1994.
6. **Lockhart J. A.:** Analysis of irreversible plant cell elongation. J.Theor. Biol., 8, 264-275, 1965.
7. **Maurel C, Reizer J, Schroeder J. I., Chrispeels M. J.:** The vacuolar membrane protein γ-TIP creates water specific channels in *Xenopus* oocytes. The EMBO Journal, 12, 2241-2247, 1993.
8. **Maurel C.:** Aquaporins and water permeability of plant membranes. Annu. Rev. Plant Physiol. Plant Mol. Biol., 48, 399-429, 1997.
9. **Michel B. E., Kaufmann M. R.:** The osmotic potential of polyethylene glycol 6000. Plant Physiol., 51, 914-916, 1973.
10. **Niedzwiedz-Siegien I., Lewak S.:** Photoinhibition of white clover seed germination at low water potential. Physiologia Plantarum, 76, 53-56, 1989.
11. **Schopfer P., Plachy C.:** Photoinhibition of radish (*Raphanus sativus* L.) seed germination: control of growth potential by cell-wall yielding in the embrio. Plant Cell and Environment, 16, 223-229, 1993.
12. **Steudle E., Peterson C.:** How does water growth through roots? J. Exp. Bot., 49, 775-788, 1998.
13. **Zhang R., van Hoek A. N., Biwersi J., Verkman A. S.:** A point mutation at cysteine-189 blocks the water permeability of ray kidney water channel CHIP28k. Biochemistry, 32, 2938-2941, 1993.

PEWNE ASPEKTY UDZIAŁU AKWAPORYN W TRANSPORCIE WODY PODCZAS PĘCZNIENIA NASION GROCHU

Grażyna Plenzler

Katedra Fizyki, Akademia Rolnicza, ul. Wojska polskiego 38/42, 60-637 Poznań
e-mail: gplenz@au.poznan.pl

S t r e s z c z e n i e. Akwaporyny to białka tworzące membranowe kanały wodne, które odgrywają fundamentalną rolę w regulacji zawartości wody w komórce. Wzrastająca ciągle liczba akwaporyn jest identyfikowana w roślinach zarówno w membranach tonoplastu jak i plazmolemie. Bezpośrednia lub pośrednia regulacja aktywności akwaporyn jest mechanizmem poprzez który rośliny kontrolują komórkowy i tkankowy przepływ wody i przystosowują się do zmieniających się ciągle warunków otoczenia. Najnowsze prace koncentrują się na dokładnym poznaniu tego mechanizmu. W tej pracy przedstawione zostały badania, zmierzające do poznania roli akwaporyn podczas przepływu wody w nasionach grochu hodowanych w warunkach stresu osmotycznego. Zaobserwowano, że w normalnych warunkach pobór wody obniża się w obecności $HgCl_2$ w podłożu na którym kiełkują nasiona. Wskazuje to na udział akwaporyn w obserwowanych efektach, ponieważ chlorek rtęci jest znany jako inhibitor kanałów wodnych. Dla nasion kiełkujących w warunkach stresu osmotycznego zmniejszony pobór wody lub jej utrata nie są zależne od obecności $HgCl_2$. Takie wyniki są zgodne z wcześniej proponowanym modelem regulacji transportu wody przez akwaporyny w następstwie ich fosforylacji lub defosforylacji przez kinazę białkową zależną od wapnia.

S ł o w a k l u c z o w e: membrany, akwaporyny (kanały wodne), kiełkowanie, nasiona grochu