A TEMPERATURE STUDY OF WATER UPTAKE BY PEA SEEDS

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Abstract. The process of germination depends on the temperature and accessibility of water and oxygen. It starts with the imbibitive absorption of water leading to an increase in the fresh mass and volume of seeds. The kinetics of water absorption by pea seeds at different temperatures and on wetting with water and water solution of mercury chloride $HgCl_2$ were studied. Mercury chloride, known as the inhibitor of water channels (aquaporins), was used for determination of water transport in the conditions of partial water channels blocking. On the basis of the temperature study of water uptake kinetics the hydration constant and activation energy of hydration were calculated. The activation energy of the hydration in the presence of water and water solution of $HgCl_2$ was found to be higher in lower temperatures (280-290K) and was 42 kJ mol^{-1} and 44 kJ mol^{-1}, respectively. At higher temperatures (290-300K), the activation energy decreased to 30 kJ mol^{-1} for the seeds in contact with water solution of $HgCl_2$ and for the seeds in contact with water. The results indicated that the water uptake by pea seeds was markedly temperature-dependent with an intersection point near 293 K. 0.2 mM $HgCl_2$ affected the energy barrier for water transport into the seeds insignificantly.

Keywords: aquaporins (water channels) pea seeds, activation energy, osmoregulation

INTRODUCTION

Inside of the molecular structure of seeds, continuous chemical processes take place. Any symptom of seed vitality is a definite complex of biochemical transformations, practically always connected with water participation.

In seed maturation a desiccation process takes place, which results in creating a glassy state of the intracellular material (Buitink and Leprince 2004). Glass is defined as an amorphous metastable state of high viscosity (10^{12}-10^{14} Pa s) that stops or slows down all chemical reactions requiring molecular diffusion. Thus, glassy state may protect quiescence and stability of seeds, slowing down both

deleterious reactions and changes in seed structure and chemical composition during storage (Leprince and Walters-Vretucci 1995).

Seeds need water for germination. It has been shown that the presence of water channels, called aquaporins, contributes to the overall water transport across the plasma membranes (Chrispeels and Maurel 1994, Maurel 1997, Tyerman *et al.*1999). Mercurial salts, such as $HgCl_2$, inhibit water flow through these channels (Henzler and Steudle 1995, Niemietz and Tyerman 1997, Maurel and Chrispeels 2001).

In the present work, the gravimetric method was used to monitor water uptake by pea seeds at various temperatures and in the presence and absence of $HgCl_2$. From those results some conclusions can be drawn regarding the hydration constant and activation energy of water transport in pea seeds.

MATERIALS AND METHODS

Seeds of *Pisum sativum* cv. Sześciotygodniowy were used in this study after pre-sorting by hand, discarding excessively small, large and damaged seeds. The seeds were placed on three layers of filter paper moistened with 3.5 ml water or aqueous solution of $HgCl_2$ (0.2 mM) in 90 mm Petri dishes. The seeds were incubated in the dark at appropriate temperature and the weight increment was measured. All the experiments were repeated at least twice, with four replications of 10 seeds per treatment. The hydration process was observed by measuring the mass increment expressed by the quotient $\Delta m/m_o = (m_t - m_o)/m_o$, where m_t is the mass of the seeds after time t from the experiment beginning, m_o – the mass of air-dry seeds. The curve describing the process can be approximated by the exponential relationship:

$$\frac{(\Delta m)_t}{m_o} = \frac{(\Delta m)_\infty}{m_o}\left(1 - e^{-kt}\right) \tag{1}$$

where $(\Delta m)_t/m_o$ is the relative mass increment determined after time t from experiment beginning, $(\Delta m)_\infty/m_o$ is the theoretically predicted relative mass increment after time $t \to \infty$. For the description of temperature dependence of the hydration coefficient the Arrhenius equation was used:

$$k = A \exp\left(-E/RT\right) \tag{2}$$

where k is the rate coefficient, A is the frequency factor, E is activation energy (per mol), R is the ideal gas law constant, T is the absolute temperature.

Electroconductivity of leakage solution from seeds after soaking in 50 ml of bi-distilled water was measured by a microcomputer conductivity meter CC-411 (Elmeton), equipped with a conductivity indicator CD-2. Measurements were expressed in mS cm^{-1} g^{-1}.

RESULTS AND DISCUSION

Water uptake isotherms for pea seeds imbibed on blotting paper moistened with water in the temperature range from 280-300 K are presented in Figure 1. As seen from the results, the moisture uptake is high in the initial phase of hydration and then declines gradually to reach maximum moisture content. The hydration rate constant reached the value from 0.0225 h^{-1} to 0.0071 h^{-1} in the studied temperature range. The Arrhenius plot of the logarithm of hydration rate versus 1/T is shown in Figure 3. The activation energy, determined from the slope of the plot, is 42 k J mol^{-1} and 30 kJ mol^{-1} in the temperature ranges of 280-290 K and 290-300 K, respectively.

The effect of the mercurial salt, recognised as a non-specific water channel inhibitor, was examined and the data obtained are presented in Figures 2 and 3. The hydration rate constant in the presence of HgCl$_2$ is, as in the case above, evidently thermally activated. The energy barrier to water movement across the membrane increases inconsiderably, reaching a value of 44 kJ mol^{-1} in the temperature range of 280-290 K and 30 kJ mol^{-1} (as in absence of HgCl$_2$) in the 290-300 K interval.
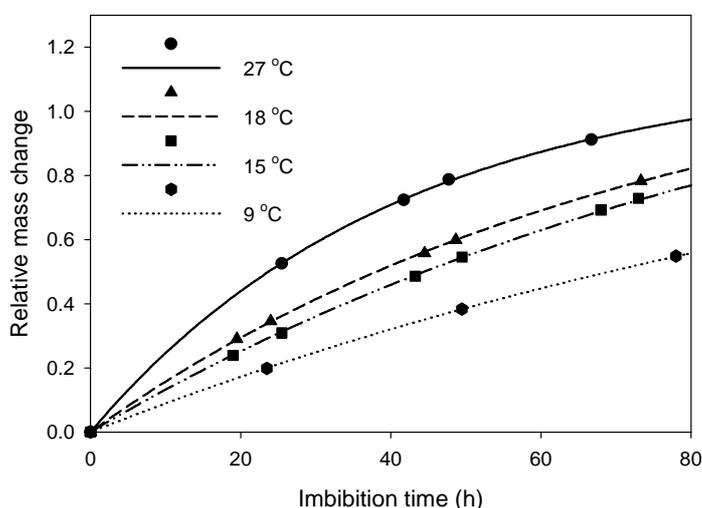
Fig. 1. Kinetics of water uptake by pea seeds imbibed on blotting paper moistened with water
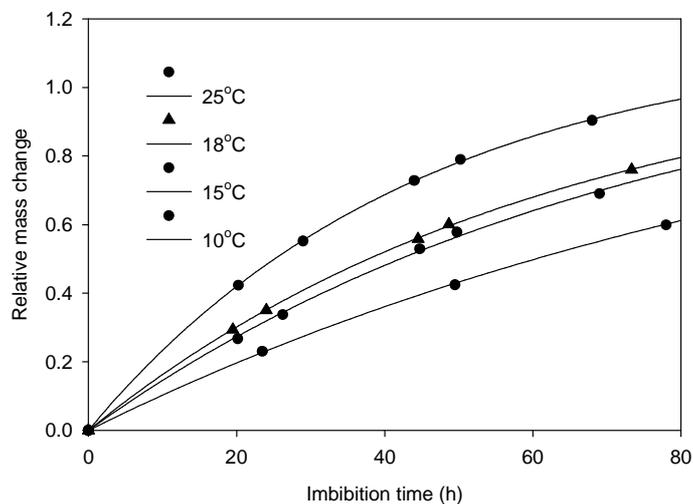
Fig. 2. Kinetics of water uptake by pea seeds imbibed on blotting paper moistened with water solution of $HgCl_2$
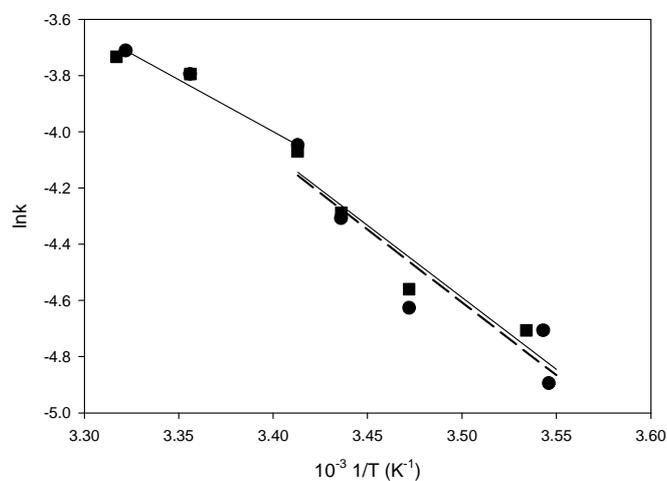
Fig. 3. The Arrhenius plot for pea seeds hydration imbibed on blotting paper saturated with water (circles, solid line) and water solution of $HgCl_2$ (squares, dotted line)

It should be emphasised that the parameters calculated using linear regression are only approximate because of the experimental error in the observed k value (Brauner and Shacham 1997).

For that reason, the obtained results indicated that $HgCl_2$ in 0.2 mM concentration insignificantly affects the water uptake by pea. This is in agreement with literature data (Veselova and Veselowsky 2006) on the influence of a mercury-containing compound (ρ-chloromercuribenzoate) on water uptake in pea seeds. From previously published results (Sigstad and Schabes 2000, Plenzler 2004) it appears that $HgCl_2$ effect on the seeds can be seen in the latter part of imbibition as a decrease in the amount of entering water. The values of activation energy and hydration coefficient relate to the whole water uptake process and therefore the observed $HgCl_2$ effect is insufficiently noticeable.

The activation energy values obtained from water uptake data are higher than those reported in literature (Henzler and Steudle 1995, Niemietz and Tyerman 1997) for water transport through plasmatic membranes in the presence of $HgCl_2$ (48 kJ mol^{-1}) and in its absence (<20 kJ mol^{-1}). The reason for this discrepancy is that the literature values concern separate isolated cells and almost exclusively the phenomenon of water transport through membranes. In such a complex system as a seed the water transport through membranes is just a part of the symplastic transport besides the apoplastic transport and is associated with water sorption by numerous seed colloids (Leprince and Walters-Vretucci 1995, Taylor *et al* 1998). Analysis of water uptake by the seed must take into account all these processes.

The effect of the temperature of seed conditioning on subsequent water uptake was also studied. The results are presented in Figure 4. As indicated by the results, practically no changes are observed in the kinetics of water uptake by the pea seeds conditioned in the air dry state at 267 K, 273 K, 282K and then subjected to water imbibition on filter paper at 294 K. These results mean that for air dry seeds the conditioning temperatures are far enough from the temperature of glass transition in the cytoplasmatic material not to affect its properties, especially its water sorption ability.

Membranes have been suggested to be the primary target for temperature perception and hence the disturbances of the membrane structure and integrity are the primary temperature effects. This coincides with an increase in leakage anions (simple ions, amino acids, proteins and saccharides) from seeds during the early phases of germination. In order to determine the solute leakage of seeds subjected to imbibition at different temperatures the electroconductivity of solutions obtained after submersion of seeds in bi-distilled water (20 seeds per 50 ml H_2O) was measured The results presented in Figure 5 show that with increasing temperature electric conductivity decreases, and only starting from 305 K it increases. This temperature may correspond to a phase transition occurring in the plasma membranes between 303 and 308 K, resulting in increased leakage (Hilhorst 1998).
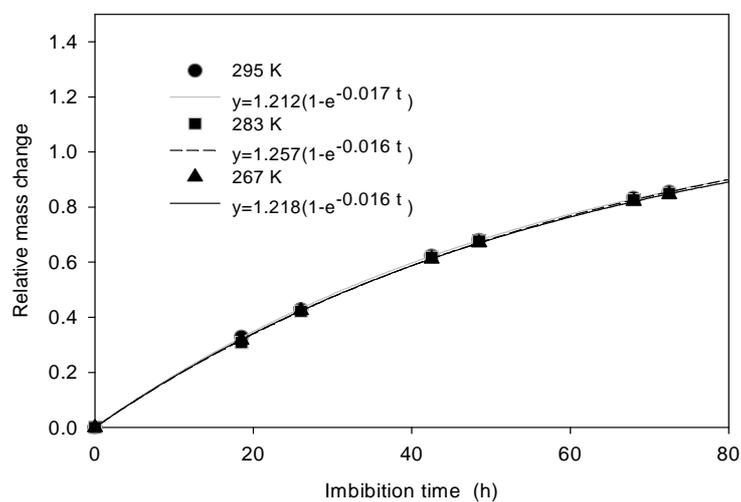
Fig. 4. The effect of air-dry storage temperature on the kinetics of water uptake
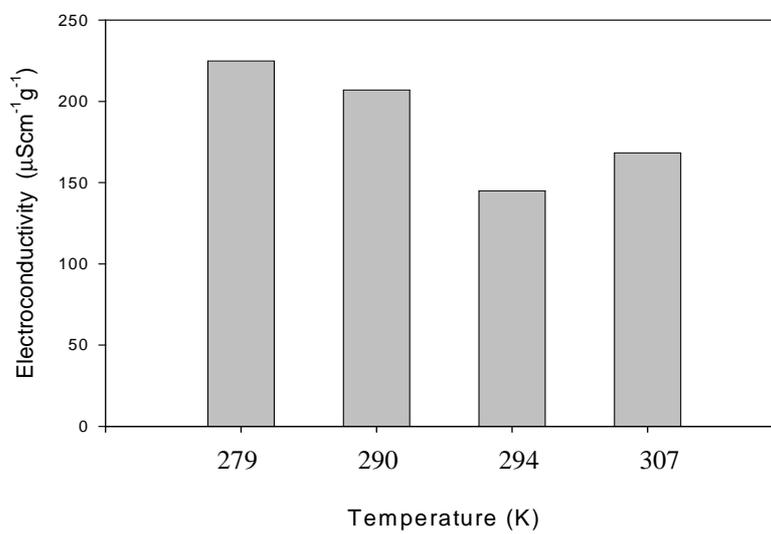

Fig. 5. The effect of air-dry storage temperature on the kinetics of water uptake

CONCLUSIONS

1. As shown by water uptake isotherms and the Arrhenius plot, the activation energy for the water movement in pea seeds is about 44 kJ mol^{-1} in the presence of HgCl$_2$ and 42 kJ mol^{-1} when pea seeds are soaked with water for the temperature range of 280-290 K.

2. The activation energy for water transport decreases to 30 kJ mol^{-1} in higher temperature range (290-300K) both in absence and presence of HgCl$_2$.

3. In air-dry pea seeds similar as in another seeds system there exists a glassy state with small molecular mobility, which ensures seeds survival in the desiccation state.

REFERENCES

Brauner N., Shacham M., 1997. Statistical analysis of linear and nonlinear correlation of the Arrhenius equation constants. Chemical Engineering and Processing, 36, 243-249.

Buitink J., Leprince O., 2004. Glass formation in plant anhydrobiotes: survival in the dry state. Cryobiology, 48, 215-228.

Chrispeels M.J., Maurel C., 1994. Aquaporins: the molecular basis of facilitated water movement through living plant cells? Plant Physiol., 105, 9-13.

Henzler T., Steudle E., 1995. Reversible closing of water channels in *Chara* internodes provides evidence for a composite transport model in the plasma membrane. Journal of Experimental Botany, 46, 199-209.

Hilhorst H.W., 1998. The regulation of secondary dormancy. The membrane hypothesis revisited. Seed Science Research, 8, 77-90.

Leprince O., Walters-Vretucci Ch., 1995. A calorimetric study of the glass transition behaviours in axes of bean seeds with relevance to storage stability. Plant Physiol., 109, 1471-1481.

Maurel C., 1997. Aquaporins and water permeability of plant membranes. Annu. Rev. Plant Physiol. Plant Mol. Biol., 48, 399-429.

Maurel C., Chrispeels M.J., 2001. Aquaporins. A molecular entry into plant water relations. Plant Physiology, 125, 135-138.

Niemietz C.M., Tyerman S.D., 1997. Characterization of water channels in wheat root membrane vesicles. Plant Physiol., 115, 561-567.

Plenzler G., 2004. Some aspects of aquaporin mediated water transport in pea seeds. Acta Agrophysica, 4(1), 141-148.

Sigstad E.E, Schabes F.I., 2000. Isothermal microcalorimetry allows detection of aquaporins in quinoa seeds. Thermochimica Acta, 349, 95-101.

Steudle E., Frensch J., 1996. Water transport in plants: Role of the apoplast. Plant Soil, 187, 67-79.

Taylor A.G., Allen P.S., Bennett M.A., Bradford K.J., Burris J.S., Misra M.K., 1998. Seed enhancements. Seed Science Research, 8, 245-256.

Tyerman S.D., Bohnert H.J., Maurel C., Steudle E., Smith J.A.C., 1999. Plant aquaporins: their molecular biology, biophysics and significance for plant water relations. Journal of Experimental Botany, 50, 1055-1071.

Veselova T.V., Veselovsky V.A., 2006. Possible involvement of aquaporins in water uptake by pea seeds differing in quality. Russian Journal of Plant Physiology, 53, 96-101.

TEMPERATUROWE BADANIA KINETYKI POBIERANIA WODY PRZEZ NASIONA GROCHU

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Streszczenie. Temperatura, dostęp wody i tlenu to podstawowe czynniki warunkujące przebieg procesu kiełkowania. Rozpoczyna je imbibicyjne pobieranie wody, co prowadzi do zwiększenia świeżej masy i objętości nasion. W pracy przedstawiono kinetyki pobierania wody przez nasiona grochu określane w różnych temperaturach przy nawilżaniu wodą oraz wodnym roztworem $HgCl_2$. Chlorek rtęci, znany jako inhibitor kanałów wodnych (akwaporyn), został wykorzystany do badania zjawiska transportu wody w warunkach ograniczenia udziału kanałów wodnych przez ich zablokowanie. Na podstawie badań temperaturowych kinetyki pobierania wody określono wybrane parametry kinetyczne: stałą hydratacji oraz energię aktywacji tego procesu Zarówno dla nasion poddanych imbibicji w obecności $HgCl_2$ jak i nasion pobierających wodę wyłącznie na podłożu wodnym energia aktywacji jest większa w przedziale niższych temperatur (280-290 K) i wynosi odpowiednio: 44 $kJ \cdot mol^{-1}$ i 42 $kJ \cdot mol^{-1}$. Dla wyższych temperatur z przedziału (290-300 K) energia aktywacji zmniejsza się i wynosi 30 $kJ \cdot mol^{-1}$ zarówno dla nasion poddanych imbibicji w obecności $HgCl_2$ jak i dla nasion kiełkujących wyłącznie na podłożu wodnym. Otrzymane wyniki wskazują, że pobieranie wody przez nasiona grochu jest silnie zależne od temperatury i ujawnia punkt przegięcia w pobliżu 293 K. O,2 mM $HgCl_2$ nieznacznie zmienia barierę energetyczną dla transportu wody do nasion.

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