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PRESENCE OF AQUAPORINS IN DESICCATION PROCESS IN PEA SEEDS

Grażyna Plenzler

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

Abstract. Desiccation of pea seeds has been re-examined by the gravimetric and spectroscopic methods in order to throw more light on the role of aquaporins in this process. Aquaporins are passive and remarkably efficient membrane channels that facilitate permeation of water in the plasma membrane in both plant and animal cells. There have been several attempts to characterise the role of aquaporins at the whole-plant level. In this paper the desiccation of pea seeds, preliminary imbibed at different time intervals and various conditions, was observed. Substantial differences were found in the short-time component of the two-exponential decay of water loss in pea seeds, which was been attributed to apoplastic water transport. No significant differences in desiccation kinetics were found in the long-time component. The effect of $HgCl_2$, which is recognised as non-specific water channel inhibitor, was checked. It was observed that the water uptake decreased in the presence of $HgCl_2$ in the imbibition medium and the presence of $HgCl_2$ did not influence the kinetics of desiccation significantly. This observation was interpreted in terms of the osmoregulation model of water transport by aquaporins.

Keywords: aquaporins (water channels), pea seeds, desiccation, osmoregulation

INTRODUCTION

Plants regulate water exchange in variable environmental conditions. In the cellular water transport the plant membranes play a significant part [1,2,7,10,13]. Small permeability of lipid bilayer on the one hand and surprisingly high rate of water transport in some cell systems (e.g. red blood cells) on the other, make it difficult to explain water transport only on the basis of simple diffusion. Therefore, for a long time, the existence of the proteinaceous water channels, or so-called aquaporins facilitating the movement of water along an osmotic gradient has been suggested. The aquaporin monomer consists of six transmembrane helices linked by five connecting loops. The N- and C-terminal tails of the protein are

always buried in the cytosol, whereas the other side either faces the apoplast or the vacuole [4].

At present there are several hypotheses elucidating the mechanisms involved in plant aquaporin regulation:

1. control of aquaporin gene expression [8,11,14],
2. alterations in subcellular relocalization as a result of osmotic stress [7],
3. control of channel gating by reversible phosphorylation of aquaporins [2,5,13].

Recently, it has been evidenced that different mechanisms by which aquaporins regulate the water exchange do not function individually and the question concerning the time and space integration of these mechanisms is left to be resolved [6,7].

The aim of the study was to identify the factors triggering the mechanisms protecting germinating seeds against the osmotic stress in which the participation of aquaporins could be experimentally verified. The initial state of the seeds studied was differentiated by the time and conditions of preliminary imbibition.

MATERIALS AND METHODS

Commercially produced pea seeds (cv. Sześciotygodniowy, purchased from CN-Poland) were used in all the experiments. The seeds were previously selected with great care to have approximately the same mass and volume. Twenty seeds in triplicate were exposed to preliminary imbibition on three layers of blotting paper, soaked with distilled water in Petri dishes at 294 K in darkness. They were subsequently transferred onto a dry bed for desiccation. Another part of the seeds was hydrated by soaking in distilled water or in water solution of $HgCl_2$ (0.1 mM or 1mM) at the some temperature and light-wanting conditions. The mass loss was estimated gravimetrically. The absorption of leakage solutions obtained after seeds soaking was measured using the Cary 300-Bio spectrophotometer and the fluorescence spectra with the Shimadzu RF-5001 PC spectrophotometer.

RESULTS AND DISCUSION

Figure 1 illustrates the kinetics of preliminary imbibition of pea seeds followed by drying. The seeds were wetted by placing them on a blotting paper soaked with water for the period of 28 or 44 h, till they reached the relative moisture content of 0.76 g H_2O /g of dry mass and 0.96 g H_2O / g of dry mass, respectively. The process of dehydration was observed by measuring the mass loss expressed by the quotient: $\Delta m/m_o = (m_t - m_o)/m_o$, where m_t is the mass of seeds after the time t from experiment beginning, m_0 – the mass of air dry seeds. The curve describing the process can be approximated by the exponential dependence being a sum of two

exponential functions: $Y = Ae^{-k_1 t} + Be^{-k_2 t}$. The constants A and B describe the contribution of each of the two processes to the total process of dehydration (mass loss). The two-exponential fit corresponds to the concurrence of two processes: (i) the process of fast drying characterised by the time constant $2 \cdot 10^{-1}$ h^{-1} and (ii) the process of slow drying characterised by the time constant 8-9 10^{-4} h^{-1}. The processes can be combined with the apoplastic and symplastic transport of water, respectively. The apoplastic transport is extracellular and takes place mainly in the cell walls and intercellular space [12]. In the pea seeds containing cotyledons as the storage material and with a low contribution of intercellular spaces this type of transport occurs to a limited degree. The process of symplastic transport occurs through plasmodesmata and cellular membranes by the mechanism of diffusion and volumetric flow with the participation of aquaporins. The relation between the constants k_1 and k_2 ($k_1 > k_2$) means that the transport of water in the cell walls is much faster than through the protoplast, while the relation between the constants A and B (A<B) means that in pea seeds the contribution of apoplastic transport is smaller than that of symplastic one.

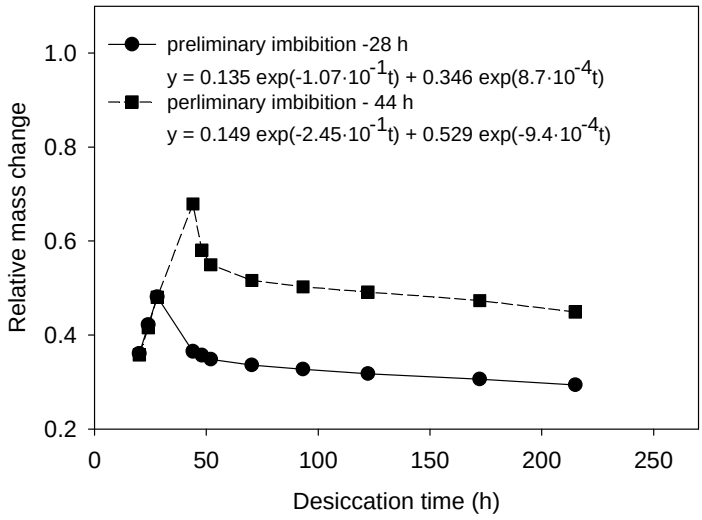

Fig. 1. Kinetics of the preliminary imbibition of pea seeds followed by their drying. The regression equations refer to desiccation kinetics

Different times of initial imbibition leading to final moisture content of 0.96 g H_2O/g of dry mass and 0.76 g H_2O/g of dry mass lead to small changes in the kinetic parameters of the component processes. Figure 2 presents the time changes in the relative mass of pea seeds subjected to preliminary imbibition by immersion in water and then dried on a blotting paper, while Figure 3 shows the

analogous dependence for pea seeds immersed in a water solution of $HgCl_2$. In the two experiments the seeds are imbibed at the extreme values of the osmotic pressure gradient, which may lead to imbibition-related damage caused by rapid penetration of water into the seeds. The extracted components of the complex dessication process presented in Figure 2 slightly differ from each other, leading – in consequence – to slightly different respective values of A, B constants and k_1 and k_2 time constants.

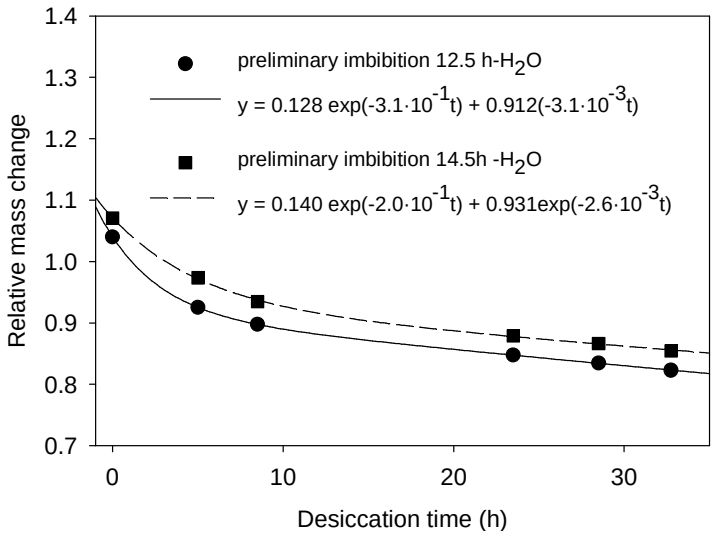

Fig. 2. Kinetics of pea seeds drying after preliminary imbibition by soaking in water

The loss of mass after placing the seeds on dry tissue is smaller if the preliminary imbibition is performed in the presence of $HgCl_2$. It is known that mercury chloride blocks water channels [3,9,10], therefore at the beginning of drying the content of water in the seeds imbibed in a water solution of $HgCl_2$ is slightly lower than that in the seeds imbibed in water. The difference depends on the concentration of $HgCl_2$, as shown in Figure 3. As follows from that figure, no significant qualitative changes take place in the process of drying and are more pronounced for higher concentrations of $HgCl_2$.

The seeds imbibition by soaking in water causes a significant outflow of such substances as simple ions, amino acids and saccharides into the surrounding water. The spectroscopic methods (absorbance and fluorescence measurements) and conductometric method (electric conductivity measurement) can detect the presence of these substances in water solution.

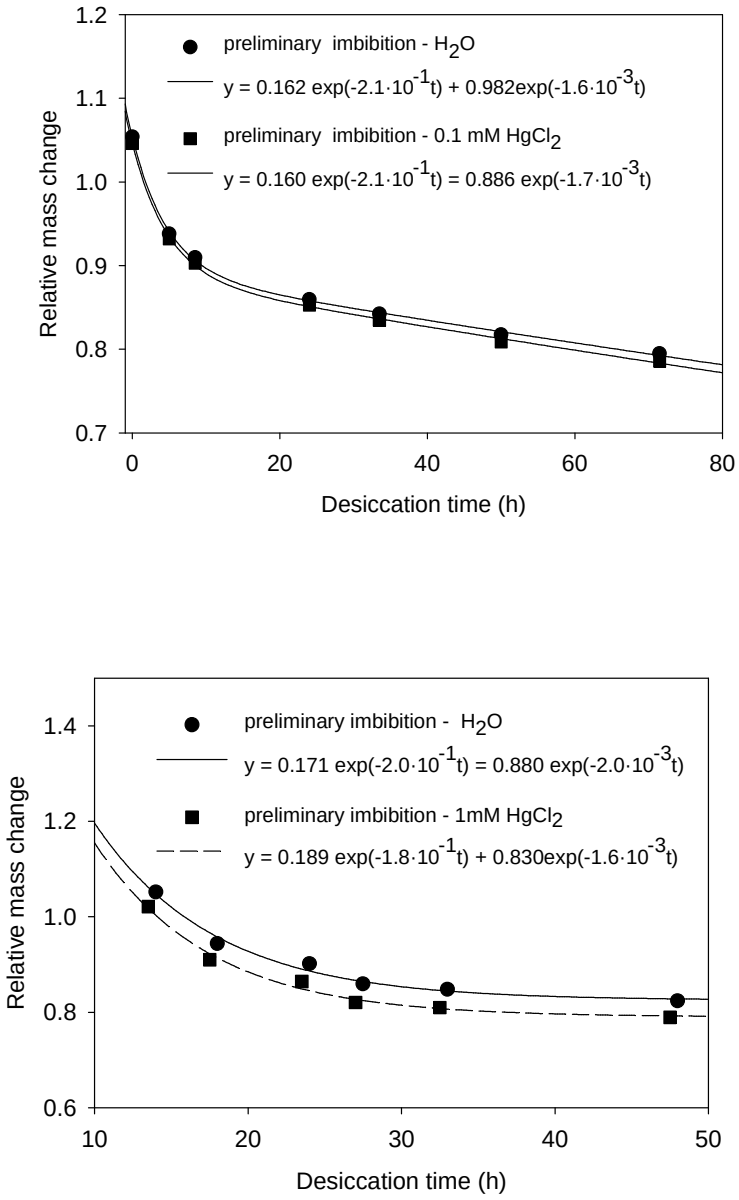

Fig. 3. Kinetics of drying of pea seeds preliminary imbibed in H_2O or solution of $HgCl_2$ in a concentration of 0.1 mM (upper figure) and 1 mM (lower figure)

The absorbance was measured at λ = 280 nm, whereas the fluorescence emission spectrum was measured in the spectral range of 300-550 nm at the excitation of λ_w = 280 nm. The results are presented in Table 1 and in Figure 4. As follows from the higher values of the absorbance A_{280}, at a higher concentration of $HgCl_2$ the outflow of different substances from the seeds is greater. At the wavelength of 280 nm, the main contribution to the absorption comes from proteins and free amino acids.

A comparison of the absorption and fluorescence spectra shows a great contribution of non-fluorescencing component, because an increase in the absorbance of the solutions is greater than the increase in the intensity of fluorescence at the maximum at the wavelength of 354 nm.

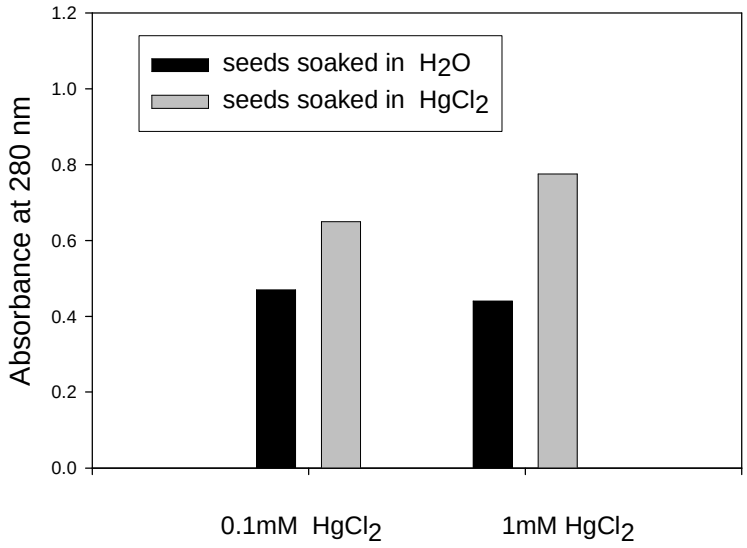

Fig. 4. Absorbance of solutions obtained as a result of outflow of substances from pea seeds imbibed in distilled water or in a water solution of $HgCl_2$

Mercurial and sulphydryl reagents block aquaporins in general, and they may target other membrane transport proteins or act as a metabolic intruder. However, mercury inhibition could be reversed by reducing agents, confirming that the blocker did not alter intratissue organisation [13]. The increase observed in absorbance value in the presence of $HgCl_2$ (Fig. 4) suggested that a greater leakage from seeds may by a result of additional effect of $HgCl_2$ on seeds coat component and other extracellular membrane proteins.

Table 1. A comparison of the absorbance and fluorescence of the solutions obtained as a result of outflow of substances from pea seeds imbibed in H_2O or in water solution of $HgCl_2$

Measurement	Preliminary imbibition H_2O	Preliminary imbibition 1 mM $HgCl_2$
Absorbance $\lambda = 280$ nm	0.440	0.775
Fluorescence $\lambda = 354$ nm	740	855

CONCLUSIONS

1. The kinetics of desiccation of the pea seeds preliminary imbibed in different conditions differs from each other only in the first phase. The experimental desiccation kinetics can be approximated by a two-exponential function characterised by two time constants. The first phase of desiccation can be related to the apoplastic process of water loss by the seeds.

2. The differentiation of preliminary imbibition conditions of pea seeds has a low influence on the slow component of desiccation kinetics. The result can be readily explained assuming that this component is related to the symplastic water transport. In this pathway some extracellular osmoregulation processes in which aquaporins are involved play the dominant part. Their aim is to appease the action of environmental disorders.

3. In the desiccation process the loss of mass of the seeds preliminary imbibed in a solution of $HgCl_2$ was smaller than of those imbibed in water, which is a result of blocking of some aquaporins by mercurial reagent. The desiccation kinetics of the seeds preliminary imbibed in water and in water solution of $HgCl_2$ does not differ significantly. It can be explained by the supposition that in the first case the aquaporins block themselves as a result of osmotic stress and in the second they become blocked by $HgCl_2$.

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OBECNOŚĆ AKWAPORYN W PROCESIE OSUSZANIA NASION GROCHU

Grażyna Plenzler

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

Streszczenie. Zbadano ponownie osuszanie nasion grochu stosując metody grawimetryczną i spektroskopowe w celu ujawnienia udziału akwaporyn w tym procesie. Akwaporyny tworzą kanały membranowe, które ułatwiają transport wody w komórkach roślin i zwierząt Podejmowano już próby scharakteryzowania roli akwaporyn na poziomie całej rośliny lub poszczególnych organów. W tej pracy zbadano proces osuszania nasion grochu nawilżanego wstępnie w zróżnicowanym czasie i warunkach. Stwierdzono istnienie różnic w krótkoczasowej składowej dwu-eksponencjalnego zaniku względnej masy nasion (ubytku masy), którą powiązano z apoplastycznym transportem wody przez nasiona. Nie stwierdzono istotnych różnic w drugiej, długoczasowej składowej, kinetyki osuszania, którą powiązano z transportem symplastycznym. Zbadano również wpływ $HgCl_2$, znanego inhibitora kanałów wodnych. Zaobserwowano, że pobór wody obniża się w obecności chlorku rtęci w ośrodku imbibicyjnym, jednak nie wpływa to istotnie na kinetykę obserwowanego później osuszania nasion. Otrzymane wyniki interpretowano opierając się na modelu komórkowej osmoregulacji stanu wody.

Słowa kluczowe: akwaporyny (kanały wodne), nasiona grochu, osuszanie, osmoregulacja